



16SrRNA-based bacterial community profiling of *Haemonchus contortus* infecting Dohne Merino sheep using Next- Generation Sequencing

T Mafuna



orcid.org 0000-0002-4978-5692

Dissertation submitted in fulfilment of the requirements for
the degree *Master of Science in Zoology* at the North-West
University

Supervisor:	Prof. MMO Thekiso
Co-supervisor:	Dr. AM Tsotetsi- Khambule
Co-supervisor:	Dr. P Soma

Graduation May 2019

29807034

DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my original work and that I have not previously in its entirety or in part submitted at any university for a degree. I furthermore cede copyright of the dissertation in favour of the North-West University.

Signature:

Date:

DEDICATION

This work is dedicated to my loving father Mpfariseni Edison Mafuna and mother Azwihangwisi Lucy Mafuna, for always believing in me and always wanting the best for me, and to my beautiful daughter Washu Sherilyn Mafuna and mother of my child Zwavhudi Laura Mpilo and my siblings Tshifhiwa Maureen Mafuna, Lufuno Mafuna and Zeldah Mafuna. You all been my source of inspiration and strength. Thank you so much for the love and support you gave me.

I LOVE YOU!!!

GOD BLESS YOU

ACKNOWLEDGEMENTS

First and foremost, all praises are due to the Almighty God who enabled me to complete this research work successfully and to submit the dissertation leading to the degree of Master of Science in Zoology.

I express my humble thanks to my supervisors, Prof. Oriel M. M. Thekiso, Dr. Pranisha Soma, Dr. Ana M. Tsotetsi-Khambule and Dr. Charles Hefer for their input and mentoring throughout the course of the study and for ensuring that this project is completed. Words cannot fully express how thankful I am. To all my supervisors, thank you for your patience, guidance, advice and encouragement throughout this research project. You have modelled what it is to be an academic and researcher, your phenomenal support in this journey is much appreciated.

To Dr. Rian Pierneef, thank you for helping me with all the Bioinformatics and ensuring that all the analysis of this project is complete, without you this project would not have been completed. Words cannot fully express how thankful I am. I express my humble thanks to Dr. Farai Muchadeyi for your assistance.

I also wish to thank my father Mpfariseni Edison Mafuna, my mother Azwihangwisi Lucy Mafuna, beautiful daughter Washu Sherilyn Mafuna, mother of my child Zwavhudi Laura Mpilo and my siblings Tshifhiwa Maureen Mafuna, Lufuno Mafuna and Zeldah Mafuna for encouragement and tremendous support throughout the entire period of my studies. Through the ups and downs of this research project my family have been a constant source of happiness and joy for that I am truly thankful. This dissertation would not have been completed without their on-going support. Special thanks to all my colleagues and staff at the ARC-BTP for your support, love and encouragement throughout this project, SALUTE.

Thank you to the many people who have assisted with this research project, the Wauldby Farm (Robbie Blaine) for supplying us with the Dohne Merino sheep and the Tandala abattoir for slaughtering the animals for us to collect samples. There are many people I would like to thank for their assistance in sample collection. I would like to pay special mention to Ms Madeleine Ramantswana and Mr Michael Rampedi. Many

thanks to the Agricultural Research Council-Biotechnology (ARC-BTP) Core team for their assistance in sample sequencing.

I thank the ARC-BTP/API/OVI for funding my research and for allowing me to use their facilities.

God Bless You All, Thank you

ABSTRACT

Gastrointestinal parasitism causes major economic losses in most livestock species including small ruminants' production throughout the world. The control of gastrointestinal nematode (GIN) parasites is mostly based on administration of anthelmintic drugs. Unfortunately, the extensive use of anthelmintic drugs leads to the evolution of drug resistance in GIN parasites. Hence, alternative control measures are needed to effectively control GIN parasites. A novel approach based on biocontrol using GIN parasites symbiotic microbiota has been suggested to limit the use of chemical based treatments. In the present study, Illumina MiSeq sequencing technology was exploited to study the bacterial communities associated with the adult *Haemonchus contortus* worms and that of its predilection site, the abomasum, with the long-term goal of manipulating them to control these GIN parasites.

The abomasum contents and adult *H. contortus* were collected from the 7 abomasum of 10 slaughtered Dohne Merino sheep collected from Wauldby farm. Adult male *H. contortus* specimens were identified and distinguished from females using morphological analysis (i.e., body length, colour, spicules and valval morphology). The bacterial community of both the adult *H. contortus* worms and the abomasum were determined with the aid of Illumina Miseq platform and metagenomics analysis.

High bacterial diversity was observed from the adult *H. contortus* and the abomasum content samples. A total of 26 bacterial phyla were found in both the adult *H. contortus* worms and the abomasum, with *Firmicutes* (45%), *Bacteroidetes* (26%) and *Actinobacteria* (7%) being the most abundant phyla present in the abomasum content. *Proteobacteria* (94%), *Firmicutes* (3%) and *Bacteroidetes* (1%) were the most abundant phyla in the adult female *H. contortus* and for adult male *H. contortus* the most abundant phyla were *Proteobacteria* (57%), *Firmicutes* (24%) and *Bacteroidetes* (8%).

The present study also elucidated the core genera *Succiniclasticum* (5%), *Rikenellaceae* RC9 gut group (4%) and *Candidatus Saccharimonas* (4%) which were the most predominant assigned genera in the abomasum.

The predominant assigned genera of the adult female *H. contortus* were *Escherichia-Shigella* (28%), *Vibrio* (11%) and *Halomonas* (6%). The dominant genera assigned in the adult male *H. contortus* were *Vibrio* (15%), *Escherichia-Shigella* (8%) and *Halomonas* (8%).

Moreover, our results revealed the bacterial genera including *Lysinibacillus* which can produce nematicidal volatile compounds with activities against nematodes. This study has pioneered detection of bacterial genera of medical and veterinary significance by metagenomics in the abomasum content of the Dohne Merino sheep and adult male and female *H. contortus* in South Africa.

Overall, the present study provides insight into the bacterial community composition in the adult male and female *H. contortus* worms and the abomasum which is highly diverse and needs to be studied further to exploit the complex interactions of different GIN parasites microbiota with the host, which has, and will continue to offer considerable potential for better understanding a wide-variety of devastating animal diseases.

Keywords: Metabarcoding, Gastrointestinal nematode parasites, *H. contortus*, metagenomics, next-generation sequencing and microbiota

TABLE OF CONTENTS

DECLARATION.....	I
DEDICATION	II
ACKNOWLEDGEMENTS	III
ABSTRACT	V
LIST OF FIGURES	XIII
LIST OF TABLES.....	XVII
ABBREVIATIONS	XVIII
CHAPTER 1	1
GENERAL INTRODUCTION AND LITERATURE REVIEW	1
1.1. Introduction	1
1.2. Bacteria.....	4
1.3. Symbiosis	5
1.4. Symbiotic bacteria.....	5
1.5. The gastrointestinal microbiota (gut symbionts).....	6
1.6. Gut bacterial symbionts of gastrointestinal nematode parasites	8
1.6.1. The microbiota-gastrointestinal nematode parasites symbiosis complexes ..	8
1.7. Symbionts of mammalian gastrointestinal nematode parasites	9
1.7.1. Filaria	9

1.7.2. <i>Ascaris suum</i>	9
1.7.3. <i>Trichuris muris</i>	10
1.7.4. <i>Haemonchus contortus</i>	10
1.8. Composition of the gastrointestinal nematode microbiota.....	11
1.9. Functions of gastrointestinal nematode microbiota	13
1.9.1. Nutrient metabolism	13
1.9.2. Immunomodulation.....	14
1.9.3. Antimicrobial protection	15
1.10. Molecular and microbiological based approach to studying the gastrointestinal nematode microbiota	16
1.10.1. Culture-dependent techniques	16
1.10.2. Culture-independent techniques.....	17
1.11. Colonization of gastrointestinal nematode parasites by microbiota.....	20
1.12. Modulation of host intestinal microbiota by gastrointestinal parasites infection	21
1.13. The importance of gastrointestinal nematode parasite-microbiota interactions for the host.....	23
1.14. Gastrointestinal nematode parasites.....	24
1.15. Biological control of gastrointestinal parasites	26
1.16. Genetic resistance to gastrointestinal nematode parasites in sheep	27
1.16.1. Genetic control of gastrointestinal nematode parasites.....	27

1.16.2. Non-specific response mechanisms to gastrointestinal nematode parasites infections	27
1.16.3. Gastrointestinal nematode parasites antigens and host antibodies.....	28
1.16.4. Immune response cells associated with resistance	28
1.17. Gastrointestinal nematode parasites of veterinary importance in Dohne Merino sheep	29
1.17.1. <i>Haemonchus contortus</i>	29
1.17.1.1. Morphology	29
1.17.1.2. Life cycle.....	30
1.17.1.2. Clinical signs, treatment and control of haemonchosis	31
1.17.1.3. Diagnosis of haemonchosis in sheep	31
1.18. History of domestic sheep and Dohne merino breed	32
1.18.1. Domestic sheep origin	32
1.18.2. Dohne merino breed.....	32
1.19. The 16s metabarcoding	33
1.20. Next-generation sequencing (NGS).....	34
1.20.1. Roche 454 sequencing technology	35
1.20.2. Ion semiconductor sequencing.....	36
1.20.3. Illumina (solexa) sequencing technology.....	36
1.20.4. Solid™ sequencing technology	36

1.20.5. Oxford nanopore minion sequencing technology	36
1.21. Bioinformatics	37
1.21.1. Bioinformatics analysis of 16s metabarcoding data.....	38
CHAPTER 2	39
PROBLEM STATEMENT AND RATIONALE	39
2.1. Problem statement.....	39
2.2. Aim, objectives and hypothesis.....	40
2.2.1. Aim	40
2.2.2. Objectives.....	40
2.2.3. Research hypothesis.....	40
2.3. Outline of dissertation	41
CHAPTER 3	42
MATERIALS AND METHODS	42
3.1. Study site	42
3.2. Study approach.....	43
3.3. Experimental animals.....	44
3.4. Procedure for collection of abomasum content and adult <i>H. contortus</i> recovery from the abomasum.....	45
3.5. Morphological identification of the adult male and female <i>H. contortus</i> worms..	46
3.6. Bacterial community profiling of abomasum content, adult <i>H. contortus</i> worms	47

3.6.1. Sterilisation of adult <i>H. contortus</i> surface	47
3.6.2. Ruling out microbial contamination on the adult <i>H. contortus</i> surfaces	47
3.6.3. Microbial DNA extraction	47
3.6.3.1. DNA extraction from abomasum content	47
3.6.3.2. DNA extraction from adult male and female <i>H. contortus</i> worms	49
3.6.4. DNA amplification and amplicon library preparation	49
3.6.4.1. Amplicon PCR	49
3.6.4.2. PCR clean-up	50
3.6.4.3. Index PCR	51
3.6.5. High-throughput sequencing	52
3.6.5.1. Library quantification, normalization, and pooling	52
3.6.5.2. Library denaturing and miseq sample loading	52
3.7. Bioinformatics analysis	53
3.8. Statistical analysis	54
3.9. Ethical approval	54
CHAPTER 4	55
RESULTS	55
4.1. Morphometrics of <i>H. contortus</i>	55
4.2. Molecular analysis of the adult male and female <i>H. contortus</i>	57

4.3. Ruling out microbial contamination associated with the adult <i>H. contortus</i> surfaces.	57
4.4. Microbial DNA amplification by Polymerase Chain Reaction	58
4.5. Diversity index analysis of bacterial community	59
4.5.1. Alpha diversity estimate of microbiota associated with abomasum content, adult male and female <i>H. contortus</i>	61
4.5.2. Beta diversity estimate of microbiota associated with the abomasum content, adult male and female <i>H. contortus</i>	64
4.6. The microbiota composition and abundance associated with abomasum content and adult male and female <i>H. contortus</i>	67
4.7. Unique and shared microbiota associated with the abomasum content, adult male and female <i>H. contortus</i>	75
4.8. The relationship between <i>H. contortus</i> microbiota and its predilection site, the abomasum content microbiota.....	81
CHAPTER 5	84
DISCUSSION, CONCLUSION AND RECOMMENDATIONS	84
5.1. Morphological and molecular identification of <i>H. contortus</i>	84
5.2. Investigation of parasite surfaces for abomasal microbial contamination and microbial dna amplification by Polymerase Chain Reaction.....	86
5.3. Microbiota associated with the abomasum content of the dohne merino sheep naturally infected with <i>H. contortus</i> field strains	87
5.4. Microbiota of <i>H. contortus</i> adult worms have a diverse and core microbiota different to their host	89

5.5. Unique and shared microbiota associated with the abomasum content and adult <i>H. contortus</i> worms.....	91
5.6. The relationship between adult <i>H. contortus</i> microbiota and its predilection site, the abomasum microbiota.....	93
5.7 conclusion.....	94
5.8 Major implications and limitations of the study.....	95
5.8. Recommendations.....	95
REFERENCES	96
APPENDIX	130

LIST OF FIGURES

Figure 1. Bar graph depicting data from study of sheep gut microbiota.....	8
Figure 2. Illustration depicting factors that may be important in defining microbiota composition.....	13
Figure 3. A simplified tree of life based on rRNA sequence comparisons.....	23
Figure 4: Pictures depicting morphological features of <i>H. contortus</i>	30
Figure 5. The life-cycle of <i>H. contortus</i> , from egg to the adult stage.....	31
Figure 6. A picture of South African Dohne Merino sheep breed raised for meat and wool purposes.....	34
Figure 7. Satellite map of the Stutterheim District, Eastern Cape, South Africa.....	43
Figure 8. Flowchart of procedures undertaken during the course of the study.....	44
Figure 9. A picture depicting adult <i>Haemonchus</i> spp. recovered from Dohne Merino sheep in Eastern Cape, South Africa.....	46
Figure 10. A picture depicting Zeiss Axio observer microscope used for morphological identification of adult <i>H. contortus</i>	47
Figure 11. A picture of depicting the 16S ribosomal RNA (rRNA) gene contains of nine hypervariable regions enclosed by regions of more conserved sequence.....	51
Figure 12. A picture of Illumina MiSeq sequencing platform used for sequencing.....	54
Figure 13. Picture depicting adult <i>H. contortus</i> characters.....	57
Figure 14. Gel electrophoresis image depicting <i>H. contortus</i> PCR amplicons at 260 bp.....	58

Figure 15. Gel electrophoresis image depicting washed and unwashed <i>H. contortus</i> PCR amplicons at 470bp.....	58
Figure 16. Gel electrophoresis image depicting abomasum content and <i>H. contortus</i> PCR amplicons at 500 bp.....	59
Figure 17. Alpha rarefaction curves of abomasum content, adult male and female <i>H. contortus</i> on 16S rRNA gene sequence of abomasum and parasite microbiota at 97% identity.....	60-61
Figure 18. Alpha diversity analysis of abomasum content, adult male and female <i>H. contortus</i> based on 16S rRNA gene sequence of abomasum and parasite microbiota at 97% identity.....	62-63
Figure 19. Principal coordinate analysis (PCoA) based on unweighted UniFrac distances and weighted UniFrac distances.....	65-66
Figure 20. Krona plot for taxonomic abundance of the abomasum content microbiota at the phylum level.....	68
Figure 21. Krona plot for taxonomic abundance of adult male <i>H. contortus</i> microbiota at the phylum level.....	69
Figure 22. Krona plot for taxonomic abundance of adult female <i>H. contortus</i> microbiota at the phylum level.....	70
Figure 23. Krona plot for taxonomic abundance of abomasum content microbiota at the family level.....	71
Figure 24. Krona plot for taxonomic abundance of adult male <i>H. contortus</i> microbiota at the family level.....	72
Figure 25. Krona plot for taxonomic abundance of adult female <i>H. contortus</i> microbiota at the family level.....	73

Figure 26. Krona plot for taxonomic abundance of the abomasum content microbiota at the genus level.....	74
Figure 27. Krona plot for taxonomic abundance of the adult male <i>H. contortus</i> microbiota at the genus level at the genus level.....	75
Figure 28. Krona plot for taxonomic abundance of the adult female <i>H. contortus</i> microbiota at the genus level at the genus level.....	76
Figure 29. Venn diagram depicting number of unique and shared OTUs associated with abomasum content, adult male and female <i>H. contortus</i>	77
Figure 30. Krona plot depicting unique bacterial OTUs of the abomasum content microbiota at the genus level.....	78
Figure 31. Krona plot depicting unique bacterial OTUs of the adult male <i>H. contortus</i> microbiota at the genus level.....	79
Figure 32. Krona plot depicting unique bacterial OTUs of the adult female <i>H. contortus</i> microbiota at the genus level.....	80
Figure 33. Microbiota abundances shared between the abomasum content, adult male and female <i>H. contortus</i>	81
Figure 34. Bar chart depicting relative microbiota abundances of the abomasum content, adult female and male <i>H. contortus</i>	83
Figure 35. Heatmap of the top 50 ranked <i>H. contortus</i> microbiota depicting relative abundance of the microbiota at the genus level.....	84

LIST OF TABLES

Table 1: Brief description of culture-dependent techniques.....	17
Table 2: Brief description of culture-independent techniques.....	19-20
Table 3. Common gastrointestinal nematode parasite in small ruminants.....	26
Table 4. List of animals which were sampled in the present study.....	45
Table 5. Summary of primers used in the current study.....	51
Table 6. Percentage of total number of <i>H. contortus</i> species identified in the present study.....	56
Table 7. Overview of alpha diversity metrics for the abomasum, adult male <i>H. contortus</i> and adult female <i>H. contortus</i> microbiota.....	64
Table 8. Overview of beta diversity for abomasum, adult male <i>H. contortus</i> and adult female <i>H. contortus</i> microbiota.....	67
Table 9. Bacterial genera with nematicidal activities against nematodes.....	82

ABBREVIATIONS

°C	: Degree Celsius
µl	: microlitre
µm	: micrometre
AMP	: Antimicrobial protein
ARC-BTP	: Agricultural Research Council-Biotechnology Platform
BHI	: Brain heart infusion
bp	: Base pairs
CDRs	: Cytosolic DNA receptors
CLRs	: C-type lectin receptors
DADA2	: The Divisive Amplicon Denoising Algorithm 2
DNA	: Deoxyribonucleic acid
ELISA	: Enzyme-linked immunosorbent assay
EPN	: Entomopathogenic nematode
FAO	: Food and Agriculture Organization of the United Nations
g	: Grams
GALT	: Gut associated lymphoid tissues
GIN	: Gastrointestinal nematode
GIT	: Gastrointestinal tract
Kb	: Kilo bases
L1	: First larval stage
L2	: Second larval stage
L3	: Infective third stage larvae
MAMPS	: Microbial-associated molecular patterns
MAP	: MinION Access Programme
Mgcl ₂	: Magnesium chloride
ng/µl	: Nano grams per microlitres
RPM	: Revolutions per minute
NGS	: Next-generation sequencing
NOD	: Nucleotide-binding oligomerization domain
PBS	: Phosphate buffered saline
PCoA	: Principal coordinate analyses
PCR	: Polymerase chain reaction
QIIME	: Quantitative Insights Into Microbial Ecology
RLRs	: RIG-I-like receptors
s	: Second
S.A.	: South Africa
SCFA	: Short chain fatty acids
TLRs	: Toll-like receptors
WHO	: World Health Organization

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

Microbiota are a complex community of microorganisms that vary in their niches occupied, species composition and their influence on various environments (Stubbendieck *et al.*, 2016). These microorganisms live in a close symbiotic relationship with multicellular eukaryotic host organisms including nematodes, insects, plants, animals and humans (Kreisinger *et al.*, 2015). The microbiota are composed and dominated by microorganisms including bacteria, fungi, archaea, protists and viruses. The symbiotic relationship between microbes and their host ranges from commensalism, mutualism, parasitism and fatal pathogenic infections (Moya *et al.*, 2008; Sinnathamby *et al.*, 2018). The most extensively researched symbionts of nematodes are those of parasitic plant nematodes and filarial worms, because of their agricultural, biological and medical significance (Kanfra, 2018; Sinnathamby *et al.*, 2018). The most researched symbionts of small ruminants are those of domestic sheep and goats although there are other nematodes-bacterial and small ruminants-bacterial symbioses of agricultural interest (Huang *et al.*, 2016; Wang *et al.*, 2017).

Eukaryotic organisms such as small ruminants and GIN parasites have a wide diversity of gastrointestinal microbiota that are important in nutrition, metabolism and immunity of their host (Kreisinger *et al.*, 2015). The gastrointestinal tract (GIT) of these organisms has a very unique ecosystem of microorganisms, which is referred to as the 'microbiota' (Kinross *et al.*, 2011; Sinnathamby *et al.*, 2018). The gastrointestinal microbiota associated with domestic animals such as dogs, cats and livestock, and their associated products as well as their faecal matter play a crucial role in the structure, diversity and development of human microbial communities when people interact with these animals in a community setting via animal husbandry activities (Mosites *et al.*, 2017). The symbiotic relationship between microbiota and their GIN parasites host has grown rapidly in recent years with recent published studies (El-Ashram & Suo, 2017; Sinnathamby *et al.*, 2018) conclusively demonstrating that GIN parasites in particular *H. contortus* harbour microbiota associated with the nematodes

egg stages, larval stages and adult worms, with each of these microbes performing respective functions in the GIN parasites life cycle stages (El-Ashram & Suo, 2017; Sinnathamby *et al.*, 2018). Apart from *H. contortus*-microbiota symbioses, there are few reports on microbiota associated with *Trichuris muris* (Hayes *et al.*, 2010) and *Ascaris suum* (Shahkolahi & Donahue, 1993).

Moreover, these GIN parasites-microbiota symbioses impacts on how food resources are utilised by the host, maintenance of the homeostasis of the digestive tract, and host health as well as GIN parasites and microbiota (El-Ashram & Suo, 2017; Fisher *et al.*, 2017). However, previous studies on GIN parasites-microbiota interactions have predominantly focused on establishing microbiota in the GIT of the host and the microbiota important functional roles in metabolism, nutrition and immunity as well as the effect of GIN parasites on host microbiota (Glendinning *et al.*, 2014). Currently, there is paucity of scientific information on the microbiota harboured by GIN parasites of livestock and their relationship to host microbiota.

The GIN parasites causes health and welfare issues on small ruminants farming because they survive on food resources they extract from their hosts as well as the surrounding host environment (Roeber *et al.*, 2013; Zvinorova *et al.*, 2016). As a result of this need to survive, the host is almost always compromised following the interaction with GIN parasites (Hale, 2006). These GIN parasites colonise the GIT of animals and humans but they may migrate to, and invade other organs, although they prefer the intestinal wall (Duarte *et al.*, 2016). The GIN parasites have a direct and indirect life cycle, in which the adult female *H. contortus* lay eggs, that pass out with faeces and hatch in the environment into first-stage larvae (L1) and then moult into L2 stage while feeding on bacteria. The L2 stage moult into an infective larval stage that infect the host. During this development stages GIN parasites interact with various environmental and gastrointestinal microbiota both pathogenic and beneficial (Lacharme-Lora *et al.*, 2009).

For example, pathogenic *Mycobacterium avium* has been isolated from GIN parasites cultured from faecal samples collected from the hosts that was simultaneously co-infected with both GIN parasites and *Mycobacterium avium* (Whittington *et al.*, 2001). Given the long evolutionary history and sympatric distribution of microbiota and GIN

parasites inside the host and in the environment, it would be surprising if GIN parasites do not interact with pathogens or interact with each other (Kreisinger *et al.*, 2015; Lacharme-Lora *et al.*, 2009 and Waterfield *et al.*, 2004). Previous studies by Lacharme-Lora *et al.* (2009) and El-Ashram & Suo, (2017) have reported that GIN parasites harbour various microbial community and can act as vectors for transmission pathogenic bacteria. *Haemonchus* spp., *Trichostrongylus* spp., *Ostertagia* spp. and *Calicophoron* spp. are amongst the economically important GIN parasites in small ruminants productions. However, the barber's pole worm called *H. contortus* is highly pathogenic and probably the most economically important nematode infecting small ruminants.

In the farming industry, economic losses caused by GIN parasites occur in a variety of ways including treatment costs, lower weight gains, lowered fertility, reduced work capacity, involuntary culling, a reduction in food intake, lower milk production and mortality in heavily parasitized animals (Tsotetsi & Mbatia, 2003; Regassa *et al.*, 2006). The underlying parasitic mechanisms of these losses result in major socioeconomic implications worldwide (Regassa *et al.*, 2006; Roeber *et al.*, 2013). According to the Food and Agriculture Organization of the United Nations (FAO) data collected in 2012, 37% and 22% of the 1.2 billion world sheep population, together with 56% and 30% of the approximately estimated 1 billion world goats population are present in Asia and Africa, respectively (FAO, 2015). This suggests that the current agriculture and financial consequences in these continents are at stake since GIN parasites can substantially impact on farm profitability. Given the limited inventory of resources in developing African countries, these parasites will continue to severely constraint production in small- and large-scale farming systems (Roeber *et al.*, 2013).

Investigations on complete microbiota associated with multicellular organisms have previously primarily relied on low throughput techniques, such as culture or cloning-based methods, which allow characterization of only a fraction of the microbiota (Mackie *et al.*, 1989; Carpi *et al.*, 2011). However, recent high throughput, next-generation sequencing (NGS) technologies have overcome the limitations of these techniques. Most importantly, NGS has shed light on microbiota composition, structure and function, understanding of underlying mechanisms driving microbiota variation, and identification of clinical implications of changes in these microbiota as well as

facilitating the complete identification of microbiota living in a symbiotic relationship with their host. This burgeon field has led to the discovery that the gastrointestinal microbiota provide crucial host services such as immune system development, protection of host from pathogenic microbes, and prevention of auto-immune disease (Kreisinger *et al.*, 2015; El-Ashram & Suo, 2017). Furthermore, NGS technologies including high-throughput shotgun sequencing has been applied for various purposes, including characterization of microbiota in complex ecosystems, such as soil, and ocean water (Venter *et al.*, 2004), and investigation of microbiota of veterinary and medical importance (Wittekindt *et al.*, 2010).

1.2. BACTERIA

Bacteria are prokaryotic single cellular microorganisms that are few micrometres in length and have various number of shapes ranging from spirals, spheres and rods (Tshikhudo *et al.*, 2013). They are categorised into two groups: Gram-negative and Gram-positive bacteria, based on their cell wall and the colour they show when stained. The Gram-negative bacteria stains red because of the thin peptidoglycan layer while Gram-positive bacteria stains purple due to their thick peptidoglycan cell wall. These microbes inhabit a wide diversity of environments such as water, soil, and eukaryotic organisms and several others. (Grenni *et al.*, 2018). There are various types of bacteria namely, coccus, bacillus, Vibrio, spirillum and spirochete (Firdich *et al.*, 2013; Van Teeseling *et al.*, 2017). These bacteria are also classified into different taxonomic groups. For example, phylum such as *Acidobacteria*, *Actinobacteria*, *Aquificae*, *Armatimonadetes*, *Bacteroidetes*, *Caldiseica*, *Chlamydiae*, *Chlorobi*, *Chloroflexi*, *Chrysiogenetes*, *Cyanobacteria*, *Deferribacteres*, *Deinococcus-Thermus*, *Dictyoglomi*, *Elusimicrobia*, *Fibrobacteres*, *Firmicutes*, *Fusobacteria*, *Gemmatimonadetes*, *Lentisphaerae*, *Nitrospirae*, *Planctomycetes*, *Proteobacteria* and *Spirochaetes* (Ciccarelli *et al.*, 2006; De Mandal *et al.*, 2017).

These microorganisms have developed a symbiotic and parasitic relationship with vertebrate, invertebrate, plants and protozoa (Chow *et al.*, 2010). The relationships between prokaryotic bacteria and the host are comprised of various symbiotic relationship types that range from mutualism, commensalism, parasitism and pathogenic (Schöttner *et al.*, 2013). Each of these relationships have its own unique

definition, as the function behind each relationship is different. (Bailey, 2017; Levitt-Barmats & Shenkar, 2018). Bacteria are commonly known for causing diseases in plants, animals and humans. However, some bacteria are beneficial including helping in digestion of feed, host health and production of medicinal drugs. For example, bacteria *Actinomycetes* which produce antibiotics such as streptomycin and nocardicin (Clardy *et al.*, 2009; Begum *et al.*, 2017). Most bacteria live symbiotically with other microbes such as fungi, archaea and GIN parasites in the GIT, plant surfaces and roots while helping in the breakdown of organic matter and digestion of food (Thursby & Juge, 2017).

1.3. SYMBIOSIS

Symbiosis is any close and prolonged biological association between two or more biological organisms of different species (Chow *et al.*, 2010). Each of these organisms are termed symbiont (Dimijian, 2000). Symbiosis can be classified in two groups based on their location or physical attachment within and on the host, namely, ectosymbiosis and endosymbiosis (Aanen & Eggleton, 2017). Ectosymbiosis is a symbiotic relationship where one symbiont resides on the body surface of the host including the inner surfaces of the GIT, examples of this include ectoparasites such as lice. Endosymbiosis is a symbiotic relationship where one symbiont live within the body of the host, examples include diverse microbiota (Fisher *et al.*, 2017). Endosymbiotic relationship is believed to have developed when bacteria inhabit gastrointestinal tract, where both bacteria and host undergo genomic and functional changes to adapt to the new environment (Moya *et al.*, 2009).

1.4. SYMBIOTIC BACTERIA

A wide diversity of bacteria colonise gastrointestinal tracts of invertebrate, vertebrate and plant surfaces (Kamagata & Narihiro, 2016; Haque & Haque, 2017). Symbiotic bacteria are commonly known as bacteria that live in a symbiotic relationship with the colonised host and sometimes with each other. Most studied symbiotic bacteria are those of animals and humans, because of their agricultural and health significance (Sinnathamby *et al.*, 2018). These bacteria play an important role in the fermentation of indigestible foods into usable nutrients for their host. Hence, the relationship is very beneficial for both species. The host in this case provides a suitable environment for

bacterial growth while the bacteria helps in food digestion and improve host immune response against pathogenic bacteria (Fisher *et al.*, 2017). Recently, GIN parasites-microbiota studies are on the increase, focusing on characterising microbial communities that are purely symbionts of GIN parasites and the ones that maybe a threat to GIN parasites and their host with long term objectives of manipulating these microbes to control GIN parasites (El-Ashram & Suo., 2017; Sinnathamby *et al.*, 2018).

1.5. THE GASTROINTESTINAL MICROBIOTA (GUT SYMBIONTS)

The gastrointestinal microbiota is made up of trillions of microbes and their genetic material that live in the GIT. These microbes are comprised of bacteria, fungi, archaea and protozoa, but mainly dominated by bacteria, which are involved in host health and well-being (Thursby & Juge, 2017). The establishment of the microbiota begins soon after birth (Dominguez-Bello *et al.*, 2010). The diversity, composition and abundance of these microbiota are influenced or affected by different factors, such as host genetics, diet composition, age, mode of delivery and geographical location (Wang *et al.*, 2017). These microbiota contribute significantly to metabolic functions, protection against pathogens, educating the immune system, and, through these basic functions, affect directly or indirectly most of our physiological functions.

The gastrointestinal microbiota help the host utilise feed rich in cellulose, hemicellulose and lignin by secreting necessary enzymes to degrade the complex polysaccharides (Holscher, 2017). These symbionts supply the host with nutrients in the form of volatile fatty acids (i.e., acetic acid, butyric acid and propanoic acid) that contribute to the host's productivity (Chaucheyras-Durand & Ossa, 2014). In the host, GIT symbionts can be classified by the respective function they perform (functional groups), such groups include, cellulolytics, amylolytics and proteolytics, that degrade different feed components and further metabolize some of the products produced by other microbes (Huang *et al.*, 2016). For example, methanogens, the methane-forming archaea; are among those that form methane by metabolizing hydrogen produced by fermentative microbes (Detman *et al.*, 2018).

The most predominant bacterial phyla are usually *Firmicutes*, *Bacteroidetes* and *Proteobacteria* (Figure 1), and their presence in the host facilitate the development of the latter's gastrointestinal tract and mediate the host physiology (Wang *et al.*, 2017).

Furthermore, microbiota play a role in the activation of toll-like receptors (TLRs) which are used by the human's intestinal tract to recognise dangers and repair damage (Nikoopour & Singh, 2014). Hence, it is crucial to understand the composition and diversity of the gastrointestinal microbiota to further enhance the growth and health of the host.

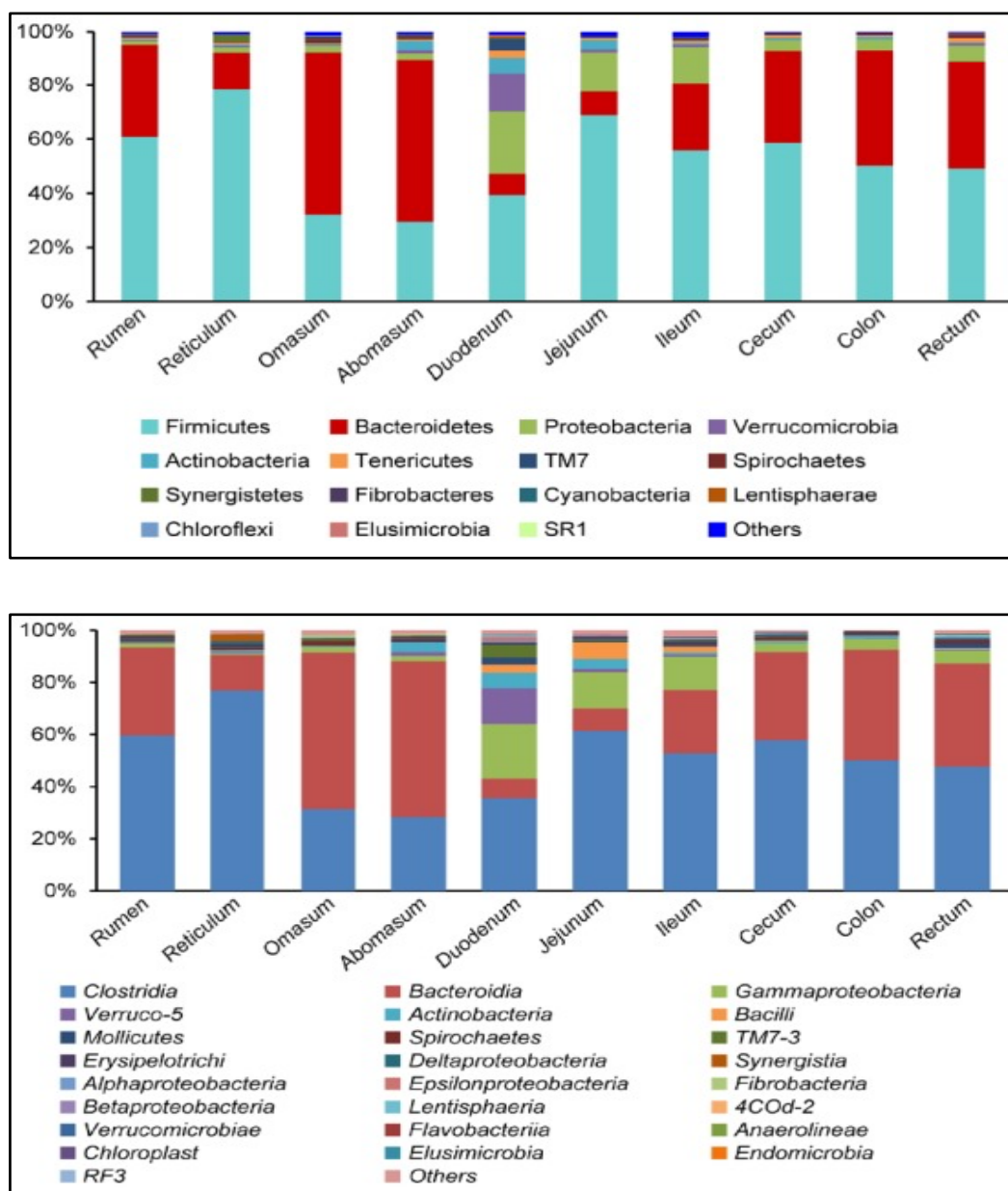


Figure 1. Bar graph depicting data from study of sheep gut microbiota. These data demonstrate the type of bacterial community that dominate the gastrointestinal microbiota at phylum and class level. Similar results has been observed in other studies by Wang et al. (2017) and Zeng *et al.* (2017)

1.6. BACTERIAL SYMBIONTS OF GASTROINTESTINAL NEMATODE PARASITES

1.6.1 THE MICROBIOTA- GASTROINTESTINAL NEMATODE PARASITES SYMBIOSIS COMPLEXES

The GIN parasites and their microbiota can live symbiotically within different environments including, marine, freshwater, soil and plant or animal host. Considering the microbiota-GIN parasites symbiosis complexes complexity and enormous diversity, it is surprising that only few microbiota-GIN parasites interactions have been studied and described in the literature as compared to that of arthropods- and amoebae-microbiota complexes (Murfin *et al.*, 2012).

The GIN parasites species feed on bacteria, fungi and protozoa, however, they avoid feeding on pathogenic species (Ryss *et al.*, 2011). The relationship between microbiota and GIN parasites is very specific, persistent and mutualistic (Murfin *et al.*, 2012). The most previous studied microbiota-GIN parasites relationship is that of terrestrial entomopathogenic whose relationship with Gram-negative *gammaproteobacteria*, *Xenorhabdus* and *Photorhabdus* bacteria are defensive, predatory and commensal relationships (Ryss *et al.*, 2011; Fukruksa *et al.*, 2017). This entomopathogenic nematode is lethal to insects and has been used as a biological control for insect pests. The *Xenorhabdus* and *Photorhabdus* bacteria colonise the GIT of the entomopathogenic nematodes (EPN) and exhibit pathogenicity to the insect prey and mutualism to the growth and development of the EPNs (Murfin *et al.*, 2012; Fukruksa *et al.*, 2017). Upon invasion of an insect host, the EPNs regurgitates its bacteria into the haemolymph, which rapidly grows and kills the insect and help degrade the insect while releasing nutrients for the EPN host (Ansari *et al.*, 2003). The studies by Bayer *et al.* (2009), Elhady *et al.* (2017) and Zhang *et al.* (2017) reported on a microbiota-nematodes symbiosis complex of plant parasitic nematodes and free-living marine nematodes inhibiting plants and marine invertebrates respectively. These nematodes were reported to harbour wide diversity of microbial communities as well

1.7. SYMBIONTS OF MAMMALIAN GASTROINTESTINAL NEMATODE PARASITES

1.7.1. FILARIA

The filaria nematode species such as *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* (Molyneux *et al.*, 2003) are responsible for a human disease called lymphatic filariasis. The first filaria endosymbiont observed was a bacteria-like structure resembling chlamydiae in the 1970's by electron microscopy (Kozek & Marroquin, 1977). Then later ignored for the next 20 years until 1994 when World Health Organization (WHO/Tropical Disease Research/United Nations Development Programme/ World Bank) established the filarial Genome Project which led to the discovery of the bacteria like structures in filaria as *Wolbachia* bacteria. This bacteria were eventually found to be obligate mutualistic endosymbionts which are responsible for nematode embryogenesis, development and adult survival (Bridgeman *et al.*, 2018). The *Wolbachia* bacteria are responsible for the supply of nutrients to this nematode and are predominantly found in lateral cords in male worms and in ovarian tissue, oocytes and developing embryos within the uterus in female worms (Slatko *et al.*, 2010; Taylor *et al.*, 2013; Bridgeman *et al.*, 2018). Furthermore, *Wolbachia* endosymbionts have now been identified in most of the filarial nematode species including *Brugia spp.* and *W. bancrofti* (Taylor *et al.*, 2005, Scott *et al.*, 2012).

1.7.2. ASCARIS SUUM

Ascaris suum is a GIN parasite that causes ascariasis in pigs. The microbiota associated with *A. suum* has been characterised with culture-based approaches, which only culture fraction of the bacteria available in the sample since most of the bacteria are unculturable (Hsu *et al.*, 1986; Shahkolahi & Donahue., 1993; Paerewijck *et al.*, 2015). A study by Hsu *et al.* (1986), reported that the bacterial species harboured by *A. suum* which were cultured in brain heart infusion (BHI) agar and in anaerobic agar were Gram-negative enterics such as *Escherichia coli*, *Enterobacter*, *Klebsiella*, *Acinetobacter*, *Citrobacter*, *Pseudomonas*, *Aeromonas*, *shigella* and Gram-positive bacteria included genera such as *Staphylococcus*, *Streptococcus*, *Corynebacterium* and *Bacillus*. Most of these bacteria isolated from *A. suum* were found to be able to secrete serotonin in vitro and they were then considered as a possible source of serotonin in the *A. Suum* as well (Hsu *et al.*, 1986; Yano *et al.*, 2015).

1.7.3. TRICHURIS MURIS

Trichuris muris is a GIN parasite that colonise the caecum and colon of the infected host. Once in the host environment, *T. muris* closely interact with the complex gastrointestinal microbiota of the host (Hayes *et al.*, 2010; Holm *et al.*, 2015). The host microbiota helps *T. muris* in eggs hatching and successful establishment of *T. muris* microbiota (Hayes *et al.*, 2010). Furthermore, *T. muris* induced changes in the host cecal microbiota following subsequent infections. Previous work done by White *et al.* (2018) has revealed that *T. muris* microbiota are dominated by bacterial phyla: *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. The distinct gastrointestinal microbiota of *T. muris* is independent of the host gastrointestinal microbiota it inhibits. Therefore, it was suggested that the GIN parasites selects for a specific subset of bacteria that may be crucial to its survival within the host (White *et al.*, 2018).

1.7.4. HAEMONCHUS CONTORTUS

The *H. contortus*- microbiota complexes are gaining attention compared to other GIN parasite-microbiota complexes because of the *H. contortus* pathogenicity to small ruminants. These studies are trying to understand these complexes with the objective of controlling this nematodes using their respective microbiota to limit the use of chemical based treatment. Previous studies by El-Ashram & Suo, (2017), Sinnathamby *et al.* (2018) have shown the microbial community which are associated with the different life-cycle stages from eggs-adult stage. The eggs-stage microbiota were dominated by bacterial phyla: *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*; the larval-stage dominated by *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Planctomycetes*; the adult-stage microbiota dominated by *Proteobacteria*, *Firmicutes*, *Tenericutes*, and *Actinobacteria* (El-Ashram & Suo, 2017; Sinnathamby *et al.*, 2018). This studies reported that the *H. contortus* free-living stages, such as L1 (*rhabditiform*), and L2 (*rhabditiform*) and L3 *filiariform* infective larvae may be a vector for environmental microbiota (El-Ashram & Suo, 2017). These results are in agreement with research finding of Aravindraja *et al* (2013), who suggested that the microbial phyla are universal and are not limited to the peculiar environment.

1.8. COMPOSITION OF THE GASTROINTESTINAL MICROBIOTA

The establishment and development of the gastrointestinal microbiota ecosystem is complex, essential for mammalian life and differ from host to host (Itani *et al.*, 2017). In the foetus, the GIT is considered sterile, but is immediately colonized by microbes during birth. The microbiota that colonize the infant mainly depend on the route of delivery (Rutayisire *et al.*, 2016). The infants delivered via vaginal birth acquire microbiota similar to that of the mother's vagina whereas the one's born via caesarean section acquire microbiota from the skin of the mother (Dominguez-Bello *et al.*, 2010; Claesson *et al.*, 2011). However, studies by Jiménez *et al.* (2008) and Dominguez-Bello *et al.* (2010) reported the presence of low numbers of bacteria in the amniotic fluid, umbilical cord blood, placental tissue and foetal membranes of healthy infants without any sign of infection which contradict the hypothesis that intestinal tract of the foetus is sterile, which is thought that this microbiota can reach the foetus through transportation via bloodstream and dendritic cells from the mother to the placenta (Jiménez *et al.*, 2008; Dominguez-Bello *et al.*, 2010).

Furthermore, meconium of premature infants have been reported to harbour complex microbiota dominated by phyla: *Firmicutes* and *Proteobacteria* with *Escherichia coli*, *Serratia marcescens* and *Klebsiella pneumoniae* as predominant species (Rodríguez *et al.*, 2015). Microbiota colonization that occurs during vaginal delivery is dominated by vaginal taxa such as *Lactobacillus*, *Bifidobacterium*, *Prevotella* whereas those acquired via caesarean section harbour skin like microbiota such as *Staphylococcus*, *Propionibacterium* and *Corynebacterium* (Dominguez-Bello *et al.*, 2010). However, the maternal microbiota does not persist indefinitely. The newborn acquires a lot more microbes as they grow and the most abundant bacterial phyla of these microbiota includes *Firmicutes* (including the families *Lactobacillaceae*, *Eubacteriaceae* and *Clostridiaceae*) followed by bacterial phyla *Bacteroidetes* (including *Bacteroidaceae*), *Proteobacteria* (including *Enterobacteriaceae*) and *Actinobacteria* (including *Bifidobacteriaceae*) (Rodríguez *et al.*, 2015). Eckburg *et al.* (2005), Ley *et al.* (2008) and Timmerman *et al.* (2017) reported that the changes in microbiota composition and development in the infant is influenced mainly by the diet; mother's breast milk is considered a major source of microbiota that colonize the breastfed infant intestinal tract and composed of a rich microbiota dominated by

streptococci, *staphylococci*, *lactic acid bacteria* and *bifidobacteria*. In contrast, formula-fed infants are composed of microbiota dominated by bacteria of the genera *Clostridium*, *Bacteroides*, *Lactobacillus* and *Escherichia* (Eckburg *et al.*, 2005; Ley *et al.*, 2008; Timmerman *et al.*, 2017). Furthermore, there are many factors that are important in defining microbiota composition depicted in Figure 2. Moreover, the predominant microbiota phyla are similar across all mammals, including mice (Nelson *et al.*, 2013).

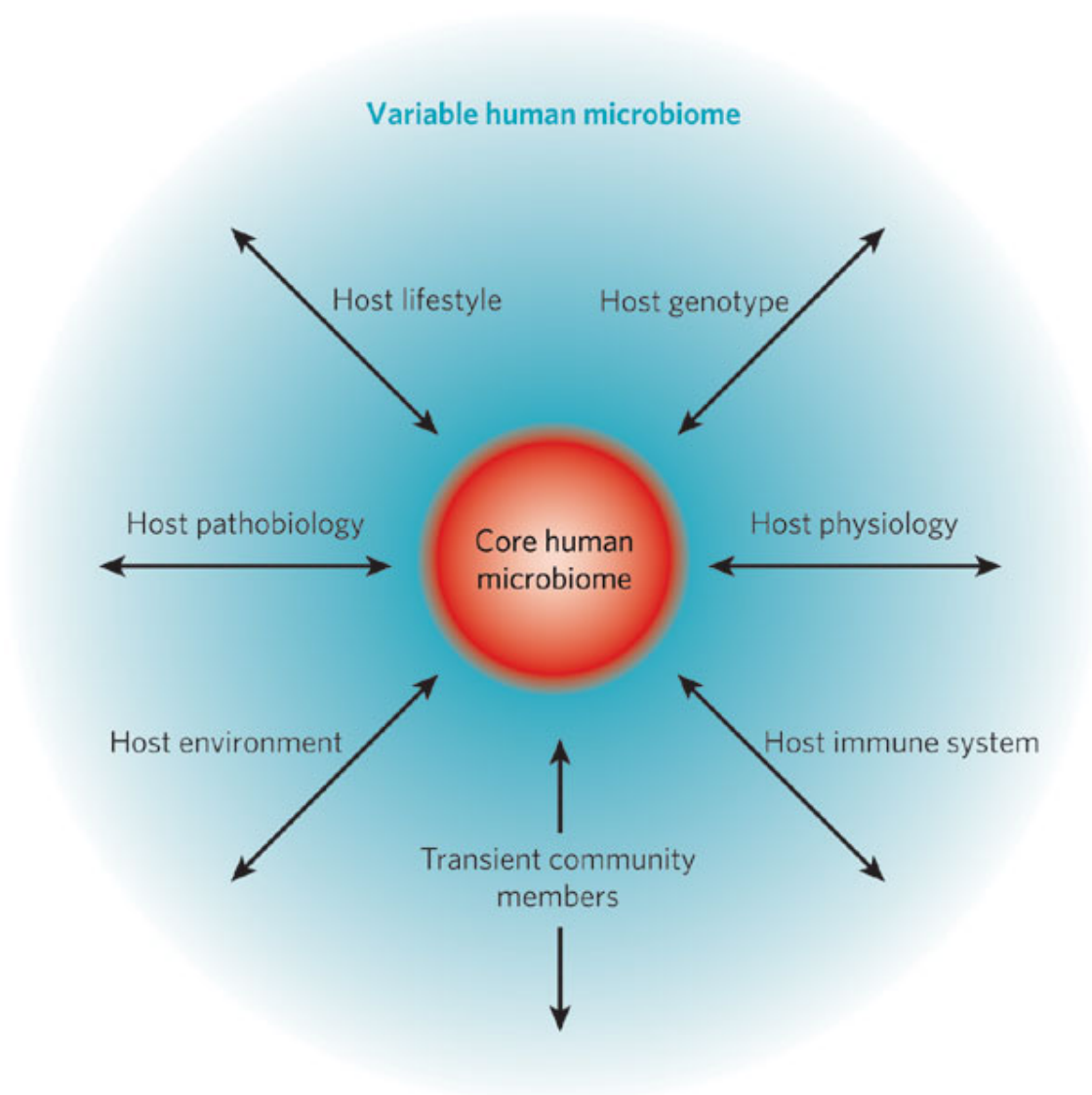


Figure 2. Illustration depicting factors that may be important in defining microbiota composition. The illustration also suggests that all individuals have a core microbiome, which provides basic functions necessary for the body, and a variable microbiome that would be defined by the influence of various factors (Turnbaugh *et al.*, 2007).

1.9. FUNCTIONS OF GASTROINTESTINAL MICROBIOTA

1.9.1. NUTRIENT METABOLISM

The gastrointestinal microbiota has a symbiotic relationship with the intestinal mucosa and has the ability to breakdown dietary carbohydrates, microbial and host products. In case of non-digestible carbohydrates, the host rely on microbiota to break them down to a simpler form, since they do not possess the necessary enzymes to digest them (Jandhyala *et al.*, 2015). Hence, the host rely on the microbiota to supply them with necessary vitamins and essential nutrients. Microbiota such as *Bacteroides*, *Roseburia*, *Bifidobacterium*, *Fecalibacterium*, and *Enterobacteria* can synthesize short chain fatty acids (SCFA) that are rich sources of energy for the host by fermenting non-digestible carbohydrates and oligosaccharides, including SCFA include acetate, butyrate and propionate and have been reported to mediate host energy by ligand-receptor interaction of the SCFAs with a G protein- coupled receptor Gpr41 (Rowland *et al.*, 2017). The carbohydrate fermentation and bacterial metabolism causes the synthesis of oxalate in the intestines of animals that results in the formation of oxalate stones in the kidney. Magwira *et al.* (2012) reported that microbiota such as *Oxalobacter formigenes* and *Lactobacillus* species can breakdown the oxalate stones, as a result, reduce risk of oxalate stones. The gastrointestinal microbiota can suppress the inhibition of lipoprotein lipase activity in adipocytes, thus help in lipid metabolism and are comprised of microbial proteinases and peptidases that help in protein metabolism (Rowland *et al.*, 2017).

1.9.2. IMMUNOMODULATION

Gastrointestinal microbiota contribute significantly to the development of the host immune system that comprises of both innate and adaptive immune responses responsible for counteracting the threat in the host health (Jandhyala *et al.*, 2015). The development and modulation of the immune responses in the GIT is thought to be influenced by colonisation of the GIT by gastrointestinal microbiota which are known to be responsible for the induction, training the host immune system, and calibrate and promote all aspects of immune system (Hasegawa *et al.*, 2010).

The GIT is the largest defense barrier of any human and animal body with more than 60% of immune cell types that identify and fight off the infections or presence of pathogenic microorganisms (Jandhyala *et al.*, 2015). The cell types that counteract the presence of potential aggressors in the body includes the gut associated lymphoid tissues (GALT), IgA producing B (plasma) cells, Group 3 innate lymphoid cells, effector and regulatory T-cells, resident macrophages and dendritic cells (Valentini *et al.*, 2014; Jandhyala *et al.*, 2015).

These cell types have the ability to distinguish pathogenic microorganisms from commensals and this ability is mediated by pattern recognition receptors (PRRs) that detect and recognise microbial antigen and microbial-associated molecular patterns (MAMPS). The PRRs include the family of toll-like receptors (TLPs), RIG-I-like receptors (RLRs), C-type lectin receptors (CLRs), toll-like receptors (TLRs), cytosolic DNA receptors (CDRs) and nucleotide-binding oligomerization domain- (NOD-) like receptors (NLRs) (Lavelle *et al.*, 2010 & Valentini *et al.*, 2014). The role of GIT microbiota in shaping a normal GALT is observed by the impaired development of Peyer's patches that results in reduction of IgA⁺ B cells and an increase in IgE⁺ B cells (Chen *et al.*, 2017). The effector T-cells responses is primarily controlled by Th2 responses and then latter mediated by Th1 and Th17 cells and gastrointestinal commensal microbiota is believed to cause TLR-MyD88 signaling which promote development of IL17. The IL17 and other cells and normal gastrointestinal microbiota are known to be crucial for the intestinal homeostasis by regulating epithelial cell functions as well as the development and function of Foxp3⁺ T regulatory (Treg) cells. (Hasegawa *et al.*, 2010; Jandhyala *et al.*, 2015).

1.9.3. ANTIMICROBIAL PROTECTION

The normal microbiota can confer resistance to pathogens and prevent overgrowth of resident pathogens without the involvement of the gut mucosal immune system (Jandhyala *et al.*, 2015). Certain normal microbiota such as Gram-positive and Gram-negative *Enterobacteriaceae* species can secrete small molecules for example, bacteriocins or microcin's with bacteriostatic or bactericidal activity. The expression of a plasmid-encoded bacteriocin by *Enterococcus faecalis* strain which is capable of inhibiting vancomycin-resistant *Enterococcus* (VRE) from the intestinal tract of mice (Ubeda *et al.*, 2017). The *Escherichia coli* strain Nissle 1917 reduces *Salmonella enterica* serovar *Typhimurium* colonization of the GIT by producing and secreting microcin's. The normal microbiota can prevent infection by producing molecules such as quorum-sensing signal AI-2 that inhibit colonization of the GIT by *Vibrio cholerae* through interference with pathogen gene expression (Hsiao *et al.*, 2014) and also suppresses growth of pathogens e.g. pathogenic *E. coli* strain O157: H7 by production of SCFA derived from fermentation of dietary fibers by bacteria (Alakomi *et al.*, 200; Ubeda *et al.*, 2017). The gastrointestinal microbiota has been reported to induce the synthesis of antimicrobial protein (AMP) such as C-type lectins and cathelicidins, and confers overgrowth of pathogenic strains by metabolizing host and bacterial produced molecules (i.e., bile acids that can inhibit overgrowth of *Clostridium difficile*) resulting in secondary metabolites that counteract pathogens overgrowth (Buffie *et al.*, 2014; Jandhyala *et al.*, 2015).

In addition, nutrient completion is a crucial factor in the antimicrobial protection in the GIT. For pathogens to colonize the GIT, they need to compete for nutrients with the normal microbiota that are already adapted to the GIT environment and are very efficient in metabolizing dietary fibers and obtain energy and utilizing host-derived nutrients. Maltby *et al.* (2013) and Ubeda *et al.* (2017) reported that mice colonized by two commensal *E. coli* strains (HS and Nissle 1917) confers resistance against *E. coli* O157:H7 infections when both commensal *E. coli* strains are present because they utilize and deplete all five sugars, thus preventing GIT colonization by *E. coli* O157:H7. However, if only one *E. coli* strain is present, it's insufficient to utilize all of the five most important sugars in the GIT. Thus, the *E. coli* O157:H7 can still exploit the remaining sugars in the GIT (Maltby *et al.*, 2013; Ubeda *et al.*, 2017).

1.10. MOLECULAR AND MICROBIOLOGICAL BASED APPROACH TO STUDYING THE GASTROINTESTINAL MICROBIOTA

1.10.1. CULTURE-DEPENDENT TECHNIQUES

Traditional culture-dependent techniques rely on selective culturing of the bacteria from clinical and environmental samples using different species specific selective media (Table 1). However, culture dependent techniques can only culture a fraction of the bacteria in the samples since 40 - 90% of gastrointestinal bacteria are unculturable under artificial laboratory conditions (Gong & Yang, 2012). The main reasons for non-cultivation of most gastrointestinal bacteria include the inability to mimic the exact GIT environment, unknown growth requirements of each of the gut bacteria and these techniques fail to simulate the associations of bacteria with other microbes such fungi, helminths and archaea in the host GIT environment (Zoetendal *et al.*, 2004). Furthermore, culturing bacteria limits the ability to determine the microbiota compositions, size and diversity. Studying microbiota using culture dependent techniques also has advantages including the ability to study living bacteria for physiological functions, their efficiency for the detection of specific intestinal pathogens and the availability of pure cultures of bacteria (Gong & Yang, 2012).

Table 1. Brief description of culture-dependent techniques.

Techniques	Description
Bacteriophage typing	Used for classification of bacteria (phage typing). Bacteria strains with a specific serotype can be distinguished from other different types of phages.
Serotyping	Useful for the direct identification of colonies without sub-culturing. performed by colony hybridization with a monoclonal antibody specific for a particular genus, species or strain.
PFGE	Highly discriminative and based on the variability of movement of large DNA restriction fragments. Performed in an electrical field of alternating polarity in an agarose gel medium. DNA fragments with similar strains are separated by comparing fingerprints.

1.10.2. CULTURE-INDEPENDENT TECHNIQUES

Culture-independent techniques are commonly used methods for studying microbiota without requiring the culturing of the bacteria. These techniques are molecular biological methods based on detection of common molecular markers found in the genome of microorganisms (Petrosino *et al.*, 2009). The use of molecular based techniques allows much more comprehensive studies of microbiota diversity and community structure (Gong & Yang, 2012). Such molecular techniques include polymerase chain reaction (PCR) based DNA profiling, quantitative PCR (q-PCR), flow cytometry, DNA microarray, fluorescent *in situ* hybridization (FISH) and DNA sequencing (Adzitey *et al.*, 2013) (Table 2).

Table 2. Brief description of culture-independent techniques.

Techniques	Description	Advantages	Drawbacks	Application/Uses
Target genes	Mostly uses molecular marker targeting the 16S ribosomal RNA gene for genetic diversity of bacteria. Because this gene is conserved in all eubacteria. Another marker for studying composition of bacteria is the gene cpn60.	• Accurate and easy-to-interpret results. • Identify variants at low allele frequencies (down to 5%).	• Inability to differentiate bacterial species that share almost the same 16S rDNA sequence.	• Clinical quantification. • Detection of uncultivable bacteria. • Novel bacterial genus and species discovery.
PCR-based DNA profiling techniques	Technique used in molecular ecology to multiply short segment of DNA/RNA generating thousands to millions of copies of a particular DNA/RNA sequence in the sample.	• Highly specific and sensitive. • Most sequencing reads will be pathogen-specific, with good coverage	• Laborious and time consuming. • Post amplification analysis.	• Genome mapping. • DNA fingerprinting. • General biology research.
Quantitative-PCR	Technique that uses DNA amplification to determine the exact/ absolute or relative concentration of target gene such as 16S rRNA gene sequences in the sample.	• Q-PCR that uses fluorescence dye-based detection that provide greater sensitivity. • Allows quantification of gene number in the sequence.	• Require expensive reagents and complexity due to simultaneous thermal cycling and fluorescence detection.	• Gene expression analysis. • Clinical quantification and genotyping. • Detection of genetically modified organisms.

FISH	Molecular cytogenetic technique that uses Oligonucleotide probes labelled with fluorescent that bind to target parts of the chromosome with a high degree of complementary 16S rRNA sequences.	• No PCR bias. • Highly sensitive. • <i>In situ</i> identification. • Semi quantitative.	• Depend on probe sequences. • Unable to identify unknown species.	• Species identification • Comparative genomic hybridization.
Flow cytometry	Originally used to count, sorting, protein engineering and evaluate mammalian cells as well as analysing bacterial population.	• Morphological, density, and metabolic analysis. • Time efficient. Highly accurate.	• Cell size bias • Complex data analysis. • Limited to liquid samples.	• Marine and plant biology. • Molecular biology and immunology.
DNA sequencing	Technique to identify composition of various species of microbiota.	• Higher throughput. • Ability to maximise the taxonomic resolution. • Most sequencing reads will be pathogen-specific, with good coverage even at low pathogen load.	• Expensive equipment. • Post sequencing analysis	• Metagenomics. • Epigenetics. • Discovery of non-coding RNAs and protein binding sites. • Transcriptomics.

1.11. COLONIZATION OF GASTROINTESTINAL NEMATODE PARASITES BY MICROBIOTA

The GIN parasites interact with multitude species including bacteria, fungi and archaea (Midha *et al.*, 2017). The host-microbiota relationship has various reciprocal relationships that play a crucial role in shaping the co-evolutionary processes of these species. The interaction of GIN parasites-microbiota influences the diversity, composition and richness of microbial environments (Walk *et al.*, 2010; Guernier *et al.*, 2017). The GIN parasites harbor own microbiota that are crucial in development, metabolism, physiology and protection against other harmful microbes (Midha *et al.*, 2017). These GIN parasites acquire most of their microbiota from the faeces and the surrounding environments during the egg hatching stage and development, and from the GIT content when the infective larvae infect the host and during development to the adult worm in the GIT of the host. However, data on GIN parasites-microbiota interaction is very limited but few studies have demonstrated the bacteria that colonize GIN parasites such as *H. contortus*, *Ascaris*, *Trichuris* and *Heligmosomoides polygyrus bakeri* (Walk *et al.*, 2010; Yano *et al.*, 2015; White *et al.*, 2018; Sinnathamby *et al.*, 2018).

A study by Walk *et al.* (2010) reported that *H. p. bakeri* harbour gastrointestinal microbiota which were confirmed when L3 larvae and adult *H. p. bakeri* was sampled and the results indicated that L3 associated microbiota was completely unique compared to the adult worm which was similar to that the GIT of the host. Walk *et al.* (2010) reported that the microbiota variations between L3 and adult worm are due to the fact that *H. p. bakeri* hatch outside of the host and interact with the external environment microbiota and when they enter their hosts with a distinct microbiota from the external environment, they are bound to change over time in the GIT when they interact with distinct microbiota from the host. Another study conducted on human co-infected with *V. cholerae* and *Ascaris lumbricoides* nematode reported that *A. lumbricoides* isolated from cholera patients were colonized by *V. cholerae* of the same serotypes that was isolated from the patients (Nalin & McLaughlin, 1976; Midha *et al.*, 2017). Thus, colonization of GIN parasites by microbiota is essential by providing them with benefits such as nutrients and protection against harmful bacteria (Lee *et al.*, 2015; Dirksen *et al.*, 2016).

1.12. MODULATION OF HOST GASTROINTESTINAL MICROBIOTA BY GASTROINTESTINAL NEMATODE PARASITES INFECTION

The mammalian GIT comprise of approximately 3.8×10^{13} microbes from different domains of life, archaea, bacteria, and eukaryotes, collectively known as the microbiota as shown in Figure 3 (Midha *et al.*, 2017). The various regions of the GIT comprises of different types, composition and diversity of microbiota (Zaiss & Harris, 2016). However, infection with GIN parasites and other factors including age, diet, genetic background and health can results in substantial shifts in the composition and diversity of the microbiota (Reynolds *et al.*, 2015; Midha *et al.*, 2017). Microbiota studies on mammals infected with GIN parasites reported that nematode infection can influence host metabolism, with an on-going implication for immune modulation. Furthermore, a study by Prevaes *et al.* (2012) conducted on mice infected with *T. muris* reported reduced metabolites that was measured in the faecal samples collected from the infected mice compared to uninfected mice. Another study by Reynolds *et al.* (2015) conducted on wild mice reported an increase in bacterial community abundance and diversity on mice that had a mixed-infection of *H. polygyrus*, *Syphacia spp.* (pinworm), and *Hymenolepis spp.* (tapeworm) as compared to uninfected mice. To date, few studies have reported the impact of GIN parasites infection on the microbiota richness and diversity. The GIN parasites-microbiota studies have reported that an increase in alpha diversity of the gastrointestinal microbiota which is generally associated with a healthy GIT homeostasis and reduced alpha diversity is associated with systemic diseases and inflammation of the GIT (Manichanh, 2006; Peachey, 2017). A study by Li *et al.* (2012) reported that pigs showed phyla *Proteobacteria* significantly increase during *T. Suis* infection. In most animal-GIN parasites system studies conducted to date, the diversity and abundance of the gastrointestinal microbiota have been reported to change or sometimes remained unchanged following GIN parasites infection, which could be due to experimental design or stage of infection during sampling. (Li *et al.*, 2011; Fricke *et al.*, 2015). Hence, the stage of infection and time of sampling are very crucial variables that may affect the results of the study (Li *et al.*, 2016). Thus, determining the impact of GIN parasites infection on gastrointestinal microbiota is a veterinary priority, in order to determine microbes that are crucial for GIT metabolism and host health.

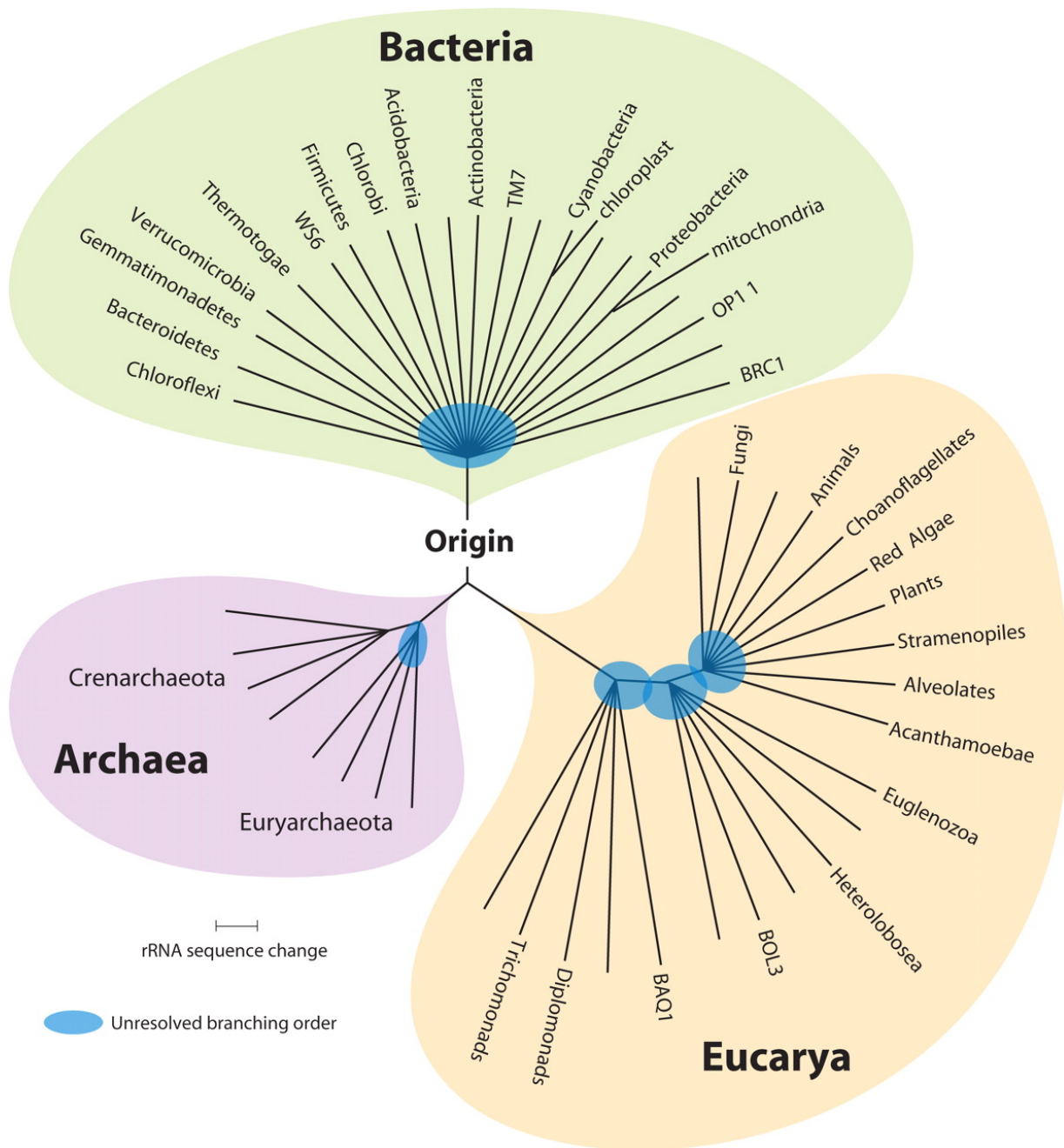


Figure 3. A simplified tree of life based on rRNA sequence comparisons. Depicting microbes from different domains of life, archaea, bacteria, and eukaryotes, collectively known as the microbiota. The grey circles represent the time points of unresolved branching order (Pace, 2009).

1.13. THE IMPORTANCE OF GASTROINTESTINAL NEMATODE PARASITES-MICROBIOTA INTERACTIONS FOR THE HOST

Gastrointestinal microbiota and GIN parasites co-evolved in the mammalian host, through a three-way interaction that has persisted for several hundred millions of years (Gause & Maizels, 2016). Growing evidence propound that microbiota-GIN parasites-host interactions have resulted in complex adaptations that have shaped the physiology of each of these organisms in disease and health. Hence, the homeostasis of mammalian host may require the presence of microbiota including nematodes (Gause & Maizels, 2016). The absence of gastrointestinal nematode parasites has been hypothesized to determine the increasing prevalence of allergic and autoimmune diseases within westernized societies (Maizels, 2005; Khan & Fallon, 2013). Sinnathamby *et al.* (2018) reported that the GIN parasites can alter the composition and diversity of gastrointestinal microbiota in the host.

Previously published studies by El- Ashram & Suo, (2017) and Sinnathamby *et al.* (2018) reported that GIN parasites infection can significantly change the composition of the gastrointestinal microbiota with respect to species abundance and composition. Different independent laboratories also reported that the proportion of *Enterobacteriaceae* and *Lactobacillaceae* increases during an infection of mice with duodenal parasite *Heligmosomoides polygyrus* (Rausch *et al.*, 2013; Reynolds *et al.*, 2014). Furthermore, alteration of gastrointestinal microbiota by GIN parasites is associated with different chronic inflammatory diseases such as obesity and diabetes (Zaiss & Harris, 2016). Moreover, previous studies conducted on pigs following *T. suis* infection is associated with campylobacteriosis in pigs (Mansfield *et al.*, 2003).

Recent data generated by Next-Generation Sequencing demonstrated that *Mucispirillum* bacteria increases in animals infected with GIN parasites and with shotgun sequencing data showing a decrease in *Ruminococcus* bacterium that are cellulolytic and as a result carbohydrate metabolism was reduced (Li *et al.*, 2012). More importantly, *campylobacter* is more prevalent in pigs infected with the *T. suis*. In addition, severe parasite infection is linked with high expression of the inflammatory genes, *arg1*, *cxcr2*, *c3arl*, *il6*, *muc5ac* and *ptgs* in the infected host (Wu *et al.*, 2012).

1.14. GASTROINTESTINAL NEMATODE PARASITES

Gastrointestinal tract parasitism is considered one of the most serious and underestimated problems, which is a severe constraint to small ruminants productivity throughout the world (Zvinorovaa *et al.*, 2016). The Gastrointestinal nematode parasitize a wide range of hosts including humans, plants and animals, both wild and domestic. The phyla *Nematoda* is the second largest in the animals' kingdom comprising of more than 500 000 species (Anderson *et al.*, 2000). Small ruminants can contract GIN parasites infection through the ingestion of contaminated food and water or the ingestion of infective larvae from the environment by accident. Such parasitic worms include *Haemonchus* spp., *Trichuris* spp., *Ostertagia* spp., *Trichostrongylus* spp. and *Ascaris suum* depicted in Table 3 (Murthy & Rao, 2014). The type and severity of nematode infection is associated with a variety of factors such as, host-related factors which include: immunity, genetic resistance, species, sex, and age; parasite-related factors which include life history, survival of larvae in the environment, their location in the host; environmental factors that include climate, weather, season, type of vegetation and microclimate (Zvinorovaa *et al.*, 2016).

Infection with GIN parasites can be asymptomatic or symptomatic depending on the host being infected or the type of worm causing the infection. However, severe GIN parasites infections can cause symptoms such as blood loss, tissue damage, spontaneous abortions, diarrhoea, weight loss, dyspnoea, congenital malformations, significant morbidity and mortality (Roeber *et al.*, 2013). GIN parasites causes major economic losses in agriculture. For example, the annual cost associated with GIN parasites in sheep has been estimated to be tens of billions of dollars worldwide, according to the sales of anti-parasitic compounds by pharmaceutical companies, excluding production losses (Roeber *et al.*, 2013). This is because the parasitic diseases in small ruminants are controlled mainly via anthelmintic treatment. Furthermore, this type of control is expensive and, in most cases, only partially effective even with timely strategic treatment (Kaplan, 2004). Consequently, the frequent and excessive use of anthelmintic has resulted in anthelmintic resistance by GIN parasites, especially *H. contortus* (Gilleard, 2006).

Table 3. Common gastrointestinal nematode parasites in small ruminants.

Family	Species	Location
Trichostrongylidae	<i>Haemonchus contortus</i>	Abomasum
Trichuridae	<i>Trichuris spp.</i>	Large intestines
Trichostrongylidae	<i>Ostertagia spp.</i>	Abomasum
Trichostrongylidae	<i>Trichostrongylus spp.</i>	Abomasum
Ascarididae	<i>Ascaris suum</i>	Bile ducts
Capillariidae	<i>Capillaria</i>	Small intestines
Trichostrongylidae	<i>Cooperia</i>	Small intestines
Molineidae	<i>Nematodirus</i>	Small intestines
Ancylostomatidae	<i>Bunostomum</i> <i>trigonocephalum</i>	Small intestines
Chabertilidae	<i>Chabertia ovina</i>	Large intestines

1.15. BIOLOGICAL CONTROL OF GASTROINTESTINAL NEMATODE PARASITES

The use of biological agents to control GIN parasites in small ruminants has gained more attention in recent decades, due to evolution of GIN parasites resistance to anthelmintics (Sahoo & Khan, 2016). Furthermore, the demand of inexpensive organic meat and milk and their products with less drug residues by consumers is increasing (Alba-Hurtado *et al.*, 2013). Hence, biological control are needed as an alternative GIN parasites control strategy.

Generally, biological control may be defined as the use of natural living microorganisms introduced into an environment to control a target microorganism such as GIN parasites (Sahoo & Khan, 2016). This method uses natural enemies to reduce GIN parasites larvae on the environment to a level below the economic threshold levels under experimental and natural conditions. For example, Nematophagous fungi have been investigated as biocontrol agents that target free living larval stages (L1 and L2) of nematodes. The fungus *Duddingtonia flagrans* and *Clonostachys rosea* have been used as biocontrol agent for livestock and plants pathogenic GIN parasites and has displayed the ability of survival in the GIT (Da Silva *et al.*, 2015). In the GIT, fungus spores germinate and looped hyphae trap the developing larval stages in the faecal environment (De & Sanyal, 2009; Da Silva *et al.*, 2015). The *D. flagrans* has the potential in the GIT of sheep and subsequently to destroy *H. contortus* larvae in faecal cultures from fungus fed sheep (Sahoo & Khan, 2016).

Moreover, bacterial species such as *Bacillus thuringiensis* (Bt), a Gram-positive, spore-forming bacterium which has the unusual property of producing a parasporal protein crystal (delta-endotoxin) which is toxic (Cry proteins) to some nematodes and insect pests (Quesada-Moraga *et al.*, 2004; Kotze *et al.*, 2005; Palma *et al.*, 2015). This bacterium has the ability to kill free-living and adult nematodes through its crystal toxin proteins. The Bt when ingested by host, the host enzymes breakdown crystal toxin proteins into the Bt insecticidal components called the delta-endotoxins (Fontenot *et al.*, 2003; Yu *et al.*, 2015). The delta-endotoxins bind on the binding sites on the GIT lining, creating pores in the membrane, leading to peritonitis as the haemolymph pours into the gut cavity, killing the nematodes in the GIT (Fontenot *et al.*, 2003; Palma *et al.*, 2015). Recently, GIN parasites metagenomics studies are

focusing on the use of symbiotic microbiota as biocontrol of GIN parasites. Focusing on understanding GIN parasites-microbiota interactions in the host and environment with the aim of manipulating these microbiota and use them as biocontrol agents of GIN parasites.

1.16. GENETIC RESISTANCE TO GASTROINTESTINAL NEMATODE PARASITES

1.16.1. GENETIC CONTROL OF GASTROINTESTINAL NEMATODE PARASITES

Genetic control of GIN parasites is when the host has an ability to control the negative effects of the worm by developing immune-mediated resistance to GIN parasites (Kelly *et al.*, 2013). The host's ability to reduce infection and parasitic load often differ from animal to animal depending on the previous exposure to the parasites, immunological responses, age, breed and physiological characteristics (Bhuiyan *et al.*, 2017). However, the immunological mechanisms by which the host acquires resistance or resilience to GIN infections are not clear. But both innate and adaptive immunities protect the host from GIN infections (Alba-Hurtado *et al.*, 2013).

1.16.2. NON-SPECIFIC RESPONSE MECHANISMS TO GASTROINTESTINAL NEMATODE PARASITES INFECTIONS

During GIN parasites inhibition of the host gut, one of the first host innate response is the complement fixation, where by the parasites activates the complement pathway as well as binds to the opsonins on their surface. This results in activation of the complement pathway where chemotactic and vasoactive peptides are generated and assemble eosinophils to the site of infection whereby eosinophils utilises its cytotoxicity against nematodes larvae in the early stages of infections (Meeusen & Balic, 2000; Reinhardt *et al.*, 2011). In other host immune system mechanisms, the host utilises to counteract GIN parasites infections includes the use of mast cells to attack the larvae before they enter the abomasum gland and the use of gastric hypersecretion, hypermotility and hyperplasia of calciform cells with the increase in mucus production (Balic *et al.*, 2002; Meeusen *et al.*, 2005). The host also utilises the presence of histamine and leukotrienes in the host abomasum mucus to restrict the motility of nematodes larvae (Alba-Hurtado *et al.*, 2013).

1.16.3. GASTROINTESTINAL NEMATODE PARASITES ANTIGENS AND HOST ANTIBODIES

The GIN parasites such as *H. contortus* has hidden antigen (enzyme complexes H11 and H- gal-GP) in the GIT that induces 2-type response and production of serum antibodies in the host (Muñoz-Guzmán *et al.*, 2006). This serum antibodies alters the nematodes digestion when the worm ingests them with blood. Serum and abomasum antibodies have been studied, although the results are variable, few studies have shown an association between serum antibodies IgG levels and genetic resistance to GIN parasites (Muñoz-Guzmán *et al.*, 2006 and Alba-Hurtado *et al.*, 2013). However, high levels of abomasum antibodies specific IgA in the abomasal mucus reduces the worm fertility and length and is known to be more important than serum antibodies in protection against GIN parasites infections (Martínez-Valladares *et al.*, 2005). GIN parasites infections also elicit 2-type response which induce specific IgE, that induces cytotoxicity in eosinophils, mast cells, and macrophages. An increase in IgE has been found to be associated with genetic resistance to GIN (de la Chevrotière *et al.*, 2011).

1.16.4. IMMUNE RESPONSE CELLS ASSOCIATED WITH RESISTANCE

Immune response cells associated with resistance in sheep include CD4+ lymphocyte, eosinophil and mast cell (Mackinnon *et al.*, 2010). Infection with GIN such as *H. contortus* elicit T-lymphocyte proliferation as well as an increase in CD4+ lymphocytes in the abomasum and peripheral blood (Alba-Hurtado *et al.*, 2013). CD4+ T-lymphocytes differentiate into two cell types, type 1 T-lymphocytes which produces IFN and IL-2 cellular response that protect against intracellular parasites and type 2 response which produces IL-4, IL-5 and IL-10, which is a humoral response associated with the presence of GINs (Andronicos *et al.*, 2010). There is also evidence that type 2 response is associated with immunity against *H. contortus* (Alba-Hurtado *et al.*, 2013). Previous study indicate that susceptible and resistant sheep's respond to the parasites infection is followed by an increase of CD4+ lymphocyte in both susceptible and resistant sheep. Hence, both groups respond to infection but in different manners (Gill *et al.*, 2000). Compared with susceptible sheep, resistant sheep is more likely to be associated with type 2 response and susceptible sheep associated with type 1 response, because susceptible animals produce more IFN and less blood eosinophils, abomasum eosinophils and specific serum antibodies (Gill *et al.*, 2000).

1.17. GASTROINTESTINAL NEMATODE PARASITE OF VETERINARY IMPORTANCE IN DOHNE MERINO SHEEP

1.17.1. HAEMONCHUS CONTORTUS

The *H. contortus* is a very common worm in small ruminants and one of the most pathogenic parasites of small ruminants. This GIN parasites is commonly known as barber's pole worm of the family *Trichostrongyloidea*. An adult *H. contortus* attaches to the abomasal mucosa of the host and causes infection, called haemonchosis that poses a major threat to the farming industry worldwide, especially in sub-tropical and tropical regions under warm and wet weather conditions (Burke, 2005). The *H. contortus* is a common blood feeder responsible for anaemia in sheep and goats (Saccareau *et al.*, 2017). It is estimated that each barber's pole worm sucks about 0.05 ml of blood per day by ingestion or seepage from lesions (Qamar *et al.*, 2009). However, the frequency and severity of these infections mostly depend on the rainfall in any particular area.

1.17.1.1. MORPHOLOGY

An adult *H. contortus* has a cylindrical shape, complete digestive system and a reddish colour or appearance because of its blood-feeding habit. The adult female *H. contortus* is longer (18 to 30 mm) compared to the adult male *H. contortus* (10 to 20 mm). The female has a vulva mostly covered by a vulva flap that is usually large and very prominent but may be smaller on other specimens whereas the male's distinct feature is a relatively developed copulatory bursa that has Y-shape dorsal ray. The eggs are thin shelled with 16 to 32 cells and are about 70–85 μm long (Rahman & Hamid, 2007).

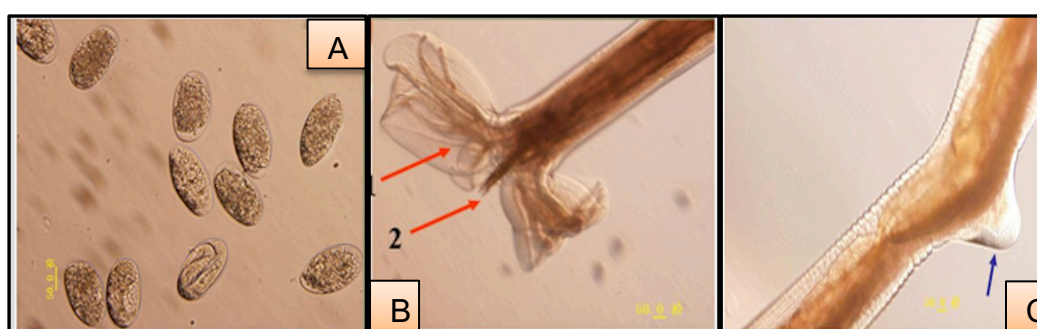


Figure 4. Pictures depicting morphological features of *H. contortus*, (A). Eggs, (B). Male, (C). Female (El-Ashram & Suo, 2017).

1.17.1.2. LIFE CYCLE

The adult female *H. contortus* is the highest egg layer of all sheep GIN parasites. This worm produces about 5000-15000 eggs daily inside the abomasum which then pass out in the faeces when the animal defecates. However, the number of eggs produced by an adult female *H. contortus* depends on the host's level of immunity against GIN parasites (Hutchinson, 2009). Once in the environment, under suitable conditions, the eggs embryonate and hatch into the first stage larvae (L1) which moult into second stage larvae (L2) while feeding on bacteria. The L2 larvae then moult into the 3 third-stage larvae (L3), which is an infective larvae stage. The larvae development process takes about 7 to 10 days depending on the environmental condition. Animals are infected when they ingest pasture that has an L3 larvae. The ingested larvae then pass to the predilection site, the abomasum. In the abomasum mucus membrane, the L3 larvae develop into L4 larvae stage and then develop into a full grown adult *H. contortus*. The adult female *H. contortus* in the abomasum start egg production in about three weeks and the cycle repeats. Figure 5, depicts the entire lifecycle of *H. contortus* which takes about 17 to 21 days, provided that the weather conditions are favourable (Junquera, 2016).

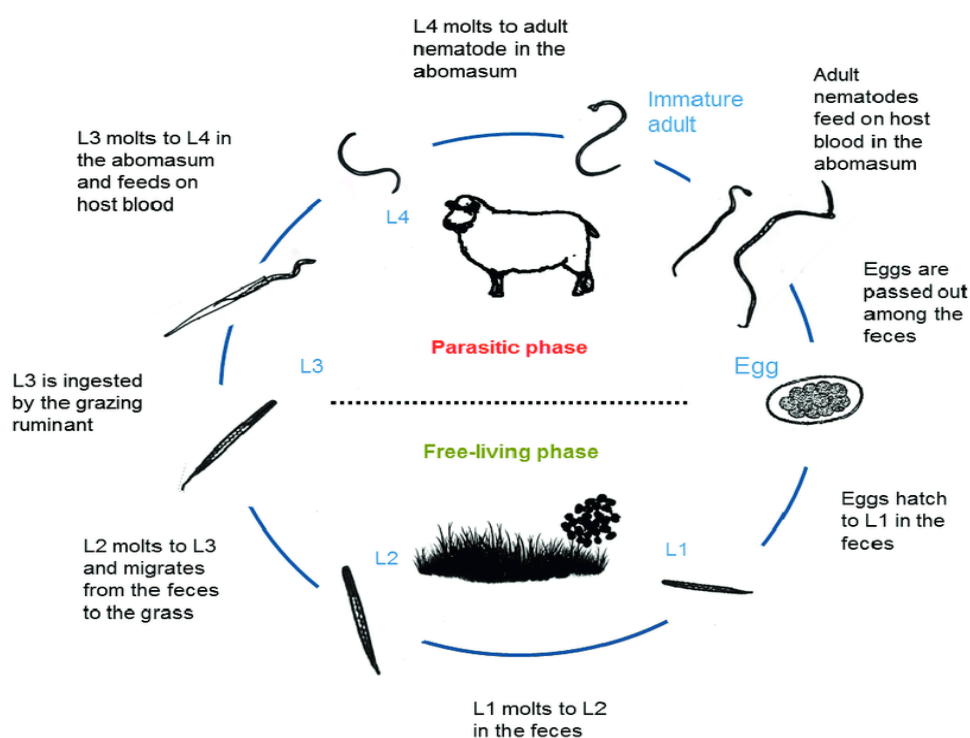


Figure 5. The life-cycle of *H. contortus*, from egg to the adult stage (Engstrom, 2016).

1.17.1.2. CLINICAL SIGNS, TREATMENT AND CONTROL OF HAEMONCHOSIS

Clinical signs of haemonchosis are due to blood loss that usually results in anaemia. In acute cases, death may be observed with no prior illness or signs of illness. Some animals may show signs of extreme weakness. Under severe infections; liver damage is often observed as well as weight loss, dehydration, poor performance, diarrhoea and mortality (Aitken, 2008; Besier *et al.*, 2016). Other signs of *H. contortus* infection include bottle-jaw: a fluid swelling beneath the jaw (Baker *et al.*, 1959). The prophylactic treatment of *H. contortus* and other parasitic infections basically depends on the use of anthelmintic (Mickael *et al.*, 2003), because they are simple and easy to administer but resistance of the parasite against these chemicals is continuously increasing (Stear *et al.*, 2007). The *H. contortus* has produced the highest resistance against anthelmintic especially benzimidazoles as well as ivermectin and the resistance against other chemical drugs is continuously growing (Burke, 2005).

1.17.1.3. DIAGNOSIS OF HAEMONCHOSIS

An accurate diagnosis of haemonchosis is essential when infection is suspected, given that *H. contortus* is one of the most pathogenic parasites of small ruminants causing on-going mortalities in animals. However, clinical diagnosis of haemonchosis primarily depends on clinical signs observed on the infected animal (Van Wyk, 2002). The infection can be confirmed by the examination of faeces for eggs using faecal egg count (FEC) (Rodríguez *et al.*, 2015). Another effective diagnostic approach is the FAMACHA system that is used to monitor anaemia in sheep. This system was developed in South Africa and involves the assessment of ocular mucous membrane colour against the FAMACHA card to identify high levels of haemonchosis in the sheep (Van Wyk & Bath, 2002). The white colours on the eyes of the sheep indicate dangerous anaemia levels whereas red colour indicates healthy sheep (Hart & Dawson, 2010). The parasite load in the infected animal can be estimated by the quantitative McMaster's technique, which allows enumeration of eggs per gram in the faeces (Van Wyk, 2002). Several laboratory diagnostic techniques exist for confirmation of the identity of GIN parasites eggs in the faeces, serology such as enzyme-linked immunosorbent assay (ELISA) and molecular techniques which includes PCR and NGS (Rodríguez *et al.*, 2015).

1.18. HISTORY OF DOHNE MERINO BREED

1.18.1. DOHNE MERINO BREED

The development of the Dohne Merino breed was initiated and executed by Mr. J.J.J. Kotze at the Dohne Agricultural Research Station situated in Eastern Cape near Stutterheim, South Africa, 27°28' longitude and 32°32'S latitude, at an altitude of 1020m above sea level and 72 km from the coast. Hence, the breed was named after the Dohne Agricultural Research Station (McMaster, 1991; Cloete & Scholtz, 1998). The breeding program was established locally in 1939 by the South Africa Department of Agriculture and the progeny was interbred and selected for high fertility, rapid lamb growth and fine merino wool production under commercial rangeland conditions (Naidoo *et al.*, 2016). The breeding program was initiated in response to challenges and limitations encountered by Merino sheep farming in the sourveld region of the Eastern Cape, South Africa. Such limitations include high mortality, low fertility, high susceptibility to intestinal nematodes and blowflies (Cloete & Scholtz, 1998). Although, Merino sheep possesses excessive skin folds which result in high level of wool production but this features also attract and increase susceptibility to blow fly and flea rot (Cloete & Scholtz, 1998; Naidoo *et al.*, 2016).

Furthermore, Merino sheep are also characterized by selective grazing habits that require intensive management strategies and higher input costs in order to maintain sustainable Merino sheep farming (van Wyk *et al.*, 2008). Hence, the farmers required a more extensive farming system that will improve profitability, reproduction rate, improved marketability of slaughter lambs and surplus breeding material. Thus, the Dohne Merino was developed in an effort to fulfil this need and overcome the above-mentioned limitations. Although, Dohne Merino was developed for semi-intensive farming, its ability to thrive under various environmental conditions resulted in breed popularity and exportation to other countries such as Australia in 1998 by means of frozen embryos (Cloete *et al.*, 1998; van Wyk *et al.*, 2008; Naidoo *et al.*, 2016).



Figure 6. A picture of South African Dohne Merino sheep breed raised for meat and wool purposes. A picture was taken at the Tandala Farm Abattoir before the sheep were slaughtered.

1.19. THE 16S METABARCODING

Metagenomics is the study of genetic material obtained directly from environmental samples. These studies have been employed to study the structure, function, and dynamics of microbiota (Thomas *et al.*, 2012) as well as allowed us to gain more understanding of the diversity of microbiota in various environments; the complex structure of the microbiota they form, and the impact they have on the surrounding environment (Huttenhower *et al.*, 2013). Earlier metagenomics-based methods such as shotgun metagenomics used to sequence many cultured microbial communities contained in environmental samples by randomly shearing DNA sequences to many short sequences, and then reconstruct them into a consensus sequence (Tyson *et al.*, 2004). Consequently, shotgun sequencing requires clone libraries to sequence genes present in the environmental samples. However, the cloning step is no longer required with advances in metagenomics high throughput sequencing technologies and

sequencing data can be obtained in greater yield without this labour-intensive bottleneck step (Nicola *et al.*, 2013).

Metagenomics offers the opportunity to facilitate research on questions and hypotheses that could not be approached before and plays a major role in the identification of new molecules and enzymes for biotechnology as well as their therapeutic applications. It is crucial in identification of the microbiota present in an environment, highlight the differences in microbiota, and determine the impact of external variables on the microbiota i.e., food, drugs, age, geography and other microbial species through sequencing of 16S rRNA gene (Nicola *et al.*, 2013). These metagenomics capabilities are particularly important when studying the association of the microbiota-disease and microbiota-GIN parasites within a given environment (Wang *et al.*, 2015). A study by Wang *et al.* (2015) reported that human gastrointestinal microbiota abundance and proportions vary between United States individuals because of long-term dietary habits. Metagenomics based methods can be used to evaluate the interactions between microbiota and the host as well as the integral role they play in each other's health. Another metagenomic study used high-throughput screening technology to investigate gastrointestinal microbiota pathways and found that *E. coli* metagenomics clones could modulate GIT mucosal proliferation by activating the nuclear factor- κ B pathway in epithelial cells (Karlsson *et al.*, 2013). Additionally, metagenomics can be applied to solve various practical challenges in agriculture, medicine, engineering sustainability and ecology (Nicola *et al.*, 2013).

1.20. NEXT-GENERATION SEQUENCING (NGS)

In the 1970s, Sanger and colleagues, and Maxam and Gilbert introduced the concept of DNA sequencing by chain termination and fragmentation techniques, respectively. In 1977, two landmark articles were published, i.e., Sanger and colleagues sequencing technique commonly known as Sanger DNA sequencing, based on determining sequences in DNA by primed synthesis with DNA polymerase and the Maxam and Gilbert DNA sequencing method based on chemical degradation DNA sequence in which terminally labelled DNA fragments were chemically cleaved at specific bases and separated by gel electrophoresis. The Sanger sequencing method was responsible for introduction of the first automated DNA sequencers, because of its high accuracy, simplicity, great availability and required less handling of toxic

chemicals and radioisotopes compared to Maxam and Gilbert's method, as results it becomes the most widely used DNA sequencing method for decades. This method was optimised and commercialized by Applied Biosystems (ABI), the European Molecular Biology Laboratory (EMBL) (Ansorge *et al.*, 1986). The commercialization of the automated Sanger sequencer led to successful completion of the human genome sequence. However, Sanger sequencing has limitations due to its low sensitivity, low throughput, time consuming and its inability to perform parallel analysis of multiple targets. Because of these limitations it was clear that faster, higher throughput and cheaper technology was required. For this reason, a series of novel Massively Parallel Sequencing (MPS) technologies were developed and termed next-generation technology (NGS) (Arsenic *et al.*, 2015).

NGS provides opportunities for analysis of multiple genomes and transcriptomes in an extremely efficient and timely manner at much lower cost than Sanger-based sequencing methods. Applications of these technologies have already benefited various research areas including; metagenomics, polymorphism discovery, non-coding RNA discovery, large-scale chromatin immunoprecipitation, gene-expression profiling, mutation mapping and whole transcriptome analysis (Buermans & den Dunnen, 2014). However, the higher throughput of NGS reactions comes with limitations as well including shorter sequences, as most sequencing platforms (Illumina, Roche, SOLiD) offer shorter read lengths (30-400 bp) than the conventional Sanger method (Ulahannan *et al.*, 2013).

Different NGS technologies have the same general processing steps with different specific technical details. Currently, five main next-generation sequencing platforms exist at the forefront of the sequencing industry, the original Roche 454 platform and the Illumina, and SOLiD sequencing technologies, and Oxford nanopore MinION.

1.20.1. ROCHE 454 SEQUENCING TECHNOLOGY

A sequencing technology developed by Mostafa Ronaghi and Pål Nyrén in 1996, based on sequencing by the synthesis principle. This technique relies on the detection of pyrophosphate release on nucleotide incorporation, rather than chain termination with dideoxy nucleotides (Nyrén, 2007).

1.20.2. ION SEMICONDUCTOR SEQUENCING

This method of DNA sequencing is based on the detection of hydrogen ions that are released during the polymerization of DNA (Rusk, 2011). This method sequences by synthesis, during which a complementary strand is built based on the sequence of a template strand (Perkel, 2011). This sequencing technology differs from other sequencing technologies because there is no modified nucleotides or optics used in the protocol. However, this technology has limitations such as the short read length compared to other sequencing methods such as Sanger sequencing or pyrosequencing. Longer read lengths are beneficial for *de novo* genome assembly (Perkel, 2011). Ion Torrent semiconductor sequencers produce an average read length of approximately 400 nucleotides per read (Rusk, 2011; Perkel, 2011).

1.20.3. ILLUMINA (SOLEXA) SEQUENCING TECHNOLOGY

This technology refers to method-based sequencing by reversible dye terminators, developed by Shankar Balasubramanian and David Klenerman in 1998 (Bentley *et al.*, 2008). This technology leverages the clonal array formation and proprietary reversible terminator technology for accurate and reliable large-scale sequencing.

1.20.4. SOLiD™ SEQUENCING TECHNOLOGY

SOLiD™ Sequencing Technology is a next-generation DNA sequencing technology developed by Life Technologies that employs emulsion PCR and flow-cell sequencing. SOLiD technology is based on sequencing by ligation. A pool of all labelled oligonucleotides of fixed length are annealed and ligated. In contrast to the other sequencing technologies, sequencing by synthesis is performed by utilizing DNA ligase, rather than polymerase (Mardis, 2008).

1.20.5. OXFORD NANOPORE MINION SEQUENCING TECHNOLOGY

MinION is a portable nanopore sequencing USB device that has been commercially available since May 2015 after having been launched initially through an early access programme, the MinION Access Programme (MAP) (Check Hayden, 2015). The MinION works on the principle of nanopore strand sequencing in real time and results

in sequence read lengths from several hundred bases to hundreds of thousands of bases (Mikheyev *et al.*, 2014). This technology has been used to successfully read the genome of a *lambda bacteriophage*, which is approximately 48,500 base pairs long, twice during one pass (Castro-Wallace *et al.*, 2017). MinION technology is fast growing, because it can read 100,000 base pairs during a single DNA capture and this has never been managed before using traditional sequencing techniques (Kerkhof *et al.*, 2017).

1.21. BIOINFORMATICS

Bioinformatics is the burgeon field that develops methods, software tools for understanding biological data, and addresses the application of computers to the collection, organization, analysis, manipulation, presentation, and sharing of biological data (Can, 2014). Bioinformatics processes are often used for initiatives that generate large data sets. Two major activities that use bioinformatics are proteomics and genomics. Proteomics refers to the field of science that focuses on studying proteins (Anderson *et al.*, 1998). Genomics on the other hand refers to a field of sciences that focuses on the analysis of genomes (Pevsner, 2009). Additionally, there are many other areas of biology that applies bioinformatics approaches (i.e., metabolomics, transcriptomics, metagenomics), with each of these important areas aiming to understand complex biological systems through bioinformatics. The use of bioinformatics in understanding biological data usually involves the following steps: collection of statistics from biological data, building a computational model, solving of a computational modelling problem and testing and evaluating a computational algorithm (Can, 2014). The primary goal of bioinformatics is to increase the understanding of the biological processes and is also used in the analysis of various processes ranging from sequence analysis to structural bioinformatics and network and systems biology. Analysis of biological data requires bioinformatics software tools, which ranges from simple command-line tools, to more complex graphical programs and standalone web-services.

1.21.1. BIOINFORMATICS ANALYSIS OF 16S METABARCODING DATA

Targeted amplicon-based analysis using 16S rRNA gene sequences is widely used for large scale studies to explore complex microbiota from different sampling sites such as gastrointestinal tract, skin, soil or water (Turnbaugh *et al.*, 2007). Microbial communities' analysis requires the use of bioinformatics tools to process the large complex amount of data generated from whole genome and amplicon-based sequencing for taxonomic assignments (Kuczynski *et al.*, 2012). The bioinformatics tools available for 16S rRNA gene sequencing data analysis includes Mothur, QIIME (Quantitative Insights Into Microbial Ecology), the RDPipeline (Ribosomal Database Project Pipeline), MG-RAST (Metagenomics Rapid Annotation using Subsystems Technology), MEGAN, METAGEN assist, VAMPS, Snowman, Genboree, EzTaxon, Pheonix2, Vegan, ade4, and ape and CloVR-16S (Plummer *et al.*, 2015; Oulas *et al.*, 2015). Only few of these tools are commonly used for 16S data analysis and denoising include QIIME, Mothur, MG-RAST and RDPipeline. Despite the vast availability of these tools, the analysis mostly requires reference database which is used to find the closest match to the OUT generated during analysis. Commonly used reference database includes Ribosomal Database Project, Greengenes and Silva (DeSantis *et al.*, 2006; Cole *et al.*, 2009; Quast *et al.*, 2013). The resultant output from the analysis can be visualized by statistical software packages such as R statistical package, Primer-E package and Metastats (Team, 2008; Clarke & Gorley, 2006; McMurdie & Holmes, 2013; Pavlopoulos *et al.*, 2013).

CONCLUSION

In summary, it is evident that there are various association between gastrointestinal parasites and microbiota that occupy different niches in the nature. This includes microbes that live in the external environment such as soil and surfaces, and within internal environment such GIT of organisms such as gastrointestinal parasites and small ruminants. These associations are of particular importance in gastrointestinal parasites and the host microbiota. The gastrointestinal parasites and host microbiota share same intestinal niche which has consequences for host, gastrointestinal parasites and microbiota. However, these consequences have not been fully investigated. The current study was therefore formulated to initiate gastrointestinal parasites and microbiota association research in South Africa.

CHAPTER 2

PROBLEM STATEMENT AND RATIONALE

2.1. PROBLEM STATEMENT

The GIN parasites infections causes severe constraints on livestock production in marginal systems (Periasamy *et al.*, 2014) and lower productivity through a reduction of feed intake, feed conversion efficiency, loss of blood and mortality (Tsotetsi & Mbat, 2003). Thus, causing substantial economic loss in developing and developed countries. In South Africa, animal production including small ruminants contributes about 48% of agricultural value. However, these parasites limit the productivity of susceptible animals through direct feeding or removing nutrients from the host by damaging the absorptive lining of the GIT (Roeber *et al.*, 2017).

Furthermore, the control of GIN is entirely based on administration of anthelmintic drugs. Unfortunately, the demand of inexpensive organic meat and milk products with less drug residues by consumers and the evolution of GIN parasites that are resistant to anthelmintic treatment is increasing (Alba-Hurtado *et al.*, 2013). Hence, new control measures are needed for effective control of GIN parasites infections such control measures includes grazing management, breeding parasite resistant animals and biological control. A novel approach could be biocontrol using bacteria pathogenic to nematodes such as *Bacillus thuringiensis* which has demonstrated promising results against GIN parasites, thus, reduce the use of chemical based treatment in livestock (Palma *et al.*, 2015; Yu *et al.*, 2015; Guo *et al.*, 2016; El-Ashram & Suo, 2017). Hence, it is important to understand the dynamics of GIN parasites infections and their respective microbiota and host microbiota through metagenomics studies which contribute in giving valuable information towards biological control of GIN parasites in small ruminants (Fukruksa *et al.*, 2017; El-Ashram & Suo, 2017; Sinnathamby *et al.*, 2018).

Recently, El-Ashram & Suo. (2017) and Sinnathamby *et al.* (2018) reported on the microbial community of *H. contortus* life-cycle stages. Most importantly, they demonstrated that Next-Generation Sequencing (NGS) platform is capable of efficiently detecting microbiota harboured by *H. contortus*. Furthermore, in South

Africa, there is a literature dearth of documented records of studies on microbiota of GIN parasites and their association to their host microbiota. Hence, there is insufficient literature of this association globally. Against this background, the current study focused on the characterization of the adult male and female *H. contortus* microbiota and their Host (Dohne Merino sheep) microbiota. Here, morphological identification and molecular techniques were used to distinguish the adult male and female *H. contortus*. Lastly, metagenomics diagnosis was employed to determine the microbiota of the sampled adult male and female *H. contortus* and their predilection site from the host, the abomasum. Data obtained from this study provides new insights into the bacterial community naturally associated with adult male and female *H. contortus* and its predilection site, the abomasum, with the long-term novel approach of manipulating them to control ruminant GIN parasites.

2.2. AIM, OBJECTIVES AND HYPOTHESIS

2.2.1. AIM

The aim of this study was to conduct comparative analysis of microbiota of the adult *H. contortus* and that of Dohne Merino sheep abomasum in order to distinguish specific GIN parasites bacterial communities.

2.2.2. OBJECTIVES

- Isolate the adult *H. contortus* from the abomasum of Dohne merino sheep based on its morphological characters using microscopy.
- To determine the bacterial communities of the adult male and female *H. contortus* using next-generation sequencing platform and metagenomics analysis.
- To determine the bacterial communities of abomasum (predilection site of *H. contortus*) from the Dohne Merino sheep using next-generation sequencing platform and metagenomics analysis.

2.2.3. RESEARCH HYPOTHESIS

- The *H. contortus* is harbouring bacterial communities which are both pathogenic and beneficial to the infected host.

2.3. OUTLINE OF DISSERTATION

Chapter 1 – Introduction and literature review: It gives background of microbiota, gastrointestinal nematodes parasites, their relation with small ruminants, their life cycle, GIN parasites-microbiota symbiosis complexes, functions of GIT microbiota, economic importance, identification and various diagnostic techniques.

Chapter 2 – Statement of the problem: It includes justification of the study, aims, objectives and hypothesis.

Chapter 3 – Materials and methods: Describes in detail the study approach including, description of the study area, materials used and methods applied, as well as how data was analysed.

Chapter 4 – Results: A full presentation of data obtained in this study.

Chapter 5 – Discussion, conclusions and recommendations: Interpretation of data with conclusions showing the aims and objectives of the study have been achieved, and recommendation of further actions and studies that needs to be undertaken with reference to data obtained from this study.

CHAPTER 3

MATERIALS AND METHODS

3.1. STUDY SITE

Experimental animals were collected from the Wauldby farm at the Stutterheim district in the Eastern Cape Province, South Africa. The farm is located at Latitude: 32°38' 0.7" (32.6335°) south, Longitude: 27° 35' 32.7" (27.5924°) east, Elevation: 761 metres (2,497 feet) in a high summer rainfall area, receiving more than 600 mm annual rain fall mostly during the summer (source: http://www.saexplorer.co.za/south-africa/climate/stutterheim_climate.asp) (Figure 7).

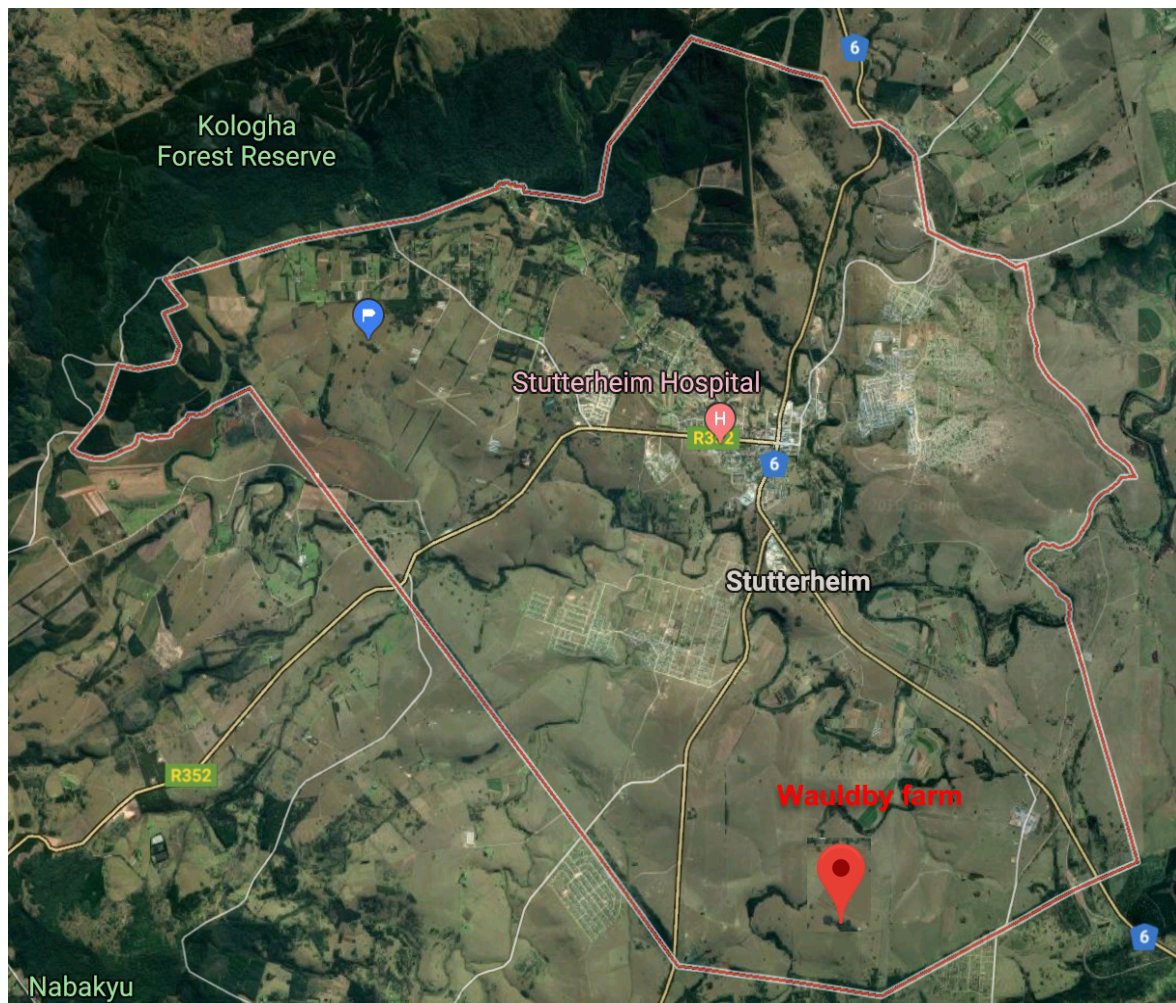


Figure 7. Satellite map of the Stutterheim District, Eastern Cape, South Africa (Google Maps, E27° 37' 32.7" S32° 35' 07"). Red lines indicate the boundary of the Stutterheim District.

3.2. STUDY APPROACH

In this study, microscopic and molecular based methods were used to determine the bacterial communities associated with adult *H. contortus* worms and their predilection site, the abomasum. Briefly, the adult *H. contortus* and the abomasum content samples were collected from sheep and the adult *H. contortus* were identified by morphological and molecular analysis. Thereafter the *H. contortus* samples were homogenized and DNA extracted from both *H. contortus* and abomasum content samples for detection of their bacterial communities by PCR. Lastly, Illumina MiSeq sequencing was conducted and data generated was analysed using bioinformatics analysis to identify bacterial species associated with adult *H. contortus* and abomasum content depicted in Figure 8.

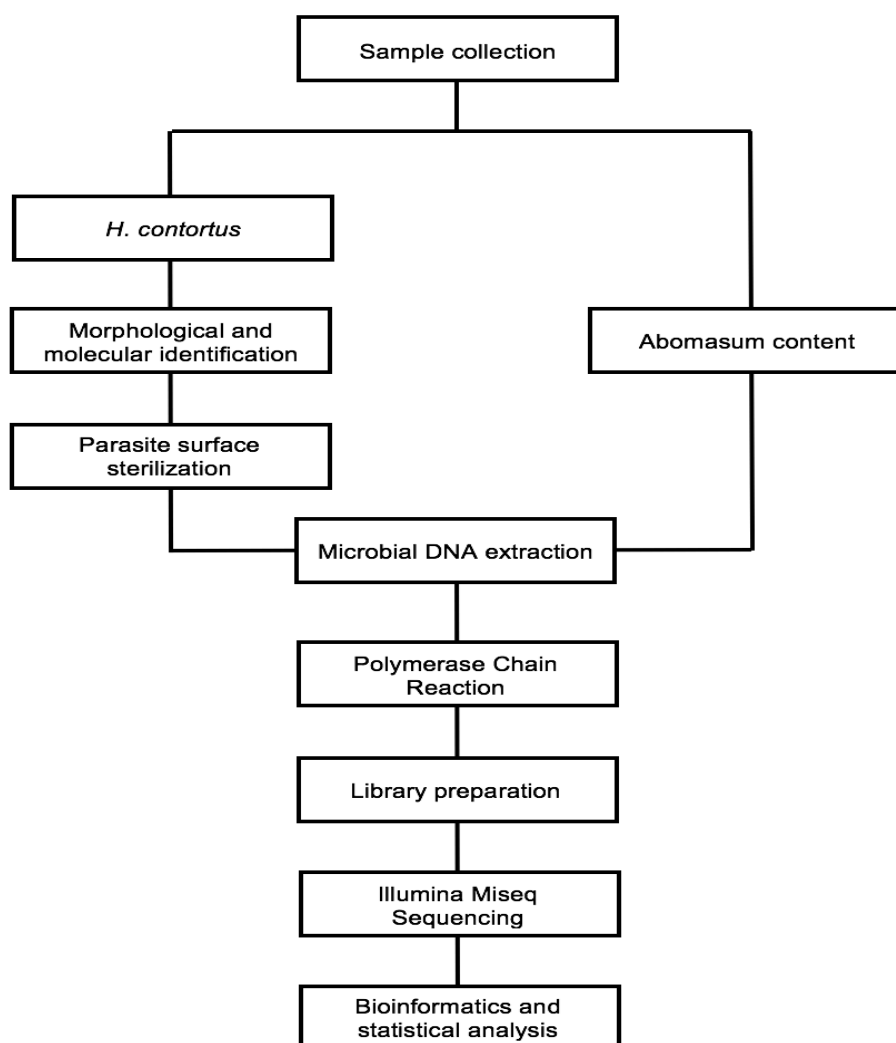


Figure 8. Flowchart of procedures undertaken during the course of the study.

3.3. EXPERIMENTAL ANIMALS

The study was conducted using 10 Dohne Merino sheep depicted in Table 4, from Wauldby farm which consist of a flock of over 450 ewes. This farm has well-documented history of heavy natural *H. contortus* infection which had been treated by dosing the flock approximately every six weeks, which inadvertently allowed the *H. contortus* to develop resistance to the anthelmintics being used. Hence, the farmer become part of long-term trial in partnership with Dr Alan Fisher (Queenstown Provincial Veterinary Laboratory) and Dr Gretha Snyman (Grootfontein Agricultural Development Institute) to research practical and effective ways to select a highly wireworm-resistant/resilient line of sheep in the Wauldby stud. The trial was done using FAMACHA, body condition scores (BCS) and faecal egg count (FEC) and indicated the heritability of the traits of resistance and resilience to parasites within these sheep.

Table 4. List of animals which were sampled in the present study.

Animals	ID	Sex	Age
Dohne Merino	B1.187	Ewe	7
Dohne Merino	B2.204	Ewe	6
Dohne Merino	B1.098	Ewe	7
Dohne Merino	B1.075	Ewe	7
Dohne Merino	B1.218	Ewe	7
Dohne Merino	B0.515	Ewe	8
Dohne Merino	B9.181	Ewe	9
Dohne Merino	B9.233	Ewe	9
Dohne Merino	B9.053	Ewe	9
Dohne Merino	B1.168	Ewe	7

3.4. PROCEDURE FOR COLLECTION OF THE ABOMASUM CONTENT AND ADULT *HAEMONCHUS CONTORTUS* RECOVERY FROM THE ABOMASUM

Naturally infected Dohne Merino sheep were slaughtered at local slaughterhouse (Tandala Farm Abattoir) under humane conditions and the GIT of the sheep were examined thoroughly especially, the abomasum. The abomasum was then opened along its greater curvature and about 5 g of the abomasum contents were collected using sterile sampling spoon into a sterile collection tube containing 95% ethanol. The rest of the contents were poured into a bucket and filtered through a sieve of 250 µm aperture that can retain worms therein. The rest of abomasal contents left on the abomasum were washed into a bucket up to a total volume of 2 liters and filtered through a sieve. The worms retained on the sieve and abomasum were collected and placed in Petri dish containing 0.05 M phosphate-buffered saline (PBS) (pH 7.4) for initial washing to remove abomasal content and allow regurgitation of abomasum content depicted in Figure 9 (Hansen, & Perry, 2018). The GIN parasite was stored in collection vials containing 70% ethanol. The collection vials containing abomasum content in 95% ethanol and GIN parasites in 70% ethanol were transported to the Agricultural Research Council-Biotechnology Platform (ARC-BTP) for laboratory analysis.



Figure 9. A picture depicting adult *Haemonchus* spp. recovered from Dohne Merino sheep in Eastern Cape, South Africa contained in a Petri dish containing 0.05M PBS (pH 7.4).

3.5. MORPHOLOGICAL IDENTIFICATION OF THE ADULT MALE AND FEMALE *HAEMONCHUS CONTORTUS* WORMS FROM SOUTH AFRICA

The recovered adult *Haemonchus* spp. from the abomasum of the infected sheep were identified and distinguished based on their morphological characters to species level as described in the literature by Lichtenfels *et al.* (1994) and Jacquet *et al.* (1997). During the study period a total of 266 *Haemonchus* spp. were examined under Zeiss dissection microscope and the pictures were taken using Zeiss Axio observer microscope depicted in Figure 10. From 20 to 50 *Haemonchus* worms per abomasum were examined and when less than 50 worms were recovered all of them were examined microscopically. The *Haemonchus* spp. males and females were identified by their morphological characters to the species level. Characters observed in adult female *H. contortus* included: body length, body colour and valval morphology. In adult male *H. contortus*, the following morphological attributes were observed: body length, spicule, left and right spicule barb length and the reddish colour on the surface of *H. contortus*. The length and width was observed based on standard body lengths of adult parasite: *H. contortus*: female (18 to 30 mm) and male (10 to 20 mm). The adult male and female *H. contortus* morphological identification was confirmed by PCR assay using species specific primers described in section 3.6.4.1.

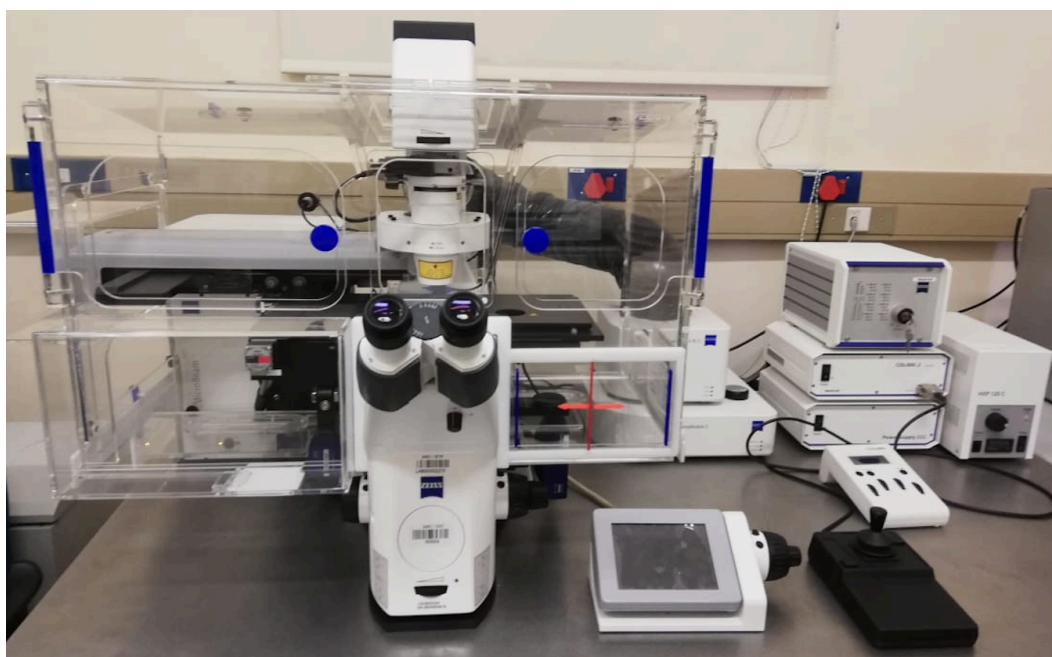


Figure 10. A picture depicting Zeiss Axio observer microscope used for morphological identification of adult *H. contortus*.

3.6. BACTERIAL COMMUNITY PROFILING OF THE ABOMASUM CONTENT, ADULT *HAEMONCHUS CONTORTUS* WORMS

3.6.1. STERILISATION OF ADULT *H. CONTORTUS* SURFACE

To remove external and abomasal contaminants the surface of adult male and female *H. contortus* stored in 70% ethanol, the samples were centrifuged at $13,000 \times g$ for 5 min and the supernatant discarded. The sample were washed with phosphate-buffered saline (PBS, pH 7.4) and incubated in an antibiotic solution (1mg/ml ampicillin/1mg/ml gentamicin) for 2 hours to kill external bacteria. Thereafter the worms were washed three times with 4% sodium hypochlorite for 20 seconds each and followed by washing five times with sterile PBS.

3.6.2. RULING OUT MICROBIAL CONTAMINATION ON THE ADULT *HAEMONCHUS CONTORTUS* SURFACES

PCR assay was employed to confirm the absence of microbial contamination on the GIN parasites surfaces from the abomasal microbial community as described in section 3.6.4. Briefly, the PBS from the final wash obtained in section 3.6.1 were used to confirm the sterility of the GIN parasite surface. DNA was extracted from the final wash and PCR assay was used to ensure the sterility of the GIN parasite surface. The sterile adult male and female *H. contortus* were stored at -20°C for downstream applications.

3.6.3. MICROBIAL DNA EXTRACTION

3.6.3.1. DNA EXTRACTION FROM ABOMASUM CONTENT

Microbial DNA was extracted from abomasum content using QIAamp DNA Microbiome Kit (Qiagen, Hilden, Germany). Sample pre-processing: Abomasum content sample preserved in 95% ethanol were centrifuged at $13,000 \times g$ for 5 min and the supernatant discarded. DNA extraction was performed according to the manufacturer's instructions with the following modifications. Samples were incubated in lysis solution composed of 20 mM Tris-HCl, pH 8.0, 2 mM sodium EDTA, 1.2% Triton® X-100, plus 20mg/ml lysozyme at 37°C for 60 min. DNA was extracted from 500 μl of the sample, as described in the manufacturer's protocol. Briefly, 500 μl

of the sample and 500 µl buffer AHL were added to 2 ml tube and incubated for 45 min at room temperature with end-over-end rotation. The sample was centrifuged at $10,000 \times g$ for 10 min and the supernatant was discarded. One hundred ninety microliters and 2.5 µl of Benzonase were added to the sample and thoroughly mixed. The mixture was then incubated for 30 min at 600 rpm in a heating block. Twenty microliters of Proteinase K was added to the mixture and incubated at 56°C for 30 min at 600 rpm in a heating block. The mixture was briefly spin down at low speed. Two hundred microliters of buffer ATL (containing Reagent DX) was added to the mixture and mixed very well. The mixture was transferred into Pathogen Lysis Tube L and then the Pathogen Lysis Tube L was placed into a 2010 Geno/Grinder® (SPEX SamplePrep LLC , New Jersey, United States) for 15 min at 1700 rpm. Thereafter, the Pathogen Lysis Tube L was centrifuged at $10,000 \times g$ for 1 min and the supernatant was transferred into fresh microcentrifuge tube. Forty microliters of Proteinase K was added to the mixture and vortexed and then incubated at 56°C for 30 min at 600 rpm in a heating block. Two hundred microliters of buffer APL2 was added and pulse vortexed for 30 s. The mixture was incubated at 70°C for 10 min and the tube was briefly spin. Two hundred microliter of ethanol was added to the lysate and mixed by pulse-vertexing for 15-30 s. Seven hundred microliters of the mixture was transferred into QIAamp UCP Mini spin column without wetting the rim. QIAamp UCP Mini spin column was centrifuged at $6000 \times g$ for 1 min and the flow-through was discarded. QIAamp UCP Mini spin column was then transferred to a fresh collection tube and 500 µl buffer AW1 was added to the column. The column was then centrifuged at $6000 \times g$ for 1 min and the filtrate was discarded. The QIAamp UCP Mini spin column was then placed in a fresh collection tube and 500 µl buffer AW2 was added, then the column was centrifuged at $20,000 \times g$ for 3 min. The column was placed in a fresh collection tube and centrifuged for $20,000 \times g$ for 1 min. The QIAamp UCP Mini spin column was placed in a fresh 1.5 ml Eppendorf tube and 50 µl buffer AVE was added directly onto the centre of the membrane, the column was incubated for 5 min. The QIAamp UCP Mini spin column was centrifuged at $6000 \times g$ for 1 min to elute the DNA. The quality of DNA was assessed by gel electrophoresis in 1% agarose gel run at 100 V for 30 min, and the sizes of the DNA were validated by comparison with a molecular size standard. The quantity and quality of the DNA were evaluated by

measuring Qubit@ 2.0 Fluorometer (Thermo Scientific). All microbial genomic DNA was stored at -20 °C before further analysis.

3.6.3.2. DNA EXTRACTION FROM ADULT MALE AND FEMALE *HAEMONCHUS CONTORTUS* WORMS

Microbial DNA together with GIN parasite DNA was extracted from sterile adult male and female *H. contortus* using the QIAamp DNA Microbiome Kit (Qiagen, Hilden, Germany). Sample pre-processing: *H. contortus* samples were homogenized and DNA extraction was performed according to the manufacturer's instructions with the following modifications. Samples were incubated in lysis solution composed of 20 mM Tris·HCl, pH 8.0, 2 mM sodium EDTA, 1.2% Triton® X-100, plus 20 mg/ml lysozyme at 37°C for 60 min. DNA was extracted from 500 µl of the sample, as described in section 3.6.3.1.

3.6.4. DNA AMPLIFICATION AND AMPLICON LIBRARY PREPARATION

3.6.4.1. AMPLICON POLYMERASE CHAIN REACTION

The 16S ribosomal RNA (rRNA) gene contains of nine hypervariable regions enclosed by regions of more conserved sequence depicted in Figure 11. The 16S ribosomal rRNA region of approximately 470 bp and 500 bp encircling the V3-V4 hypervariable region within the 16S rRNA gene was amplified employing a set of commonly used primers for the analysis of bacterial communities and 260 bp of the *H. contortus* gene was also amplified employing species specific primers for confirmation of *H. contortus* depicted in Table 5. Briefly, each 25 µl of PCR assay reaction contains 5 µl of genomic DNA as the template, 12.5 µl 2x KAPA HiFi HotStart ReadyMix (KAPABIOSYSTEMS, United States) and 5 µl of 10 µM of each primer. PCR reactions was carried out on BIO-RAD T100™ Thermal Cycler (Bio-Rad Laboratories, United Kingdom) using the following protocol: (1) an initial denaturation step performed at 95 °C for 5 min followed by 30 cycles of denaturation (95 °C, 30 s), annealing (56 °C, 30 s) and extension (72 °C, 40 s), and a final elongation of 10 min at 72 °C. PCR amplicon were assessed by gel electrophoresis in 1% agarose gel run at 100 V for 45 min, and the sizes of the products were validated by comparison with a molecular marker.

Table 5. Summary of primers used in the current study

Primer pairs	Primer sequence (5'-3')	Targeted regions	Reference
338F 806R	5'-CCTACGGGNGGCWGCAG-3' 5'-GACTACHVGGGTATCTAATCC - 3'	V3-V4 region	Klindworth <i>et al.</i> , 2013
HcBotuF1 HcBotuR2	5'-TGTCGAACACGAAACTCGTC-3' 5'-TGTGTCTCTACCGCCCGAGT-3'.	<i>H. contortus</i>	Amarante <i>et al.</i> , 2017

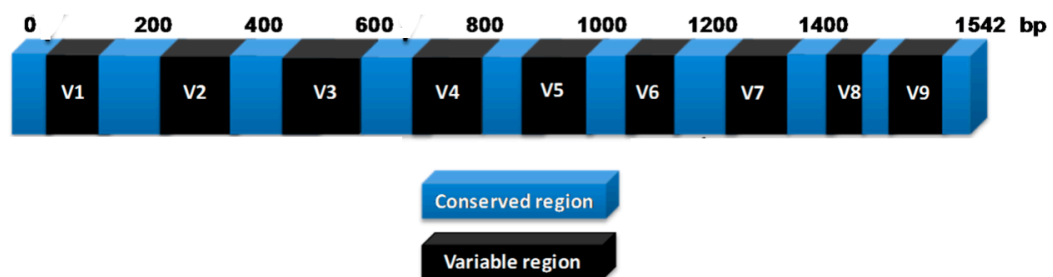


Figure 11. A picture of depicting the 16S ribosomal RNA (rRNA) gene containing the nine hypervariable regions enclosed by regions of more conserved sequence.

3.6.4.2. POLYMERASE CHAIN REACTION CLEAN-UP

The PCR clean-up was conducted using AMPure XP beads (Beckman Coulter, United States). Briefly, PCR amplicons were centrifuged at $1,000 \times g$ at 20°C for 1 min to collect condensation. The AMPure XP beads were Vortexed for 30 s to make sure that the beads are evenly dispersed. Twenty microliters of the AMPure XP beads were then added to each PCR amplicon. The mixture was mixed gently with a pipette and mixed at 1800 rpm for 2 min. Thereafter, the mixture was incubated at room temperature without shaking for 5 min. The tube containing the solution mixture was placed on a magnetic stand for 2 min or until the supernatant has cleared. While the amplicon tube on the magnetic stand, the supernatant was removed and discarded. Two hundred microliters of freshly prepared 80% ethanol was added to each sample tube and incubated on the magnetic stand for 30 s. The supernatant was carefully removed and discarded. This step was repeated twice and the excess ethanol was therein removed. While the Amplicon tube still on the magnetic stand, the beads were allowed to air-dry for 10 min. Fifty five micro liters of 10 mM Tris pH 8.5 were added to each amplicon tube and mixed at 1800 rpm for 2 min while ensuring that beads are fully resuspended. The mixture was then incubated at room temperature for 2 min and after the tube was placed on the magnetic stand for 2 min or until the supernatant has cleared. Fifty micro liters of the clean DNA was transferred into a new 96-well PCR plate.

3.6.4.3. INDEX POLYMERASE CHAIN REACTION

Index PCR assay was employed attaches dual indices and Illumina sequencing adapters using the Nextera XT Index Kit (Illumina, USA). Briefly, each 50 μl of PCR reaction contains 5 μl of microbial genomic DNA as the template, 25 μl 2x KAPA HiFi HotStart ReadyMix (KAPABIOSYSTEMS, United States), 5 μl of each Nextera XT Index Primers (N7XX) from the Nextera XT Index kit and 10 μl PCR Grade water. PCR reactions was carried out using the following protocol: (1) an initial denaturation step performed at 95°C for 3 min followed by 10 cycles of denaturation (95°C , 30 s), annealing (55°C , 30 s) and extension (72°C , 30 s), and a final elongation of 5 min at 72°C . The PCR amplicon was cleaned, as described in section 3.6.4.2.

3.6.5. HIGH-THROUGHPUT SEQUENCING

3.6.5.1. LIBRARY QUANTIFICATION, NORMALIZATION, AND POOLING

The Library Quantification, Normalization, and Pooling were done as per Illumina 16S Metagenomic Sequencing Library Preparation guide. Briefly, The library from index PCR were quantifying using a fluorometric quantification method that uses dsDNA binding dyes. The DNA concentration in nM, based on the size of DNA amplicons were calculated as follows:

$$:(\text{concentration in ng/}\mu\text{l}) \times 10^6 = (660 \text{ g/mol} \times \text{average library size})$$

The concentrated final library were diluted using Resuspension Buffer or 10 mM Tris pH 8.5 to 4 nM. Thereafter, 5 μl of diluted DNA were aliquoted from each library and mixed for pooling libraries with unique indices.

3.6.5.2. LIBRARY DENATURING AND MISEQ SAMPLE LOADING

In preparation for cluster generation and sequencing, pooled libraries were denatured with NaOH, diluted with hybridization buffer, and heat denatured before MiSeq sequencing. Each run included a 5% PhiX to serve as an internal control for these low-diversity libraries. The prepared pooled libraries were then loaded into the Illumina MiSeq sequencing platform (ARC-BTP, S.A.) (Figure 12).

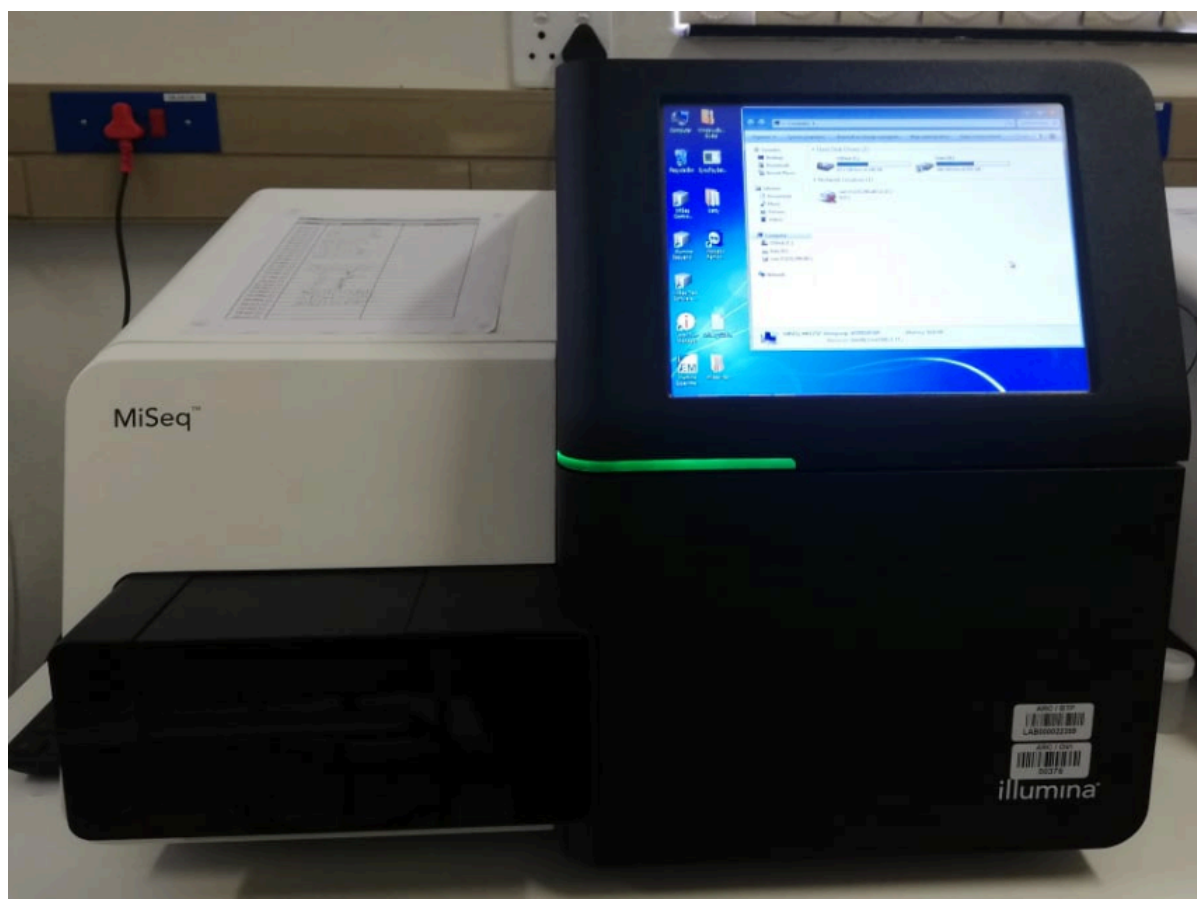


Figure 12. A picture of Illumina MiSeq sequencing platform used for sequencing.

3.7. BIOINFORMATICS ANALYSIS

Demultiplexed paired-end fastq reads and a mapping file or metadata file were imported into the Quantitative Insights Into Microbial Ecology 2 program (QIIME2, ver. 2018.6.0, <https://qiime2.org/>). Sequencing reads were pre-processed, quality filtered and analysed using QIIME2 version 2018.6.0. The Divisive Amplicon Denoising Algorithm 2 (DADA2) software package, wrapped in QIIME2, was used for quality filter, trim, de-noise, and merge the data including removal of chimeras with the “consensus” method and sequence variant calling of the Illumina amplicon sequences. Due to the decreasing quality scores of the sequences at the end, especially for the reverse reads, forward and reverse reads were truncated at 270 bp and 230bp of the 300 bp respectively, corresponding to a quality score >20. This step was done by the QIIME dada2 denoise-paired command. Resultant feature sequences were summarized and then annotated using the QIIME2 q2- feature-classifier plugin and Silva 132 99% OTUs (full-length, seven-level taxonomy) classifier pre-trained to the full-length Silva

database to assign taxonomy to all ribosomal sequence variants. QIIME2 taxa barplot command was used to visualise the classified taxonomic composition of the samples. Contaminating mitochondrial and chloroplast sequences were filtered out of the resulting feature table. The remaining representative sequences were aligned with MAFFT and used for phylogenetic reconstruction of FastTree.

3.8. STATISTICAL ANALYSIS

Alpha and beta-diversity analyses were performed with the q2-diversity plugin wrapped in QIIME2 at a sampling depth of 15000 sequences. There was no sample excluded from these analyses because the lowest sequence count was 15,031 of all the samples. To calculate diversity indexes, alpha diversity was calculated by alpha diversity metrics including observed OTUs, Shannon diversity index, Simpson diversity index, Pielou's measure of species evenness and Faith's Phylogenetic Diversity with statistical significance determined using Kruskal-Wallis tests. The Beta diversity was calculated by Bray-Curtis and Jaccard distances, unweighted and weighted UniFrac. Thereafter, Principal coordinate analyses (PCoA) was performed based on unweighted and weighted UniFrac distances in QIIME2 and visualized with QIIME2 emperor visualizer (ver. 2018.6.0). Permutational multivariate analysis of variance (PERMANOVA) was used to analyse statistical differences in beta diversity within QIIME2. Additional analysis was conducted with krona plot, ggplot2 and VennDiagram packages in the R software (R version 3.5.0 (2018-04-23)) to construct the heatmap and Venn diagram in order to visualise the shared and unique OTUs between the groups.

3.9. ETHICAL APPROVAL

The study was conducted after the approval of the Onderstepoort Veterinary Institute Animal Ethics Committee (AEC) (AEC No: 36.18). The study was also approved by the scientific committee of the Unit for Environmental Sciences and Management of the North-West University, Potchefstroom Campus.

CHAPTER 4

RESULTS

4.1. MORPHOMETRICS OF *HAEMONCHUS CONTORTUS* FROM SOUTH AFRICA

A total of 266 GIN parasites belonging to the genus *Haemonchus* were recovered from 7 out of 10 (70%) abomasum of the Dohne Merino sheep sampled from Eastern Cape province, South Africa depicted in Table 6. These recovered adult *H. contortus* were selected for the characterization of their microbiota in the present study as presented in Figure 13. The specimens were first examined in detail with the assistance of an experienced helminthologist to identify adult *H. contortus* using morphological characters described in literature by Lichtenfels *et al.* (1994) and Jacquiet *et al.* (1997). Of the 266 adult *Haemonchus* spp analysed, 169 females (64%) and 97 males (36.5%) were identified as *H. contortus* based on the selected morphometrics characters: reddish colour, body length, copulatory bursa, vulval and spicules morphologies to the species level using Zeiss dissection microscope.

Table 6. Percentage of total number of adult male and female *H. contortus* species identified in the present study.

Animals ID	No of <i>H. c</i>	Observed male <i>H. c</i>	%Male	Observed female <i>H. c</i>	%Female
B1.187	26	9	35%	17	65%
B2.204	-	-	-	-	-
B1.098	-	-	-	-	-
B1.075	42	11	26%	31	73%
B1.219	50	21	42%	29	58%
B0.515	50	23	46%	27	54%
B9.181	50	20	40%	30	60%
B9.233	28	7	25%	21	75%
B9.053	-	-	-	-	-
B1.168	20	6	30%	14	70%
Total	266	97		169	
Overall %			36.5%		64%

H. c= *Haemonchus contortus*. No of *H. c*= Number of *H. contortus* recovered.

Male

The adult male *H. contortus* were morphologically identified by reddish colour, body length and the tail end which bears a copulatory bursa that consists of three lobes, two large lateral lobes and a poorly developed dorsal lobe and two spicules. Dorsal ray is asymmetrical and bifurcated. External dorsal ray is thin and long. Lateral rays arise from a common trunk; ventral rays are fused proximally and separated dorsally depicted in Figure 13A.

Female

The adult female *H. contortus* were morphologically identified by its reddish colour, body length and vulva which is situated in the posterior third of the body at a distance of 2.11- 4.55 mm from the posterior end. The vulval lips are inconspicuous but a linguiform process is invariably present. Vulva is covered by valves depicted in Figure 13B.

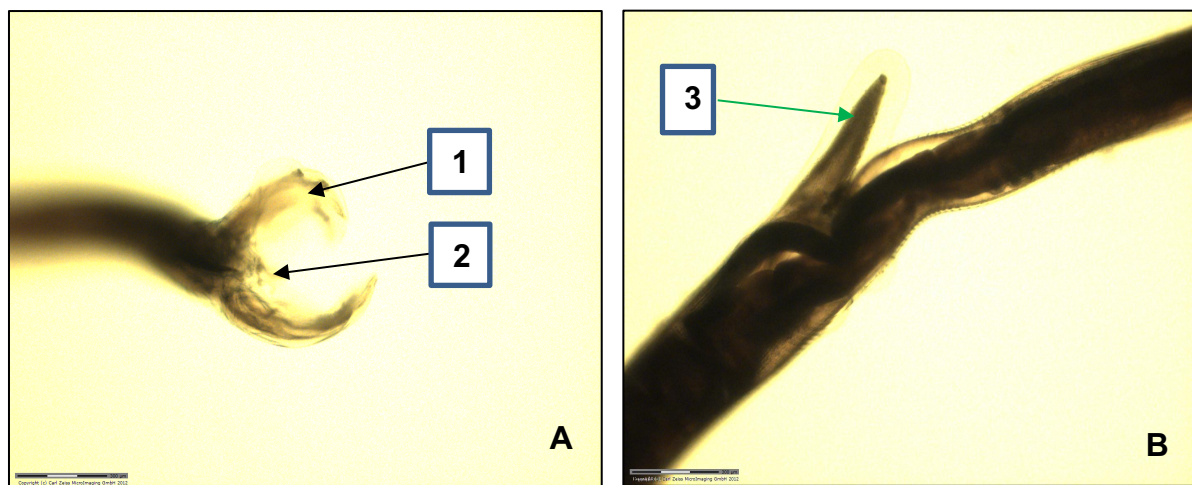


Figure 13. Picture depicting adult *H. contortus* characters (A). Male bursa and (B). Female vulva flap. The black arrows are pointing to the male copulatory bursa (1) and spicules used to hold open the female worms genital opening (2), the green arrow is pointing to the female vulval flap (3).

4.2. MOLECULAR ANALYSIS OF THE ADULT MALE AND FEMALE *H. CONTORTUS*

A total of 14 pooled adult male and female *H. contortus* samples identified by their morphometric characteristics were confirmed by the PCR assay to be *H. contortus*. An amplification band was obtained with all *H. contortus* samples using the species specific HcBotuF1/R2 primer pair and the band was present at 260 bp in Figure 14.

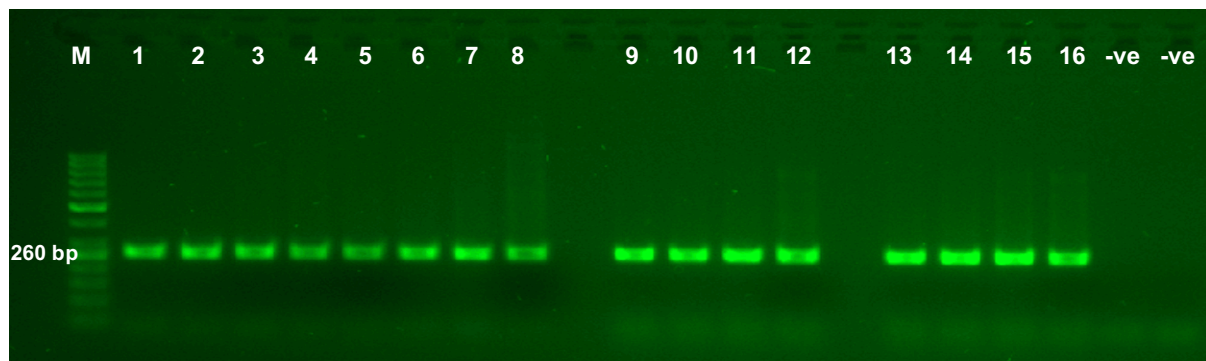


Figure 14. Gel electrophoresis image depicting *H. contortus* PCR amplicons at 260 bp. (M) as 50 bp Marker . Line (1-16) *H. contortus*. (-ve) double distilled water used as negative control.

4.3. RULING OUT MICROBIAL CONTAMINATION ASSOCIATED WITH THE ADULT *H. CONTORTUS* SURFACES.

There was no contamination (i.e. intact bacteria and their DNA products) originating from the abomasal microbiota, as there were no bands observed during gel electrophoresis on washed samples as depicted in Figure 15.

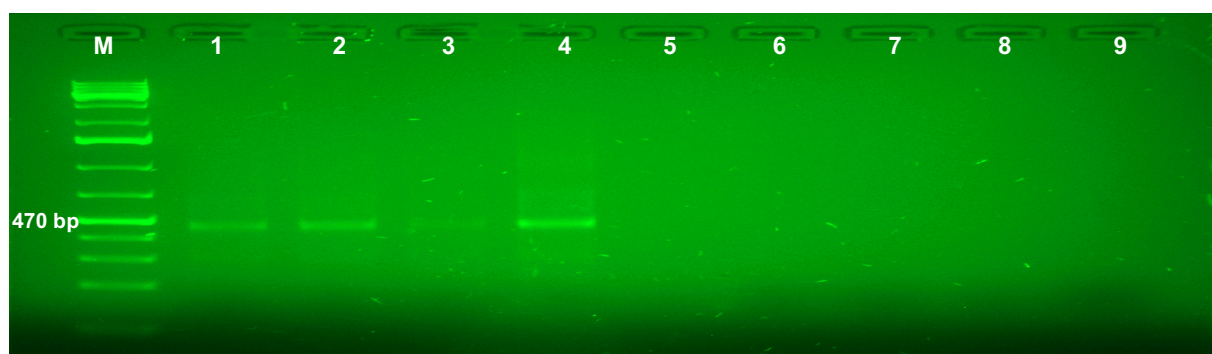


Figure 15. Gel electrophoresis image depicting washed and unwashed *H. contortus* PCR amplicons at 470 bp. (M) as 100 bp Marker (gene ruler). Line (1-4) unwashed. Line (5-9) washed *H. contortus*.

4.4. MICROBIAL DNA AMPLIFICATION BY POLYMERASE CHAIN REACTION

A total of 24 pooled samples including 10 abomasum content and 14 adult male and female *H. contortus* worms which were identified by their morphometric characters and confirmed by molecular analyses using PCR assay. The absence of the external microbial contamination on the surface of the *H. contortus* was confirmed by PCR assay and gel electrophoresis. The extracted microbial DNA from both the abomasum content and *H. contortus* samples were amplified by PCR assay employing 16S-F/16S-R primer pair which has been suggested to be suitable for microbial sequencing on Illumina 16S Metagenomic Sequencing Library Preparation guide. An amplification band was obtained with all the abomasum content and *H. contortus* samples. The amplification product was assessed and visualised by gel electrophoresis (Bio-Rad Ultraviolet image system) with the band present at 500-bp in Figure 16. All the PCR amplicons were cleaned up and sequenced using Illumina Miseq Sequencing Platform (ARC-BTP, S.A).

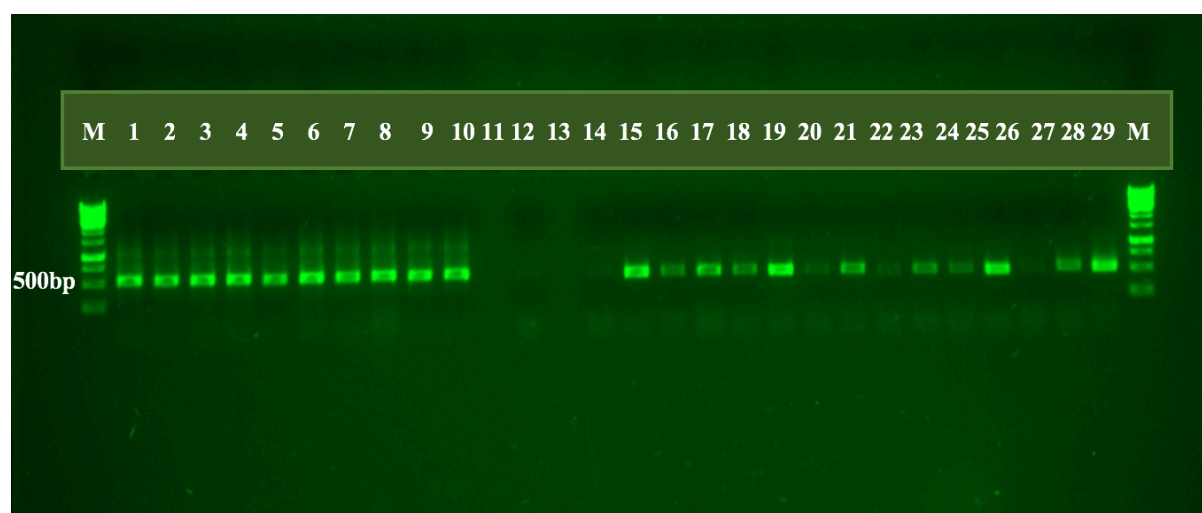
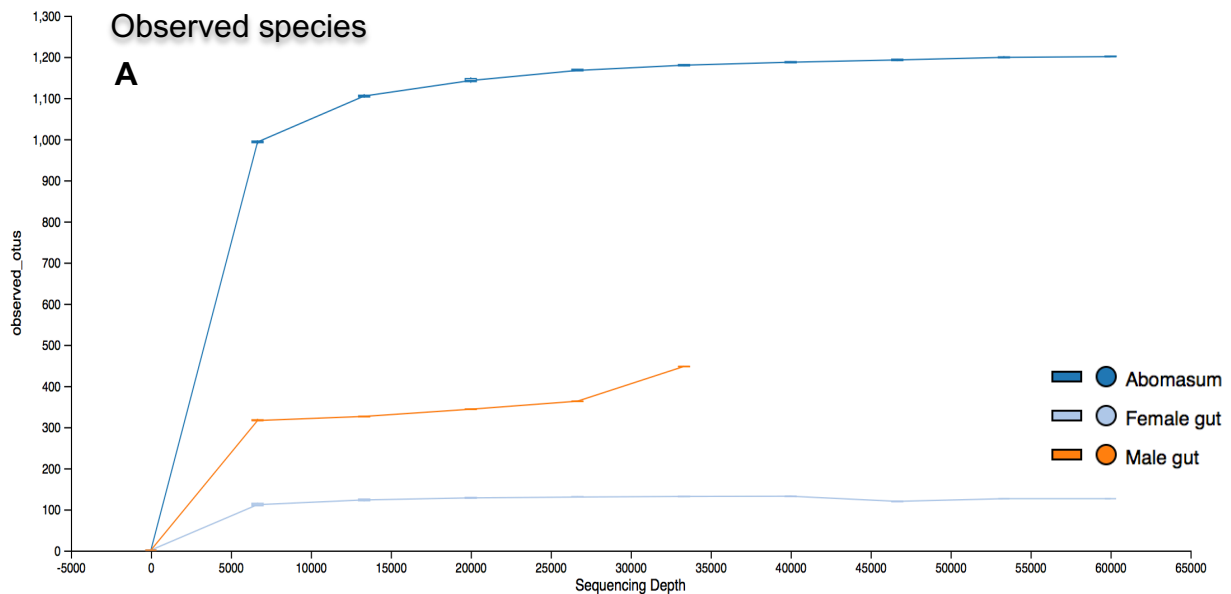


Figure 16. Gel electrophoresis image depicting the abomasum content and *H. contortus* microbiota PCR amplicons at 500 bp. (M) as 1500 bp Marker (gene ruler). Lines (11-13) double distilled water used as negative control. Lines (1-10) abomasum content PCR amplicons. Lines (14-29) *H. contortus* PCR amplicons.

4.5. DIVERSITY INDEX ANALYSIS OF BACTERIAL COMMUNITY

In total, 5157491 high quality paired-end sequence reads were obtained from 10 abomasum content and 14 adult male and female *H. contortus* samples. The demultiplexed sequence counts ranged from 47194 to 510257 reads per sample with the sequence reads quality trimmed at 270 bp forward read and 230 bp reverse read. The subsequent reads were normalized to 15000 sequences with rarefaction curves to determine that this depth was sufficient to describe the sample diversity. The total number of OTUs identified were 7,003 OTUs across the rarefied dataset with frequency of 1,486,820 at 97% identity. The illustrated rarefaction curves primarily determines if the OTUs richness of the samples has been fully observed or sequenced at a random pool of sequences in different depths. The rarefaction curves for the abomasum content, adult male and female *H. contortus* reached the near plateau phase depicting satisfying sampling depth. The rarefaction estimators, such as observed species, Shannon and Faith_pd showed that OTUs abundance was higher in the abomasum content compared to adult male and female *H. contortus* across all three matrices as depicted in Figure 17.



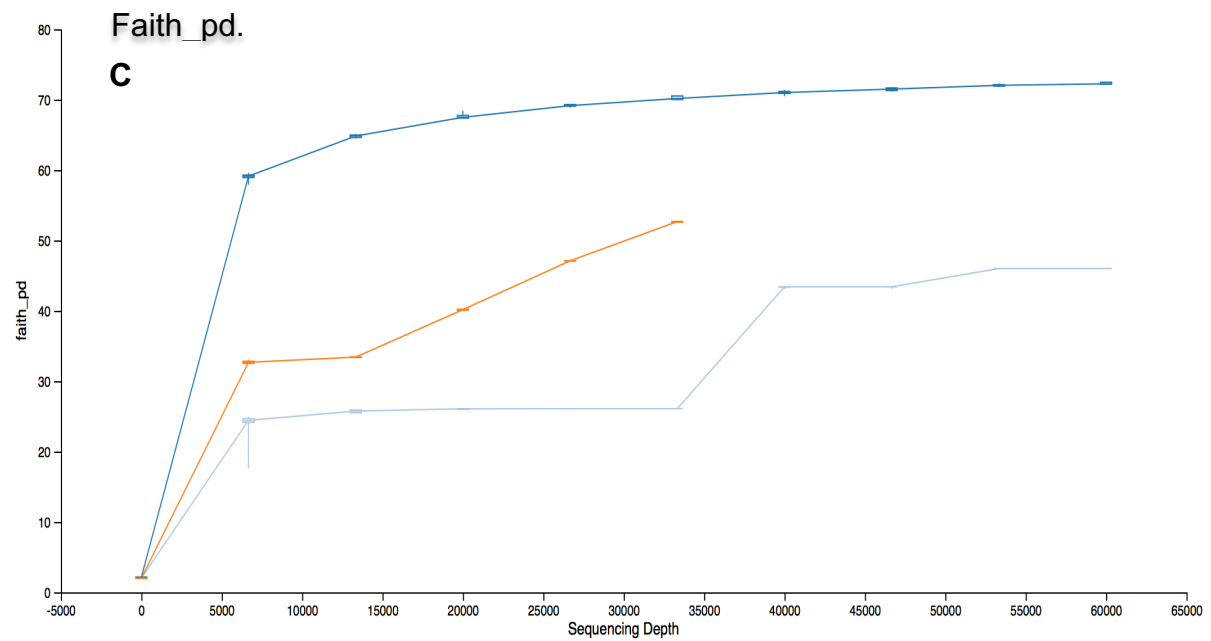
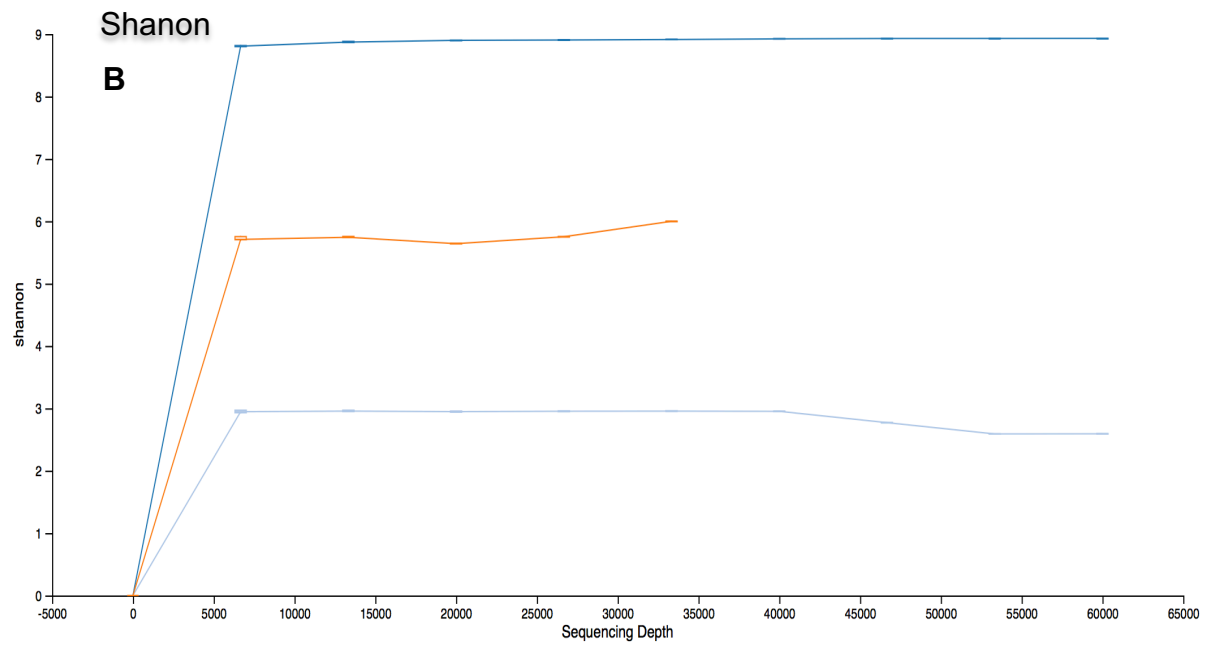
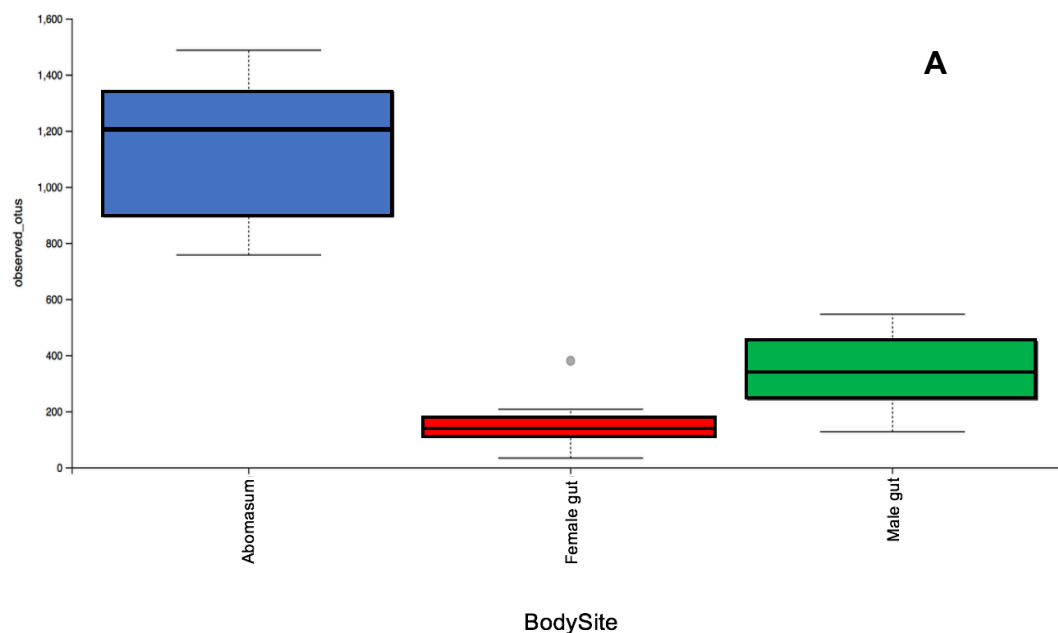


Figure 17. Alpha rarefaction curves (**A–C**) of the abomasum content, adult male and female *H. contortus* on 16S rRNA gene sequence (V3-V4 region) of abomasum and parasite microbiota at 97% identity. Rarefaction curve (**A**). Observed species (**B**). Shannon, (**C**). Faith_pd. Data represent average values from pooled populations.

4.5.1. ALPHA DIVERSITY ESTIMATE OF MICROBIOTA ASSOCIATED WITH THE ABOMASUM CONTENT, ADULT MALE AND FEMALE *HAEMONCHUS CONTORTUS*

To determine the bacterial diversity within samples, the alpha diversity was examined using various alpha matrices such as Observed, Shannon, Simpsons, Faith_pd and Pielou's Evenness. As depicted in Table 7 below, the highest OTU richness was found in the abomasum content followed by the adult male *H. contortus* while the adult female *H. contortus* had lowest OTU richness. The average number for the observed OTUs were between 159.714 ± 110.045 to 1144.4 ± 268.091 ; the average number for the Shannon index varied from 3.274 ± 1.049 to 8.906 ± 0.421 ; the average number for the Simpson index were between 0.777 ± 0.080 to 0.994 ± 0.003 and the average Faith_pd varied from 33.260 ± 22.530 to 70.50 ± 15.930 . Furthermore, In Figure 18, the abomasum content was compared to the adult male *H. contortus* microbiota, which were later compared to the adult female *H. contortus* microbiota and displayed a significant differences employing Observed OTUs, Shannon, Simpson and Pielou's Evenness diversity, p-value was $P < 0.05$. Similarly, the adult male and female *H. contortus* were compared and there was a consequential difference between the adult male *H. contortus* microbiota in comparison with the adult female *H. contortus* microbiota across all alpha diversity matrices except for Faith's Phylogenetic Diversity (PD) which did not show any significant differences, p-value was $P > 0.05$ in Table 7.



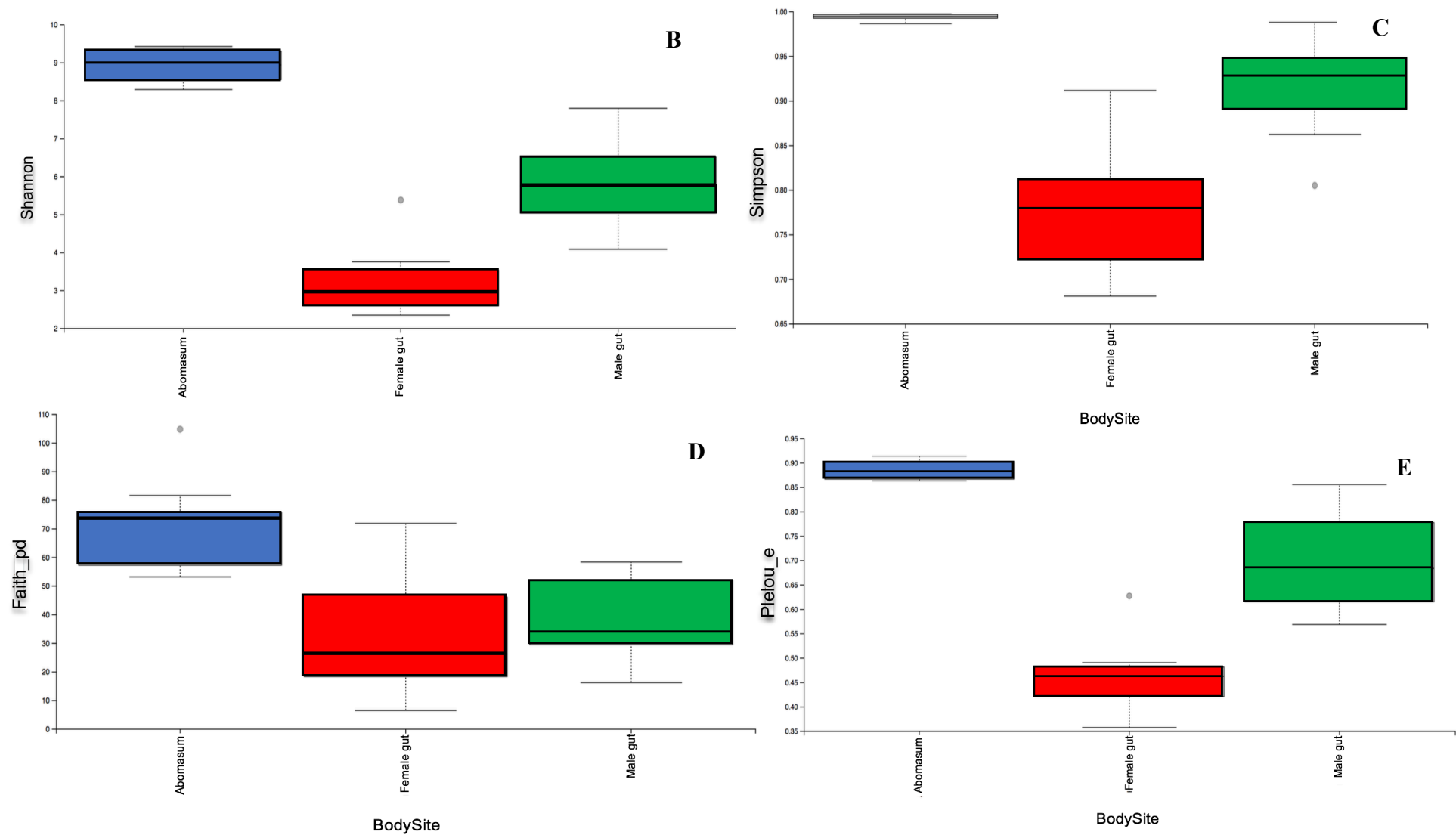


Figure 18. Alpha diversity analysis (A-E) of the abomasum content, adult male and female *H. contortus* based on 16S rRNA gene sequence of abomasum and parasite microbiota at 97% identity. Alpha diversity matrices (A). observed OTUs, (B). Shannon, (C). Simpson, (D). Faith_pd, (E). Pielou's Evenness. Data represent average values from pooled populations.

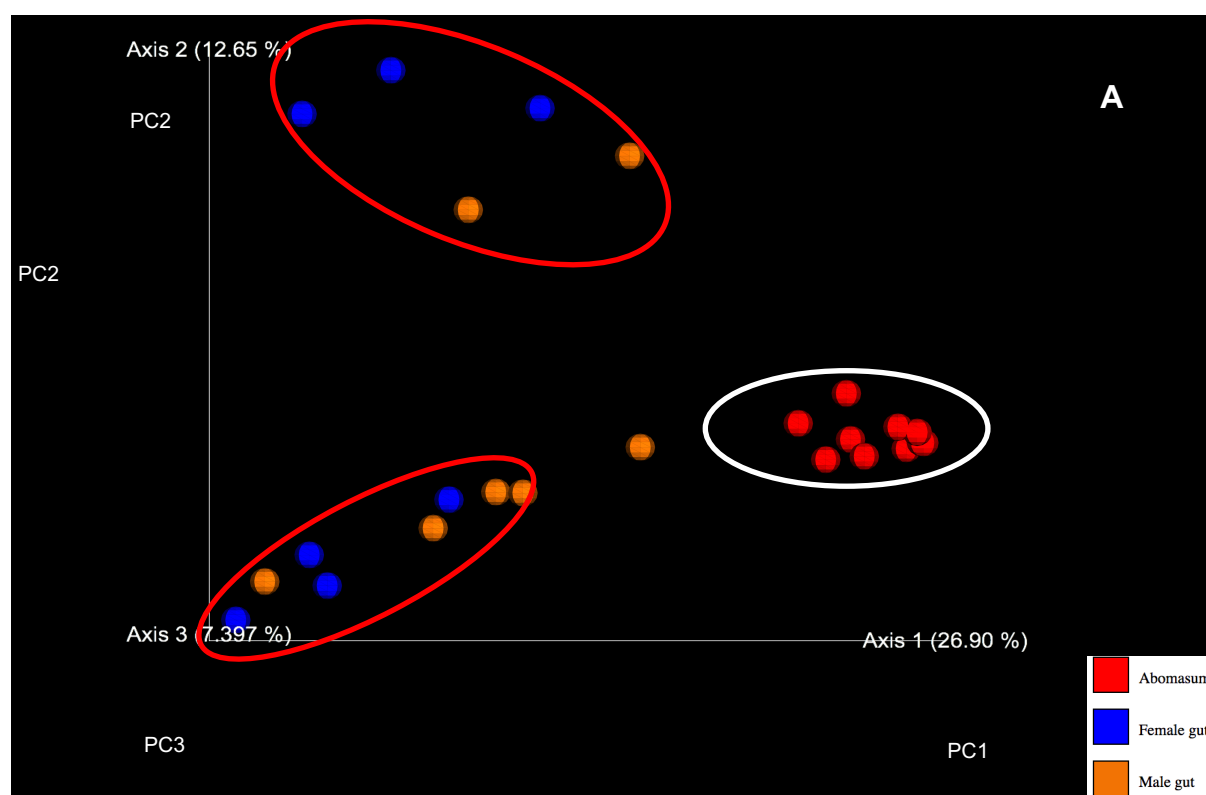
Table 7. Overview of alpha diversity metrics for the abomasum content, adult male and female *H. contortus* microbiota

Diversity matrices	Abomasum microbiota ^a	<i>H. contortus</i> male Microbiota ^a	<i>H. contortus</i> female microbiota ^a	<i>Abomasum</i> vs <i>H. contortus</i> male		<i>Abomasum</i> vs <i>H. contortus</i> female		<i>H. contortus</i> male vs <i>H. contortus</i> female	
	Average; STD	Average; STD	Average; STD	H-value	P-value	H-value	P-value	H-value	P-value
Observed OTUs	1144.4±268.091	339.571±155.680	159.714±110.045	12	0.000636	12	0.000636	4.4	0.035006
Shannon-index	8.906±0.421	5.815±1.267	3.274±1.049	12	0.000636	12	0.000636	8.3	0.004041
Simpson's index	0.994±0.003	0.913±0.062	0.777±0.080	12	0.000906	12	0.000636	6.9	0.008809
Faith_pd	70.50±15.930	38.639±15.790	33.260±22.530	8	0.004653	9	0.003415	0.5	0.482203
Genus evenness (equitability)	0.884±0.0196	0.698±0.1147	0.464±0.0854	12	0.000636	12	0.000636	8.3	0.004041

P-values were calculated using Kruskal-Wallis test. The level of significance was determined at *P* < 0.05. ^aData represent average and standard deviation (STD) values from pooled populations.

4.5.2. BETA DIVERSITY ESTIMATE OF MICROBIOTA ASSOCIATED WITH THE ABOMASUM CONTENT, ADULT MALE AND FEMALE *HAEMONCHUS CONTORTUS*.

The diversity differences between samples were calculated exploiting the default beta diversity metrics of Jaccard distance, Bray-Curtis distance, weighted and unweighted UniFrac distance. The unweighted UniFrac distance (presence/absence of bacterial taxa) and weighted UniFrac distance (based on presence-absence and relative abundance of bacterial taxa) were applied to examine variations in the microbiota composition and structure across all three hosts. Principal Coordinate Analysis (PCoA) was plotted using the result of UniFrac distance matrices to determine the similarity between groups of samples. The three-dimensional PCoA plots were visualized employing the QIIME2 Emperor tool. An unweighted and weighted UniFrac distance analysis indicated that the microbiota composition and structure in the abomasum content was significantly different ($P = 0.001$) from that of the adult male and female *H. contortus*. Furthermore, the beta diversity analysis showed one very distinct cluster (abomasum) separated from adult male and female *H. contortus* which clustered together in Figure 19. Whereas the adult male *H. contortus* were more similar to adult female *H. contortus*.



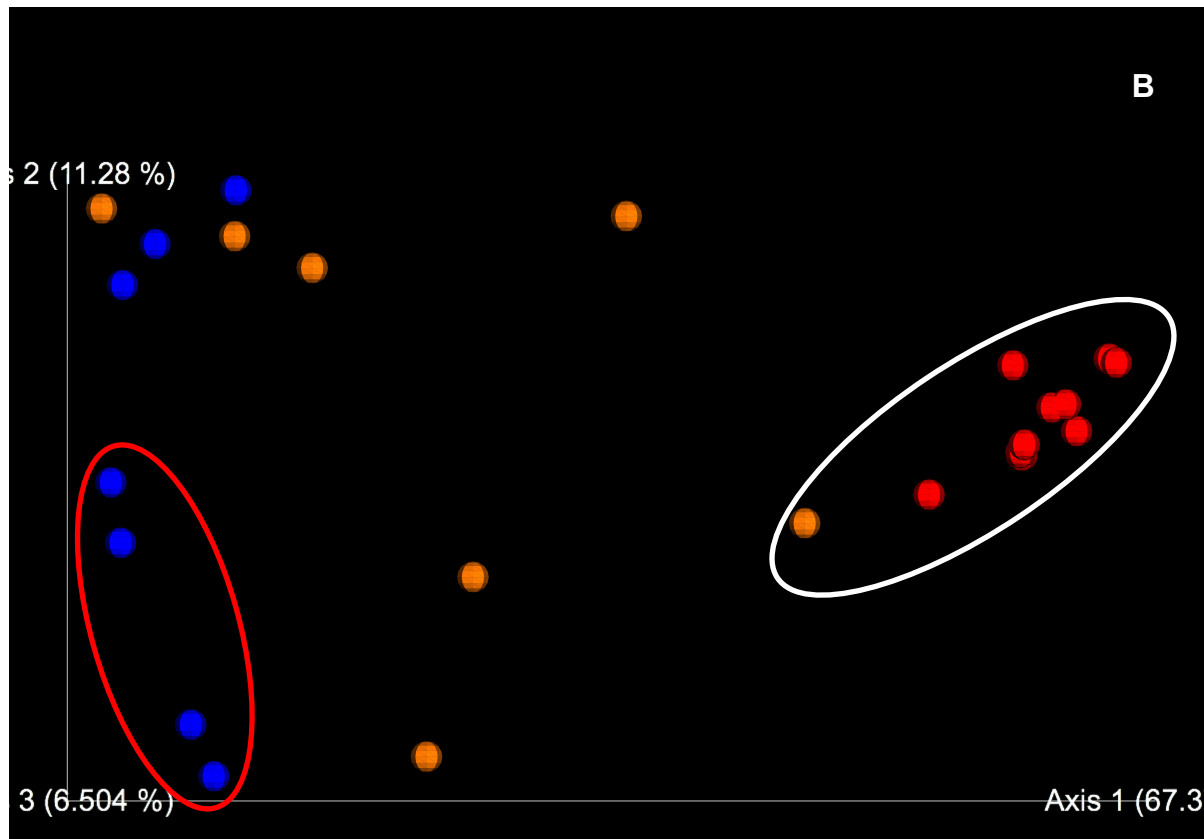


Figure 19. Principal coordinate analysis (PCoA) based on (A). unweighted UniFrac distances (Measures the fraction of unique branch length), (B). weighted UniFrac distances (Takes into account taxa abundance). The percentage variation explained with first three principal components (PC1, PC2 and PC3). Data represent average values from pooled populations.

These results demonstrated that the microbiota composition and structure was significantly different between the abomasum content and adult female *H. contortus*, the distance between the two cluster was significant ($F = 43.357699$, $P = 0.001$). The composition and structure between the abomasum content and adult male *H. contortus* was also significantly different ($F = 14.711808$, $P = 0.001$), although the distance between the two clusters indicate that they are separated but not far apart. The adult male and female *H. contortus* showed least level of significance and their microbiota clustered together indicating that they share similar microbiota, as shown by the Pseudo- F value (indicate the separated clusters and close-knit) was very low ($F = 2.850805$, $P = 0.029$) in Table 8.

Table 8. Overview of beta diversity for the abomasum content, adult male and female *H. contortus* microbiota

Pairwise distances	Permutations	Pseudo- F	P-value	q-value
Abomasum to Male gut	999	14.711808	0.001	0.0015
Abomasum to Female gut	999	43.357699	0.001	0.0015
Male to Female	999	2.850805	0.029	0.0290

P -values and pseudo- F were calculated using PERMANOVA test (Pseudo- F). The level of significance was determined at $P < 0.05$.

4.6. THE MICROBIOTA COMPOSITION AND ABUNDANCE ASSOCIATED WITH ABOMASUM CONTENT, ADULT MALE AND FEMALE *HAEMONCHUS CONTORTUS*

The microbiota composition at the phylum level

In order to explore the microbiota composition and abundance associated with the abomasum content, adult male and female *H. contortus*, we carried out high-throughput Illumina paired end sequencing. The results of the taxonomic abundances of all the OTUs at the phylum level were visualised in a Krona_plot that is based on relative abundances depicted in Figure 20 - 22. A total of 20 assigned bacterial phyla were obtained in the abomasum content. The five most abundant bacterial phyla in the abomasum content were *Firmicutes* (45%) followed by *Bacteroidetes* (26%), *Actinobacteria* (7%), *Patescibacteria* (4%) and *Planctomycetes* (3%). The remaining bacterial phyla were relatively low ranging from 1% to 0.0003%. The least abundant bacterial phyla included *Fusobacteria* (1%), *Verrucomicrobia* (0.01%) and *Chlamydiae* (0.005%) depicted in Figure 20.

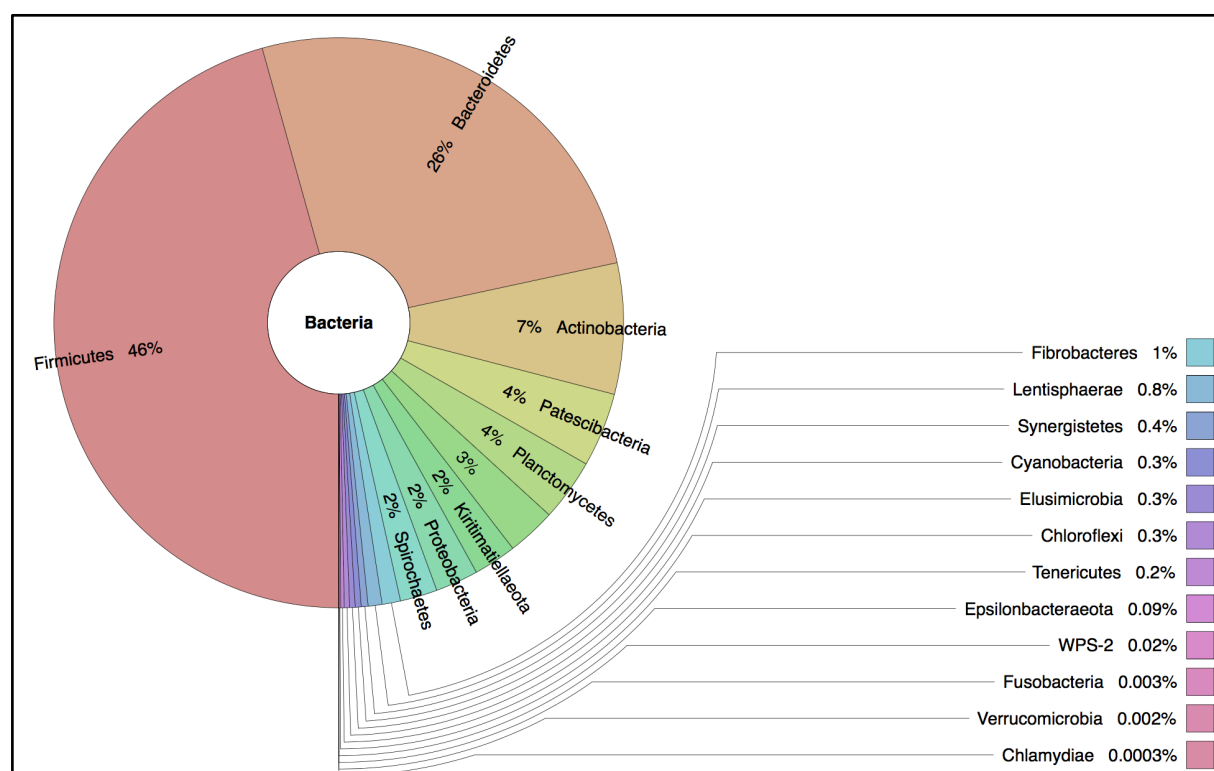


Figure 20. Krona plot for taxonomic abundance of the abomasum content microbiota at the phylum level. Data represent average values from pooled populations.

A total of 23 assigned bacterial phyla were obtained in the adult male *H. contortus*. The adult male *H. contortus* microbiota were predominantly dominated by bacterial phyla: *Proteobacteria* (57%) followed by *Firmicutes* (24%), *Bacteroidetes* (8%) and *Actinobacteria* (6%). However, 0.8% of the bacterial phyla in the adult female *H. contortus* were unclassified. The remaining bacterial phyla were relatively low ranging from 1% to 0.003%. The least abundant bacterial phyla included *Verrucomicrobia* (0.008%), *Deinococcus-Thermus* (0.008%) and *Chlamydiae* (0.003%) depicted in Figure 21.

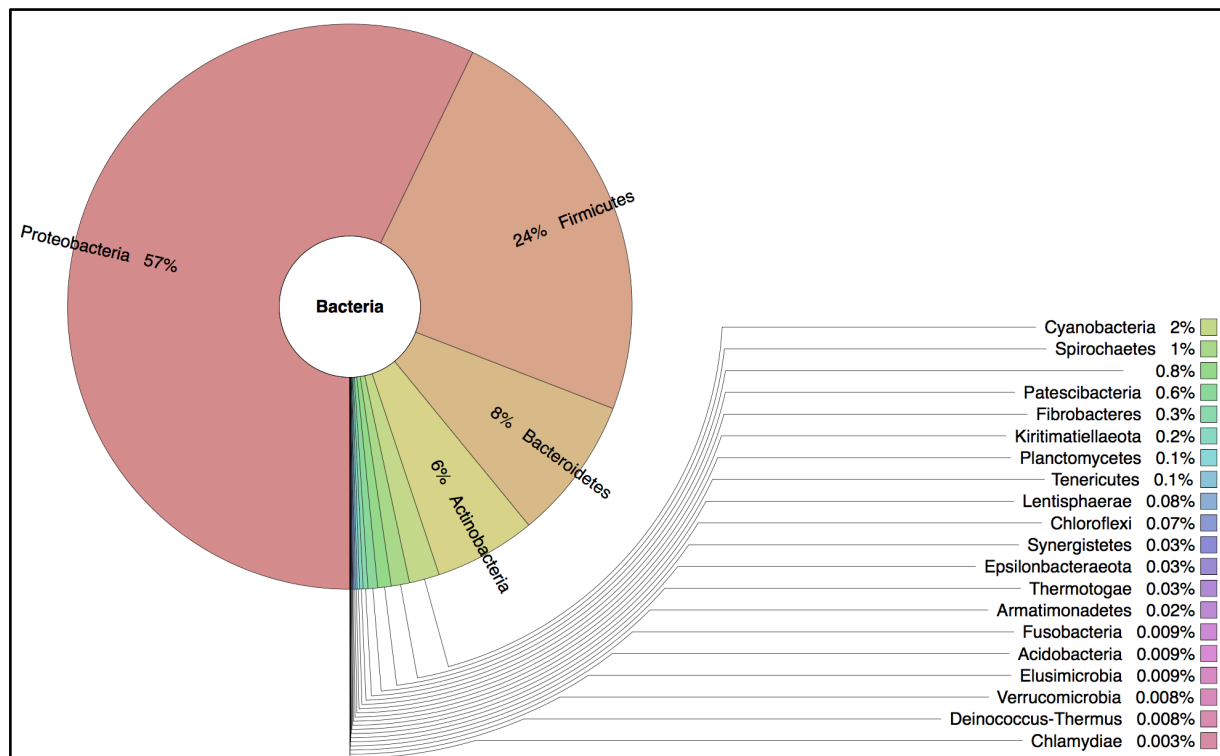


Figure 21. Krona plot for taxonomic abundance of adult male *H. contortus* microbiota at the phylum level. Data represent average values from pooled populations.

A total of 17 assigned bacterial phyla were obtained in the adult female *H. contortus*. The adult female *H. contortus* microbiota were predominantly dominated by bacterial phyla: *Proteobacteria* (94%) followed by *Firmicutes* (3%), *Bacteroidetes* (1%), *Actinobacteria* (0.6%) and *Cyanobacteria* (0.3%). However, 0.09% of the bacterial phyla in the adult female *H. contortus* were unclassified. The remaining bacterial phyla were relatively low ranging from 0.6% to 0.005%. The least abundant bacterial phyla included *Elusimicrobia* (0.01%), *Synergistetes* (0.01%) and *Thermotogae* (0.005%) depicted in Figure 22.

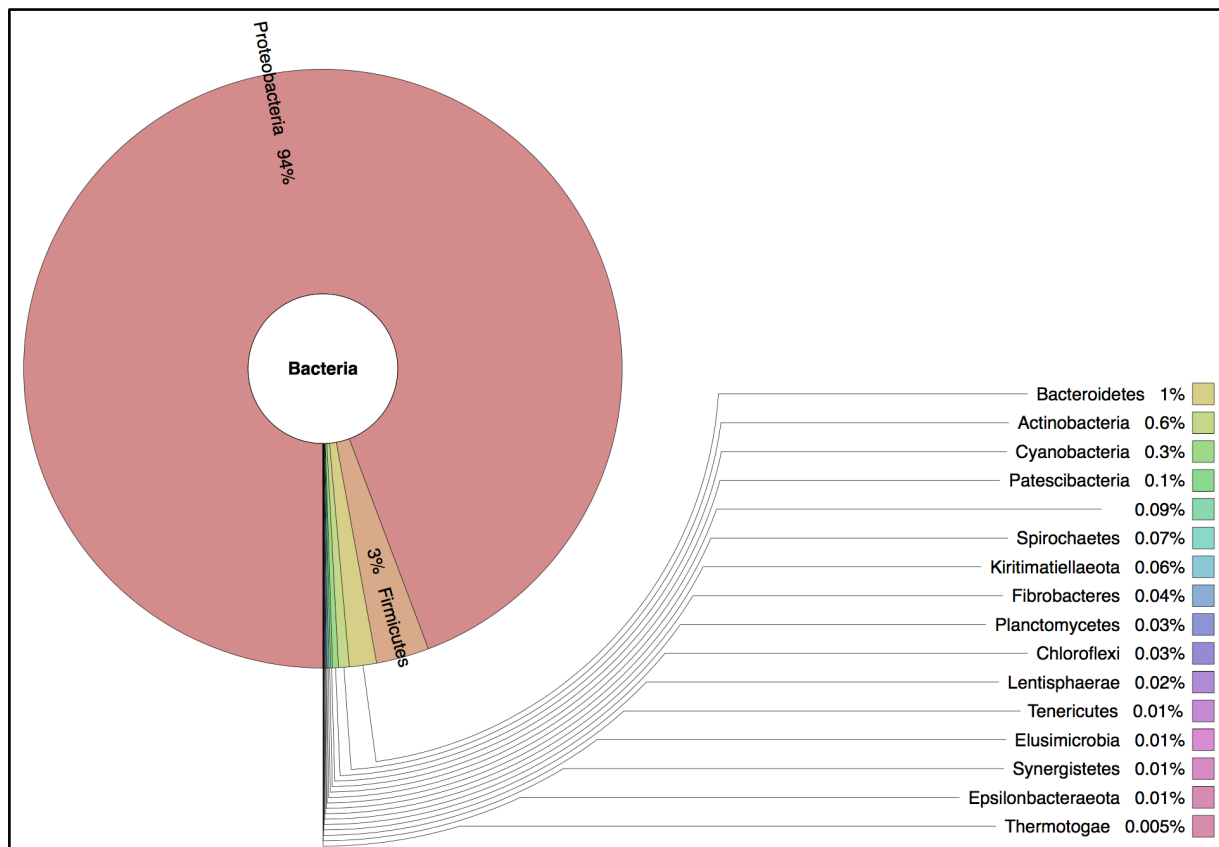


Figure 22. Krona plot for taxonomic abundance of adult female *H. contortus* microbiota at the phylum level. Data represent average values from pooled populations.

The microbiota composition at the family level

The microbiota composition associated with the abomasum content at the family level was as follows: The abomasum content was dominated by bacterial family: *Lachnospiraceae* (15%) followed by *Ruminococcaceae* (13%), *Prevotellaceae* (9%), *Acidaminococcaceae* (5%), *Rikenellaceae* (5%) and *Bifidobacteriaceae*. The least assigned abundant bacterial family in the abomasum content included *Veillonellaceae* (2%), *Coriobacteriales* (2%) and *Spirochaetaceae* (2%) depicted in Figure 23 .

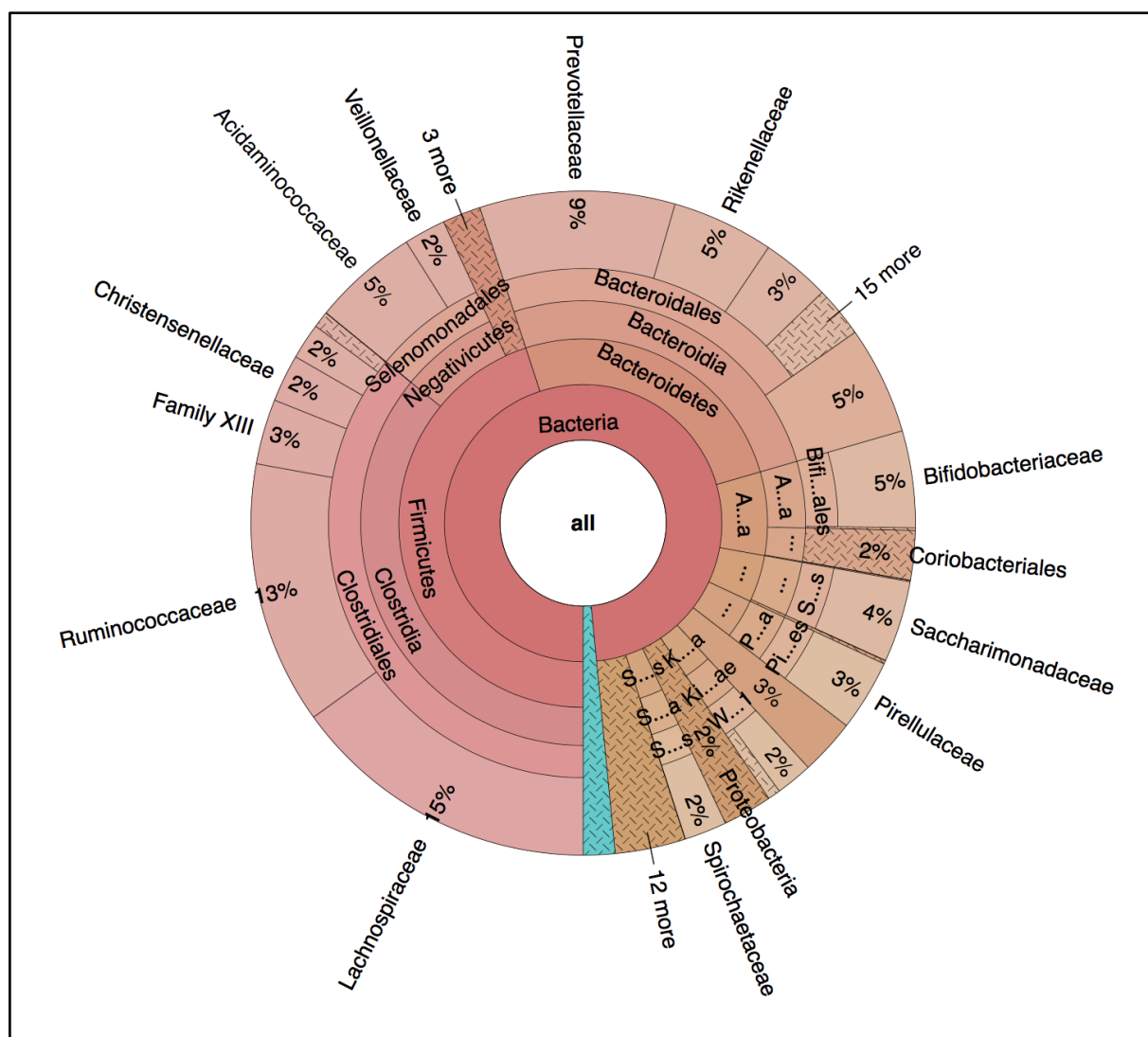


Figure 23. Krona plot for taxonomic abundance of abomasum content microbiota at the family level. Data represent average values from pooled populations.

The microbiota composition associated with the adult male *H. contortus* at the family level was as follows: The adult male *H. contortus* was dominated by bacterial family: *Vibrionaceae* (15%) followed by *Enterobacteriaceae* (9%), *Halomonadaceae* (8%), *Lachnospiraceae* (8%) and *Ruminococcaceae* (6%). The least assigned abundant bacterial family in the adult male *H. contortus* included Family XII (2%), *Pseudoalteromonadaceae* (1%) and *Alteromonadaceae* (1%) depicted in Figure 24.

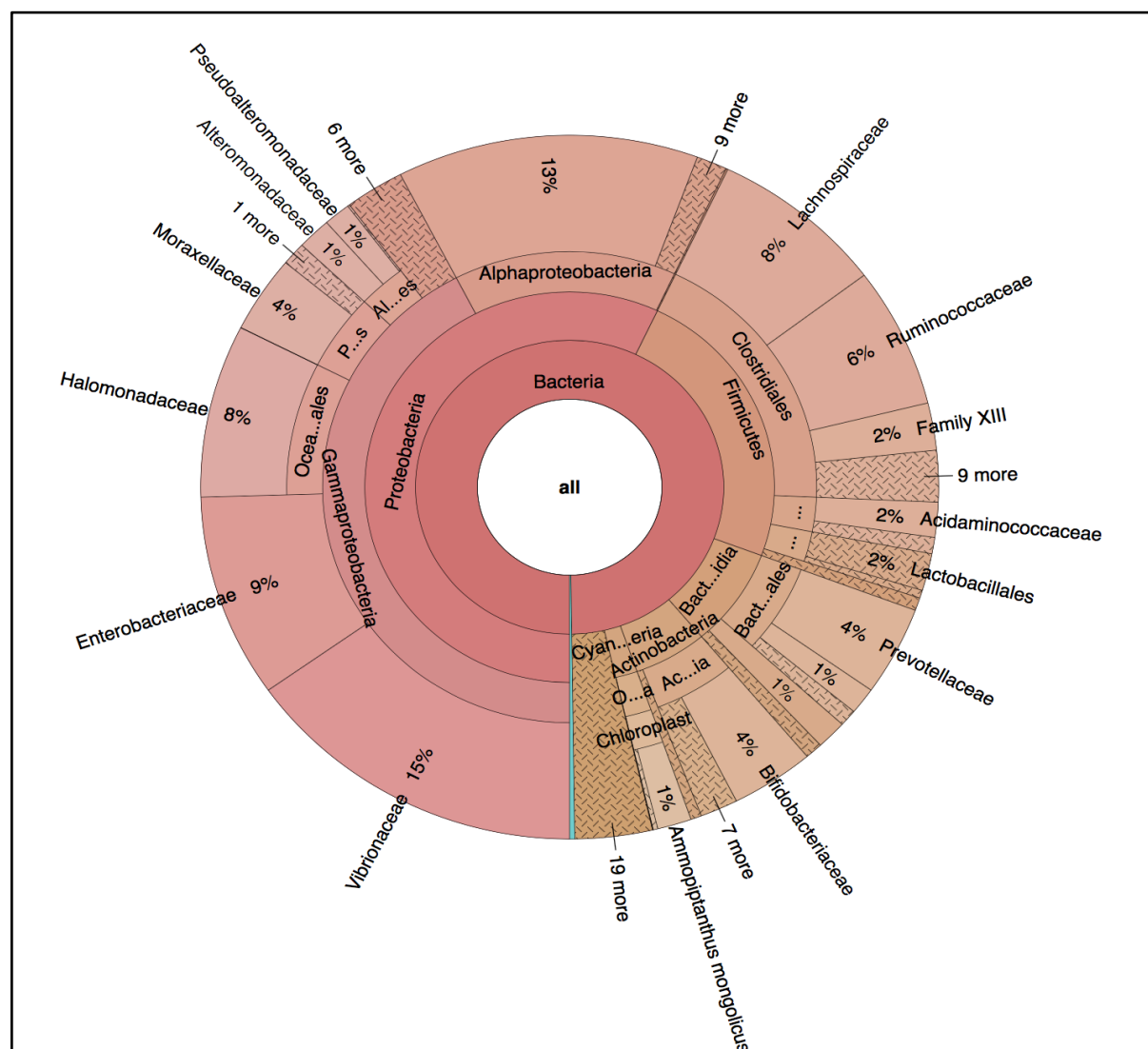


Figure 24. Krona plot for taxonomic abundance of adult male *H. contortus* microbiota at the family level. Data represent average values from pooled populations.

The microbiota composition associated with the adult female *H. contortus* at the family level was as follows: The adult female *H. contortus* was dominated by bacterial family: *Enterobacteriaceae* (29%) followed by *Vibrionaceae* (11%), *Halomonadaceae* (6%), *Moraxellaceae* (2%), *Clostridiales* (1%) and *Lactobacillales* (1%). The least assigned abundant bacterial family in the adult male *H. contortus* included *Clostridiales* (1%), *Lactobacillales* (1%) and *Bacteroidia* (1%) depicted in Figure 25.

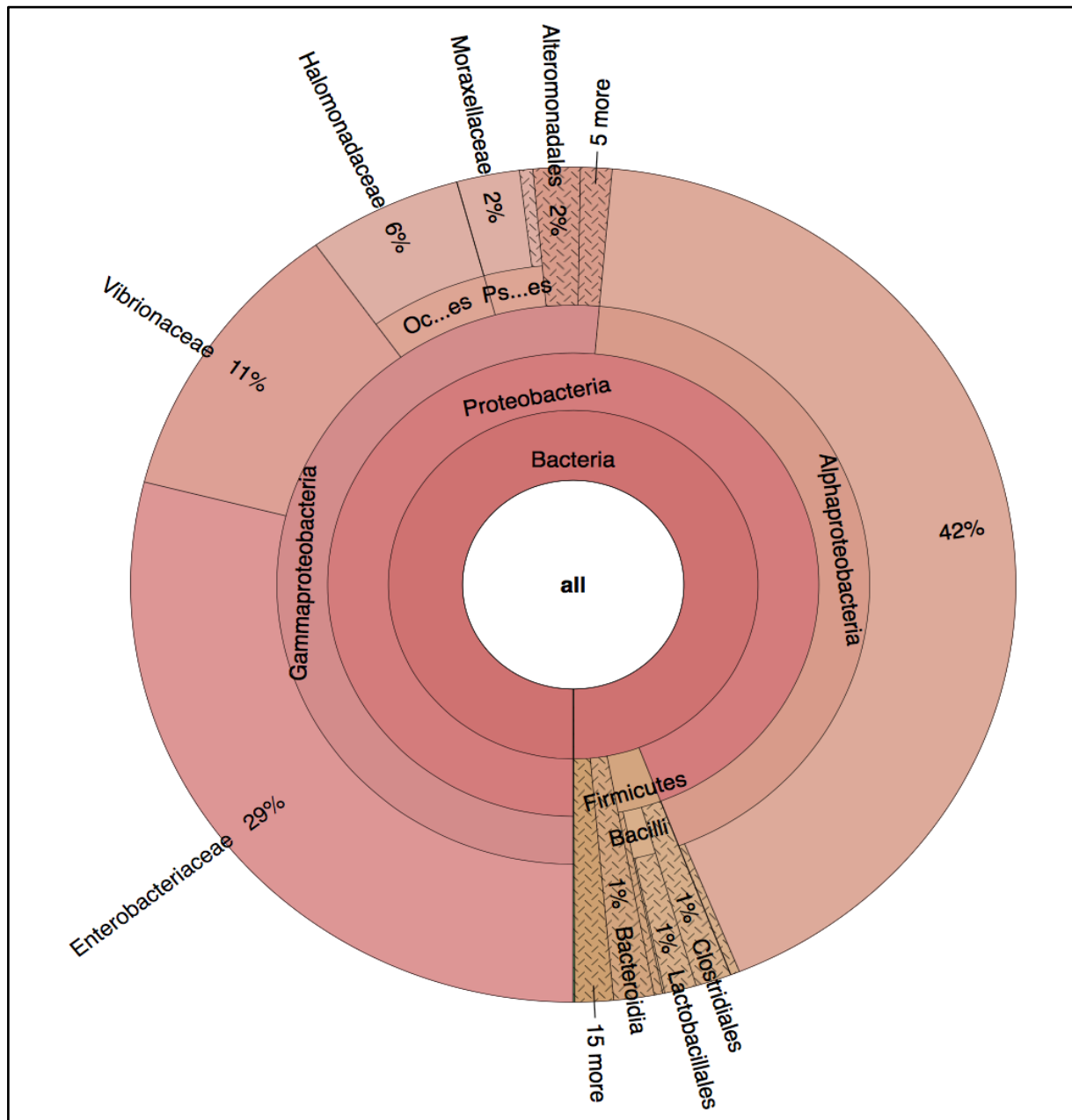


Figure 25. Krona plot for taxonomic abundance of adult female *H. contortus* microbiota at the family level. Data represent average values from pooled populations.

The microbiota composition at the genus level

The microbiota composition associated with the abomasum content at the genus level where assessed for the relative distribution of the abomasum content microbiota. *Succiniclasticum* (5%) followed by *Rikenellaceae* RC9 gut group (4%), *Candidatus Saccharimonas* (4%), *Ruminococcaceae* UCG-011 3%, p-1088-a5 gut group (3%) and *Prevotella* 1 (3%) were the most predominant assigned bacterial genera in the abomasum content depicted in Figure 26.

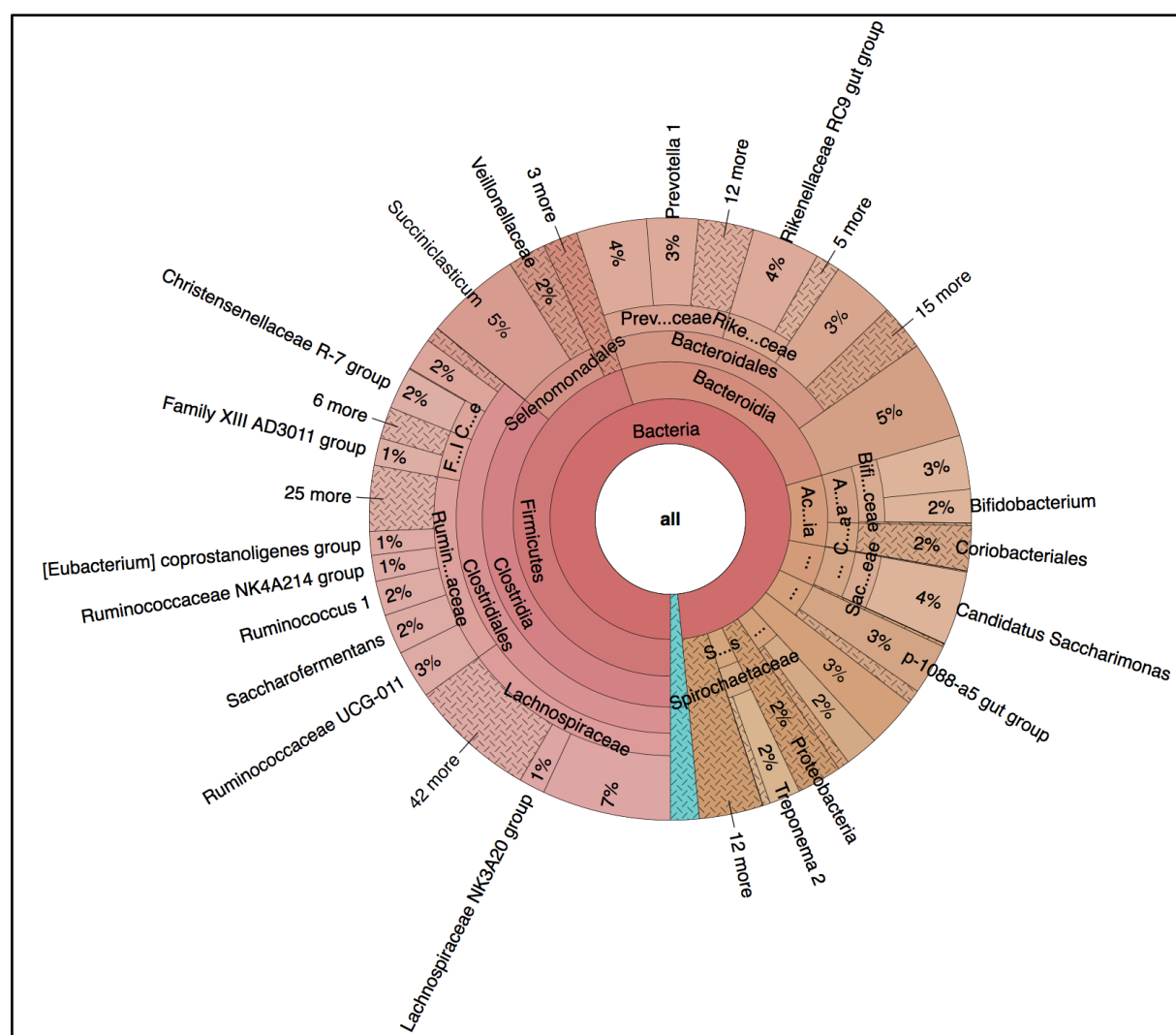


Figure 26. Krona plot for taxonomic abundance of the abomasum content microbiota at the genus level. Data represent average values from pooled populations.

The microbiota composition associated with the adult female *H. contortus* at the genus level where assessed for the relative distribution of the adult female *H. contortus* microbiota. The predominant assigned bacterial genera in the adult female *H. contortus* were dominated by *Escherichia-Shigella* (28%), *Vibrio* (11%) followed by *Halomonas* (6%), *Acinetobacter* (2%) and *Alteromonadales* (2%) depicted in Figure 28.

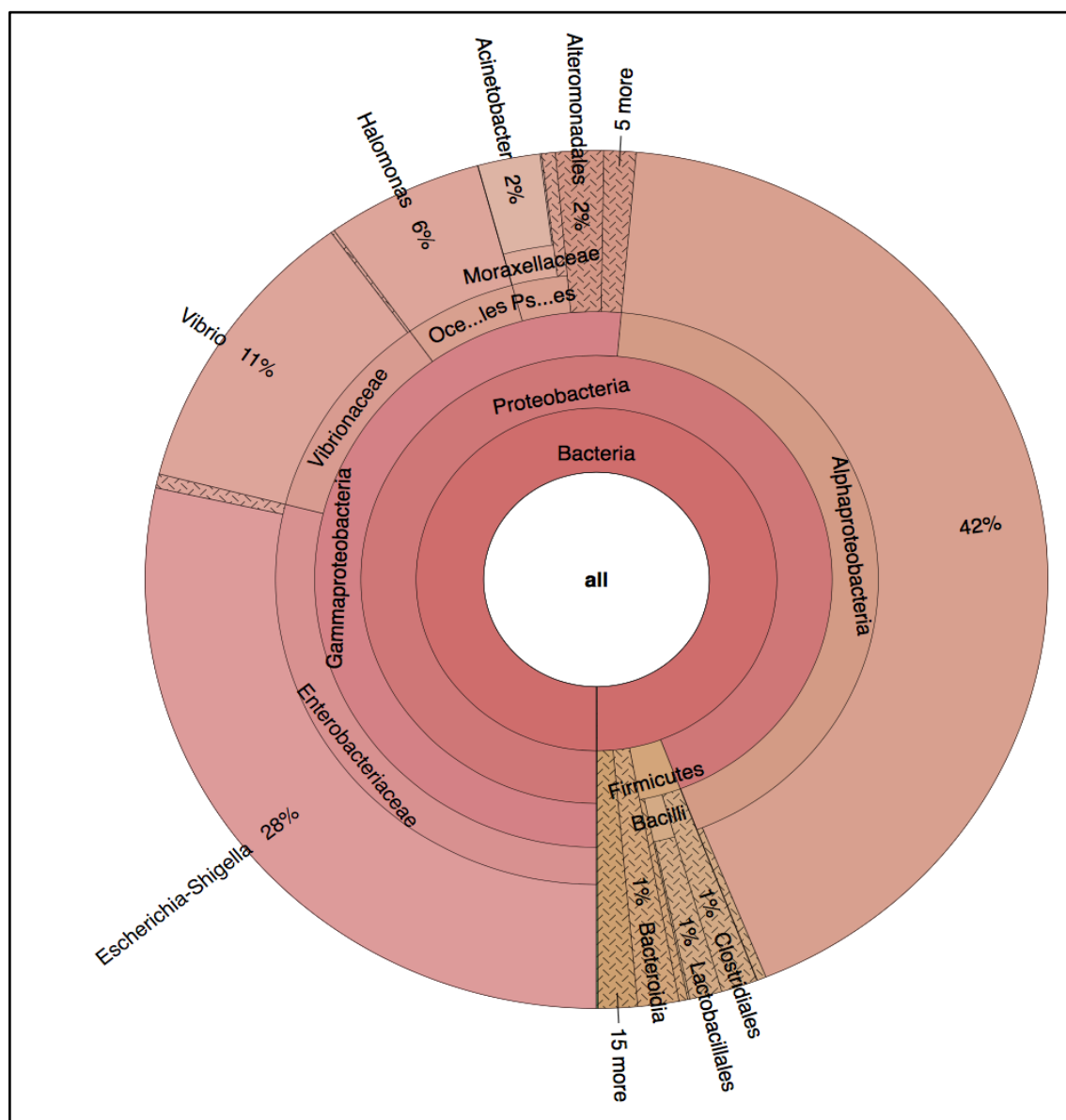


Figure 28. Krona plot for taxonomic abundance of the adult female *H. contortus* microbiota at the genus level at the genus level. Data represent average values from pooled populations.

4.7. THE UNIQUE AND SHARED MICROBIOTA ASSOCIATED WITH THE ABOMASUM CONTENT, ADULT MALE AND FEMALE *HAEMONCHUS CONTORTUS*

The results showed unique and shared microbiota associated with the abomasum content, adult male and female *H. contortus* at the genus level. In total, 660 unique bacterial OTUs across the three hosts were identified (APPENDIX II). The unique and shared OTUs between abomasum content, adult male and adult female *H. contortus* are depicted in the Venn diagram in Figure 29 and krona plot below in Figure 30-32. The results showed that the abomasum content and adult male *H. contortus* shares 113 OTUs with each host having 169 and 98 unique OTUs present, respectively. A total of 16 bacterial OTUs were shared between abomasum content and adult female *H. contortus* with the adult female *H. contortus* having 46 unique OTUs present, and a total of 57 bacterial OTUs were shared between adult male *H. contortus* and adult female *H. contortus* as depicted in Figure 29. Interestingly, the adult male *H. contortus* shared more OTUs with the abomasum content despite being the same *Haemonchus* spp. with the adult female *H. contortus* which shared much less bacterial OTUs with the abomasum content, although both adult male and female *H. contortus* shares similar environmental condition both inside and outside the host.

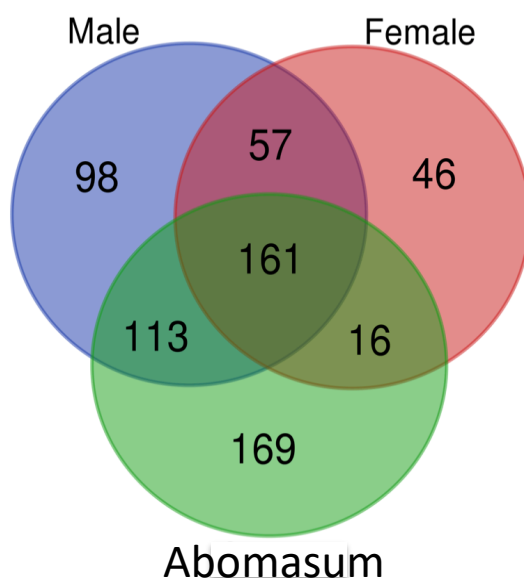
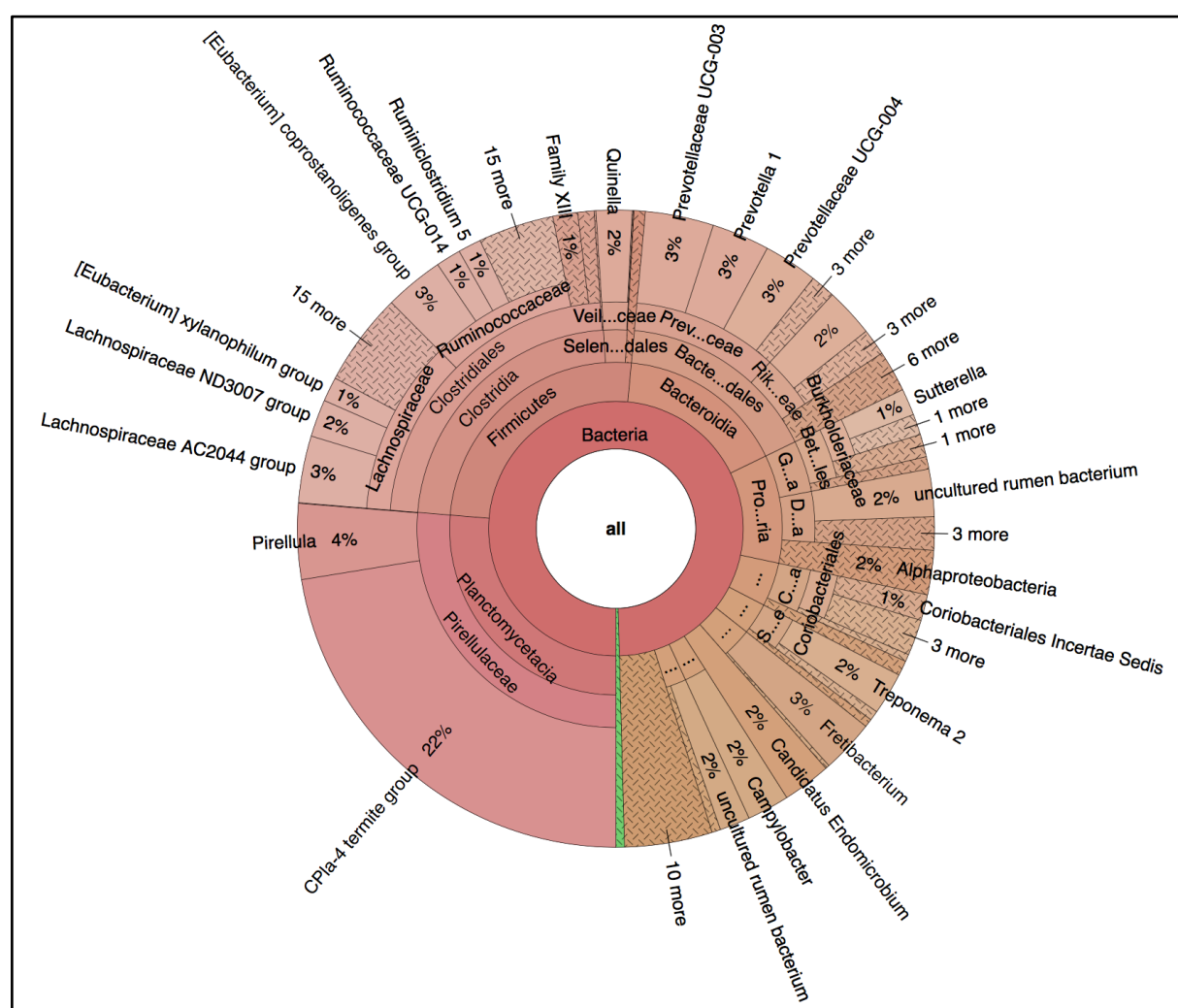


Figure 29. Venn diagram depicting number of unique and shared OTUs associated with abomasum content, adult male and female *H. contortus*. Data represent average values from pooled populations.

The unique microbiota composition associated with the abomasum content at the genus level were assessed for the relative distribution of the abomasum content microbiota. The unique microbiota associated with the abomasum content were predominantly dominated by unique bacterial genera: CPlA-4 termite group (22%) followed by *Pirellula* (4%), *Lachnospiraceae* AC2044 group (3%), *Prevotellaceae* UGG003 (3%), *Prevotellaceae* UGG004 (3%), *Prevotella* (3%) and *Coprostanoligenes* group (3%) depicted in Figure 30.



77

The circular phylogenetic tree illustrates the distribution of 16S rDNA sequences from the human gut. The tree is organized into three main phyla: Bacteria (red), Eukarya (green), and Archaea (blue). The Bacteria phylum is further divided into Actinobacteria, Firmicutes, Proteobacteria, and Chloroflexi. The Eukarya phylum is divided into Eukaryota. The Archaea phylum is divided into Euryarchaeota and Crenarchaeota. The tree shows the relative abundance of each taxon, with percentages indicated next to the labels. The total number of sequences is 100.

Phylum	Class	Order	Family	Genus	Percentage
Bacteria	Actinobacteria	Corynebacterales	Corynebacteriaceae	Corynebacterium 1	10%
				3 more	
				Luteimonas	2%
				2 more	
				Shewanella	1%
	Firmicutes	Bacillales	Bacillaceae	Bacillus	4%
				Sporosarcina	1%
				Lactococcus	4%
				2 more	
				Empedobacter	3%
	Proteobacteria	Alphaproteobacteria	Rhizobiales	Azospirillum	7%
				2 more	
				Pelagibacterium	1%
				Microvirga	1%
				Acetobacter	4%
Chloroflexi	Chloroflexales	Chloroflexaceae	Chloroflexus	2%	
			2 more		
			Pirellula	2%	
			uncultured	1%	
			3 more		
Eukarya	Eukaryota	Eukaryota	all	100%	
			8 more		
			2 more		
			uncultured	1%	
			3 more		
			Porphyrromonas	1%	
			1 more		
			Empedobacter	3%	
			2 more		
			Lactococcus	4%	
Archaea	Euryarchaeota	Methanococcales	Methanococcaceae	Methanococcus	4%
				Sporosarcina	1%
				Lactococcus	4%
				2 more	
				Empedobacter	3%
				1 more	
				Porphyrromonas	1%
				3 more	
				uncultured	1%
				2 more	
				Pirellula	2%
				2 more	
				uncultured	1%
				3 more	

The unique microbiota composition associated with the adult female *H. contortus* at the genus level where assessed for the relative distribution of the adult female *H. contortus* microbiota. The unique microbiota associated with the adult female *H. contortus* were predominantly dominated by unique bacterial genera: *Lactobacillus* (15%) followed by *Bradyrhizobium* (7%), *Pseudonocardia* (5%), *Prevotella* (5%), *Anaplasma* (4%) and *Treponema2* (4%) depicted in Figure 32.

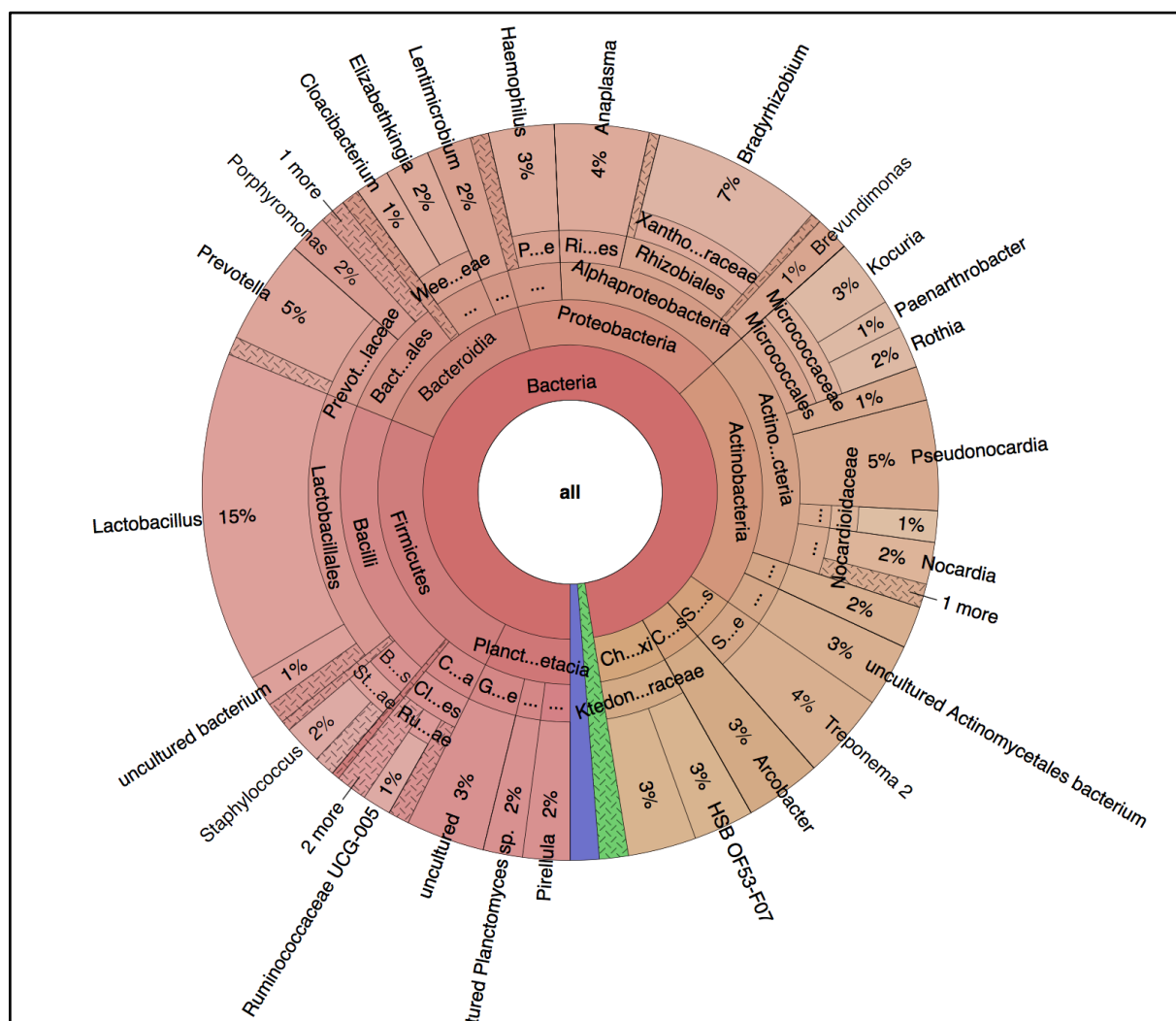


Figure 32. Krona plot depicting unique bacterial OTUs of the adult female *H. contortus* microbiota at the genus level. Data represent average values from pooled populations.

Composition and abundances of shared OTUs between the abomasum content, adult male and female *H. contortus*

Furthermore, a total of 161 bacterial OTUs shared between the abomasum content, adult male and female *H. contortus* worms and their abundances are depicted in Figure 29. The composition and abundances of shared bacterial OTUs between the three host are also depicted in APPENDIX I. The abomasum content (66%) had higher shared microbiota abundance followed by adult female *H. contortus* (23%) while the adult male *H. contortus* (11%) displaying the least number of shared microbiota abundances. The abundances of the microbiota in all three host are depicted in pie chart in Figure 33, constructed based on the shared bacterial OTUs abundances average values.

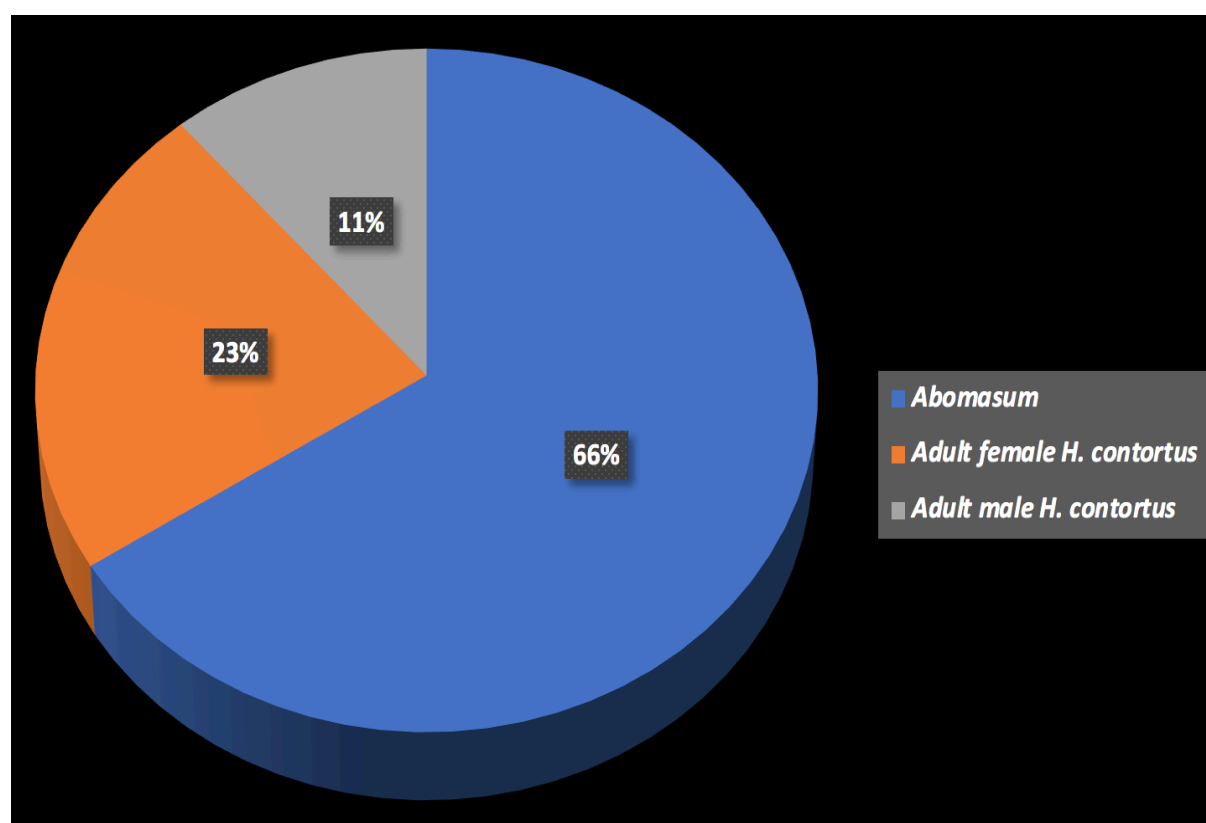


Figure 33. Microbiota abundances shared between the abomasum content, adult male and female *H. contortus*. Pie chart depicting shared bacterial OTUs between the three host. Data represent average values from pooled populations.

4.8. THE RELATIONSHIP BETWEEN *HAEMONCHUS CONTORTUS* MICROBIOTA AND ITS PREDILECTION SITE, THE ABOMASUM CONTENT MICROBIOTA

To determine the relationship between *H. contortus* microbiota and abomasum content microbiota. A heatmap were constructed to depict the relative percentage of each OTUs (variables clustering on the X-axis) within the abomasum content, adult male and female *H. contortus*. As depicted in the heatmap analysis below in Figure 35, the adult male *H. contortus* microbiota did not cluster together indicating that the bacterial diversity of the adult male *H. contortus* is varied and shared some OTUs with the abomasum content and some with the adult female *H. contortus*. The abomasum content microbiota clustered together indicating that the abomasum content harbour similar and diverse microbiota compared to adult male and female *H. contortus* depicted in Figure 19 and 35. As depicted in Table 8, there was significant difference between the adult male and female *H. contortus* microbiota suggesting impact of gender on the composition and abundances of the adult *H. contortus* microbiota, $P=0,029$. The gastrointestinal microbiota of abomasum content were compositionally significantly different from the adult *H. contortus* worms. Furthermore, in terms of total bacterial OTUs abundances (660 OTUs generated between abomasum, adult *H. contortus* worms), abomasum content had higher bacterial OTUs abundances followed by adult female *H. contortus* while the adult male *H. contortus* had the lowest bacterial OTUs abundances depicted in Figure 34.

Furthermore, there were number of bacterial genera that has been previously characterised to produce nematicidal volatile compounds with activities against a nematodes which were also detected in the present study. A summary of those bacterial genera are provided in Table 9.

Table 9. bacterial genera with nematicidal activities against nematodes

Bacterial genera	Nematicidal activities against
<i>Lysinibacillus</i>	Root-knot nematode <i>Meloidogyne incognita</i>
<i>Pseudomonas</i>	Root-knot nematode <i>Meloidogyne incognita</i>
<i>Pseudoalteromonas</i>	Pine wood nematode
<i>Vibrio</i>	Pine wood nematode

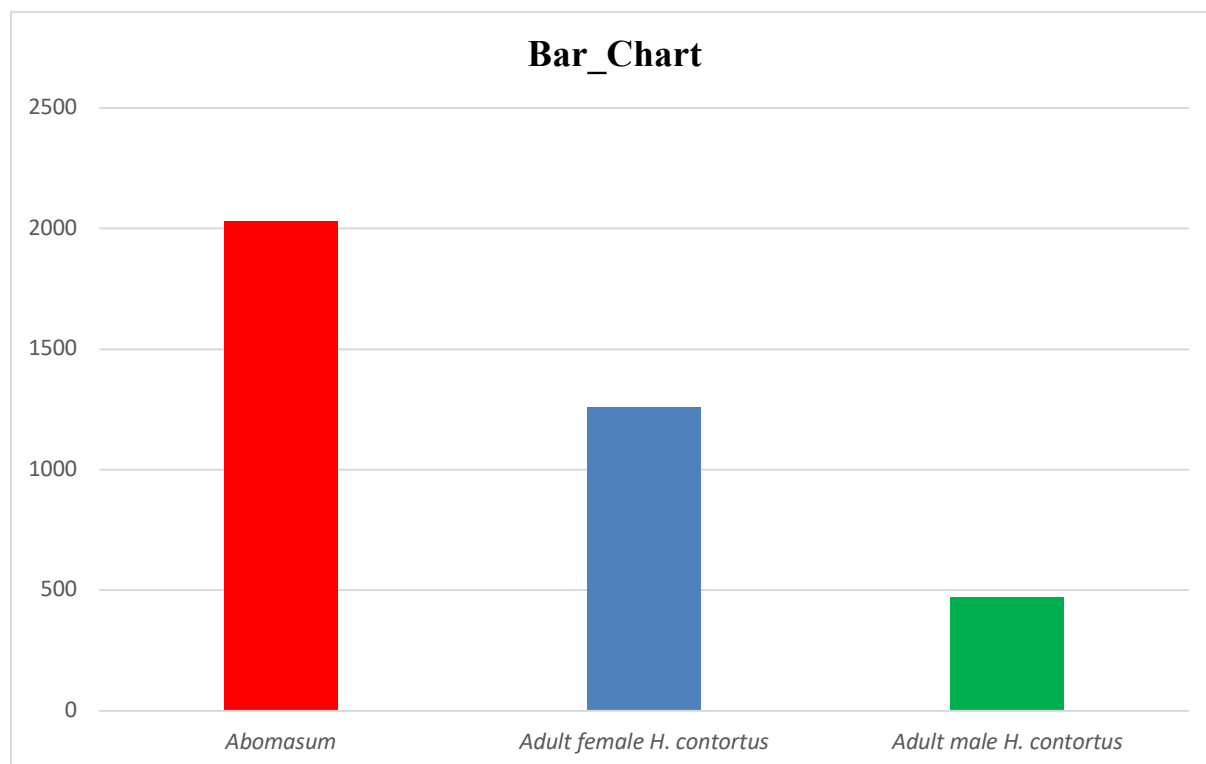


Figure 34. Bar chart depicting relative OTUs abundances of the abomasum content, adult female and male *H. contortus*. Data represent average values from pooled populations.

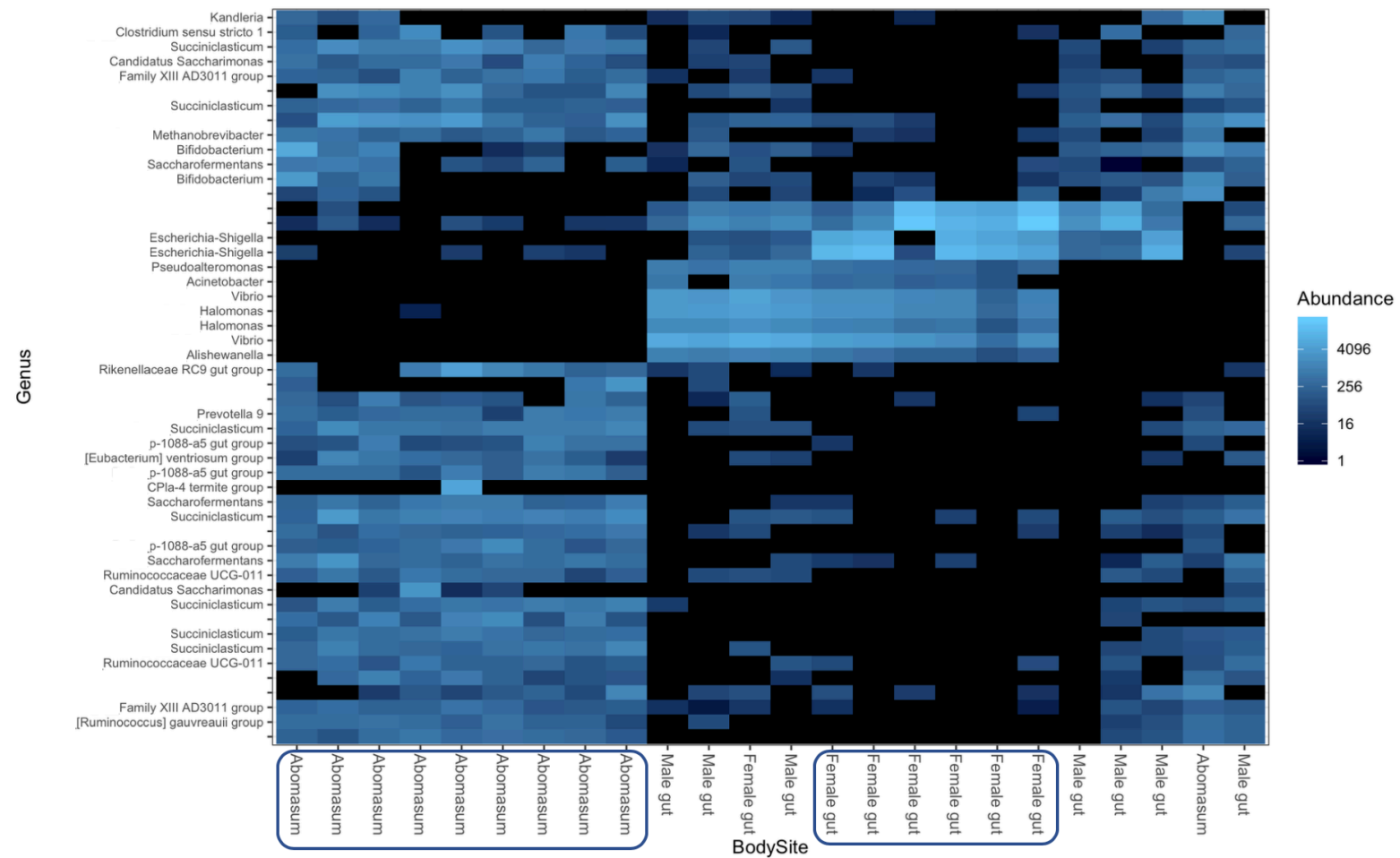


Figure 35. Heatmap of the top 50 ranked *H. contortus* microbiota depicting relative abundance of the bacterial community at the genus level. The relative values for bacterial species are depicted by color intensity, with legend indicated at the bottom of the figure. Data represent average values from pooled populations.

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Summary

The impact of gastrointestinal parasites infection on host microbiota and immune response is well documented in the literature. Recent studies are questioning and focusing on the impact of gastrointestinal microbiota on gastrointestinal parasites infection as a new biological control measures for effective control of gastrointestinal parasites infection in livestock, thus, to limit the use of chemical based treatment in livestock.

The current research has focused on characterising the gastrointestinal microbiota and demonstrated microbiota associated with the adult *H. contortus* worms and its predilection site, the abomasum, with long-term goal of manipulating microbiota to control GIN parasites. Further analyses of the gastrointestinal microbiota demonstrated the bacterial genera which can produce nematocidal volatile compounds with activities against a nematodes including *Lysinibacillus*, *Pseudomonas*, *Pseudoalteromonas* and *Vibrio*. Therefore, biological control of gastrointestinal parasites infection with their respective microbiota and host microbiota is possible.

5.1. MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF *HAEMONCHUS CONTORTUS* FROM SOUTH AFRICA

The *H. contortus* is a very common nematode infecting small ruminants and is one of the most pathogenic gastrointestinal nematodes of small ruminants (Tsotetsi & Mbat, 2003; Regassa *et al.*, 2006; (Roeber *et al.*, 2017). Identification of common stomach worms of small ruminants has been previously based on recognized morphological characteristics that make it possible to morphologically differentiate between *Haemonchus* species, particularly, *H. contortus* and *H. placei*. Furthermore, Lichtenfels (Lichtenfels *et al.*, 1986; Lichtenfels *et al.*, 1994) reported that the distribution of surface cuticular ridges or synlophe can be used to distinguish populations of *H. contortus* from *H. placei*. For this reason, researchers have primarily used subventral and subdorsal ridges to differentiate these two species. However, misidentification can occur when synlophe of hybrids is used to distinguish *H. contortus* from *H. placei*. Lichtenfels *et al.* (1988) and Gibbons, (1979) indicated that spicule lengths and vulvar flap provide the quickest and easiest character used for separating most populations of *H. contortus* from *H. placei*.

In the present study, the recovered *Haemonchus* spp specimens were distinguished as the adult male and female *H. contortus* based on their morphological characters as suggested by Lichtenfels *et al.*, (1988) and Gibbons, (1979). The adult female *H. contortus* were recognised mainly by their reddish colour and vulvar flap morphologies which are also used for the characterisation of the nematode population, which occurs in ruminant hosts in certain areas, than for species identification. The adult male *H. contortus* were distinguished based on the low variability of spicule morphology within species. Do Amarante *et al.* (2011) reported that male morphological characters are more reliable and suitable for species identification than their female characters.

These adult male and female *H. contortus* distinguished based on morphological characters were confirmed by PCR using species specific *H. contortus* primers. The present study indicated that the use of HcBotuF1/R2 primer pair is able to specifically amplify *H. contortus* DNA with the amplification product size of 260 bp. These results corroborate those of Amarante *et al.* (2016) who reported that species specific primers HcBotuF1/R2 primer pair are able to differentiate between *Haemonchus* species from various parts of the world.

5.2. INVESTIGATION OF PARASITE SURFACES FOR ABOMASAL MICROBIAL CONTAMINATION AND MICROBIAL DNA AMPLIFICATION BY POLYMERASE CHAIN REACTION

The *H. contortus* gets exposed to environmental and abomasal microbiota during its development stages (Sinnathamby *et al.*, 2018). As a result, in the present study, a set of measures were employed to ensure that there is no cross-contamination of abomasal microbial community on the *H. contortus* surfaces. Previous studies by Berg *et al.* (2016) and Dirksen *et al.* (2016) have showed that several washing steps with gentamycin or media containing Triton X-100 can be used to remove microbial contaminants on the parasite surfaces. However, this protocol is not sufficient to completely remove all microbial contaminants on the parasite surface. Hence, false detection of gastrointestinal microbiota of the GIN parasites maybe documented. Therefore, in the present study, following recovery of the parasite from the abomasum, we used a more rigorous treatment consisting of the antibiotic solution (1mg/ml ampicillin/1mg/ml gentamicin) and 4% sodium hypochlorite to ensure removal of all microbial contamination on the parasite surface. The PCR assay results with 16S rRNA gene primers showed that there is no contamination on the parasite originating from the abomasal microbial community. These results correlate with the previously published results by White, (2016) and El-Ashram & Suo, (2017) who reported that sterilisation with antibiotic solution (1mg/ml ampicillin/1mg/ml gentamicin) and 4% sodium hypochlorite followed by 5 washes with sterile (PBS, pH 7.4) or double distilled water (H₂O) is sufficient to remove abomasal microbial contaminants on the surface of the parasite. The final washes with (PBS, pH 7.4) ensures that all antibiotic solution and sodium hypochlorite residues that might be left on the surface of the parasites are removed. Thus, prevent PCR inhibition or false negative results.

Microbial DNA from abomasum content and sterile adult *H. contortus* was successfully extracted using the QIAamp DNA Microbiome Kit and amplified by conventional PCR with previously published 16S primers targeting region V3-V4 of the 16S ribosomal RNA (rRNA) gene. Our results showed PCR amplification with 16S primers targeting the V3-V4 regions of bacterial rRNA gene. These results correspond with previously published studies which reported that PCR amplification with V3-V4 primer pairs can amplify microbial DNA from metagenomics samples. As a result, this makes sequencing and classification of gastrointestinal tract and environmental microbiota possible (Klindworth *et al.*, 2013; Fischer *et al.*, 2016; Thijs *et al.*, 2017).

5.3. MICROBIOTA ASSOCIATED WITH THE ABOMASUM CONTENT OF THE DOHNE MERINO SHEEP NATURALLY INFECTED WITH *HAEMONCHUS CONTORTUS* FIELD STRAINS

Dohne Merino sheep breed is dual purpose breed which is bred in response to challenges and limitations encountered by Merino sheep farming in the sourveld region of the Eastern Cape, S.A. Such limitations include high mortality, low fertility, high susceptibility to intestinal nematodes and blowflies. However, the Dohne Merino in S.A. are facing heavy challenges of *H. contortus* infections. although some of these sheep are developing resistance to *H. contortus*, majority are still susceptible to the *H. contortus*. Furthermore, it is known that *H. contortus* infection in the GIT can induce significant changes to the host microbiota. Hence, in this study we characterised the abomasum content microbiota of the Dohne Merino sheep naturally infected with *H. contortus* field strains to gain insight into the impact that GIN parasites infection have on the host microbiota.

In the present study, the abomasum content microbiota were characterised and were primarily dominated by the following bacterial phyla: *Firmicutes* (45%), *Bacteroidetes* (26%), *Actinobacteria* (7%), *Patescibacteria* (4%) and *Planctomycetes* (3%). These results paralleled those of other studied microbiota of the abomasum content in small ruminants by Wang *et al.*, (2016) who reported that the abomasum content were predominantly dominated by bacterial phyla: *Bacteroidetes* followed by *Firmicutes*, *Actinobacteria* and *proteobacteria*; and Zeng *et al.* (2017) reported similar results of the abomasum content which were dominated by bacterial phyla: *Firmicutes* followed by *Bacteroidetes*, *proteobacteria* and *Bacteroidetes*. However, with regard to the abundance of these predominant phyla, there were some variations found across these studies. Interestingly, the phyla *Patescibacteria* and *Planctomycetes* were found to be abundant in the abomasum content in the present study. However, none of these phyla *Patescibacteria* and *Planctomycetes* were reported in other previously published studies (Zeng *et al.*, 2015; Wang *et al* 2016; Zeng *et al.*, 2017). These variations between the present study and previously published studies could be due to known factors that affect microbiota composition such as worm burden, breed type, diets and different environmental condition. Moreover, the relative abundances of *Firmicutes*, *Bacteroidetes* and *Actinobacteria* in the sheep GIT might be due to the ability of some members to degrade fibre and cellulose (Wang *et al* 2016); and digestion of complex carbohydrates, and ferment organic matter (Zeng *et al.*, 2017); and production of secondary metabolites, of which many are potent antibiotics (Wu *et al.*, 2012) respectively.

The predominant assigned bacterial genera observed in the abomasum content in the present study were *Succiniclasicum* (5%), *Rikenellaceae* RC9 gut group (4%), *Candidatus Saccharimonas* (4%), *Ruminococcaceae* UCG-011 3%, p-1088-a5 gut group (3%) and *Prevotella* 1 (3%). These genera belong to the bacterial phyla: *Firmicutes*, *Bacteroidetes* and *Patescibacteria*. The predominant genera in the present study was *Succiniclasicum* which specializes in fermenting succinate from starch degradation and converting it to propionate, an important glucose precursor of ruminants, and a propionic acid fermentation that provide great amount of energy for the host (Zhang *et al.*, 2018). The stomach is the main place for the absorption of nutrients. Hence, large numbers of *Succiniclasicum* in the abomasum is normal. Guarner, (2006) and Zhang *et al.* (2018) reported the presence of *Succiniclasicum* in sheep. However, these studies about *Succiniclasicum* focused on rumen and propionic acid fermentation by *Succiniclasicum* in the small intestines. This study demonstrated that genera *Succiniclasicum* occurs in high numbers in the abomasum as well. *Rikenellaceae* RC9 gut group, *Ruminococcaceae* UCG-011 and p-1088-a5 gut group are major degraders of complex polysaccharides, such as starch and cellulose, and contributes in the metabolism of dietary fiber in ruminants. However, shortage of dietary substrates in the host might cause their decline in their relative abundance (Huws *et al.*, 2011; Wu *et al.*, 2012; Su *et al.*, 2014; Tanca *et al.*, 2017). *Prevotella* and *Candidatus Saccharimonas* are regularly found in the stomach of small ruminants and are capable of degradation of complex carbohydrates in the rumen. Li *et al.* (2012), Malmuthuge *et al.* (2015) and Zeng *et al.* (2017) reported that the *Prevotella* and *Lactobacillus* genera have a unique capability of mucin glycoprotein, hemicelluloses and xylans degradation, as a results promotes the digestion of the feed. Moreover, an increase in *Prevotella* is related to grain-based diet (Li *et al.*, 2012; Malmuthuge *et al.*, 2015; Zeng *et al.*, 2017). These results of the abomasum content microbiota at genus level are in agreement with observations reported by Zeng *et al.* (2017), Tanca *et al.* (2017) and Zhang *et al.* (2018) whereby predominant genera in the GIT depend on the worm burden, diet and environmental condition. Hence, there was variations in the microbiota observed in our study and other published studies (Zeng *et al.*, 2017; Tanca *et al.*, 2017; Zhang *et al.*, 2018).

5.4. MICROBIOTA OF *HAEMONCHUS CONTORTUS* ADULT WORMS HAVE A DIVERSE AND CORE MICROBIOTA DIFFERENT TO THEIR HOST

The adult male and female *H. contortus* worms has different life cycles stages in which they get exposed to the microbiota of rumen and abomasum in mammalian host, whilst the environmental stages including egg and larval (L1, L2 and L3) stages are exposed to soil and pasture microbiota (Lacharme-Lora *et al.*, 2009). Hence, the nematode gastrointestinal microbiota may reflect the internal microorganisms from microhabitat (abomasum and rumen) and macrohabitat (pasture and manure).

In the present study, *H. contortus* adult worm's microbiota were characterised in the absence of external microbial contaminants on the surface of the parasite. The results demonstrated that the adult female *H. contortus* were dominated by bacterial phyla: *Proteobacteria* (94%) followed by *Firmicutes* (3%), *Bacteroidetes* (1%), *Actinobacteria* (0.6%) and *Cyanobacteria* (0.3%); and the adult male *H. contortus* were dominated by bacterial phyla: *Proteobacteria* (57%) followed by *Firmicutes* (24%), *Bacteroidetes* (8%), *Actinobacteria* (6%) and *Cyanobacteria* (2%). These results are similar to those observed in earlier studies on *T. muris* by White, (2016) who reported that the adult *T. muris* were dominated by bacterial phyla: *Bacteroidetes* followed by *Firmicutes* and *Proteobacteria*. El-Ashram & Suo, (2017) and Sinnathamby *et al.* (2018) reported that the adult *H. contortus* were dominated by bacterial phyla: *Proteobacteria* followed by *Firmicutes*, *Tenericutes* and *Actinobacteria*, and *Firmicutes* followed by *Proteobacteria* and *Bacteroidetes* respectively. Relatedly, the microbiota composition at phylum level observed in the current study on the adult *H. contortus* worms were similar to those observed previously on *T. muris*, *A. suum* and *H. contortus* but there was variations in abundance of these microbiota under the same phyla which are usually caused by type of host they inhabit and environmental condition (White, 2016, El-Ashram & Suo, 2017). Previous studies by White, (2016), El-Ashram & Suo, (2017) and Sinnathamby *et al.* (2018) compared the differences and similarity of microbial community of the different parasite life-cycle stages but not of the different genders of the adult stages. As a result, in the present study, we compared the adult male and adult female *H. contortus* microbiota as well.

The *H. contortus* adult worms microbiota at the genus level were dominated by bacterial genera: *Escherichia-Shigella* (28%) followed by *Vibrio* (11%), *Halomonas* (6%), *Acinetobacter* (2%) and *Alteromonadales* (2%) in the adult female *H. contortus*. The

dominant assigned bacterial genera in the adult male *H. contortus* were *Vibrio* (15%) followed by *Escherichia-Shigella* (8%), *Halomonas* (8%) and *Acinetobacter* (3%). Furthermore, the predominant bacterial genera in the adult male *H. contortus* worms were similar to adult female *H. contortus* but with variation in the genera abundances. The female showed higher genera abundance than male *H. contortus* worms, although the males had more diversity than female *H. contortus*. Among the aforementioned genera, *Escherichia-Shigella*, *Vibrio*, *Halomonas* and *Acinetobacter* were predominantly shared by both adult male and female *H. contortus* worms. These results are different from those reported by El-Ashram & Suo, (2017) who indicated that the predominant bacterial genera of the adult *H. contortus* were *Escherichia-Shigella* (87.5%), *Pseudomonas* (5.91%) and *Ochrobactrum* (5.06%).

The gastrointestinal microbiota of adult male and female *H. contortus* worms showed similarity and variations in terms of abundances and diversity in the present study. Furthermore, our results indicated that the adult *H. contortus* harbour distinct microbiota to their host. Hence, *H. contortus* selects for specific microbiota from the host that are important for their development. Moreover, there was significant difference between adult male and female *H. contortus* microbiota abundances suggesting there is GIN parasites gender preferences which ultimately influences the composition of the predominant microbiota ($P = 0.029$). Furthermore, the studied worms in this study are from the same sheep breed and which is fed same diet under similar environmental conditions. Hence, the variations in the microbiota of adult male and female *H. contortus* may be caused by hormonal changes, immune response and reproductive needs including egg development in females which does not occur in males. However, the functions of GIN parasites microbiota are not characterised as yet in the literature. Previous studies by Haro *et al.* (2016), Morimoto *et al.* (2017) and Fransen *et al.* (2017) reported that the variations of microbiota in mammals is related to their hormonal changes and immune response. Future studies, will have to assess the relationship of GIN parasites microbiota to the above mentioned factors.

5.5. THE UNIQUE AND SHARED MICROBIOTA ASSOCIATED WITH THE ABOMASUM CONTENT AND ADULT *HAEMONCHUS CONTORTUS* WORMS

In this study, we examined how the overlap in environment and natural history of the adult *H. contortus* worms and its predilection site, the abomasum, interactions affected the composition of their respective microbiota.

The results showed that the adult male *H. contortus* harbour higher number of unique bacterial OTUs compared to adult female *H. contortus* and shared higher number of bacterial OTUs with the abomasum content than adult female *H. contortus*. This results suggest that there are various selective measures at play within the *H. contortus* digestive tract, different to that of the host gastrointestinal tract. Therefore, some species are unable to persist. The metabolic needs of the adult *H. contortus* might vary to that of its host and therefore microbiota selection will depend on what GIN parasite needs for its own fitness and survival within the host. These results are in agreement with Sinnathamby *et al.* (2018) who reported that the adult *H. contortus* harbour distinct/unique microbiota from its host that are important in their well-being.

Furthermore, the present study indicated that the abomasum content harbour higher OTUs abundances compare to adult *H. contortus* worms, which would be due to its higher metabolic needs. This observation is similar to previous studies on *T. muris* and *H. contortus* by White *et al.* (2018) and Sinnathamby *et al* (2018) which reported that the parasite mammalian host harbour more diverse microbiota than the parasite life-cycle stages.

In the present study, the most unique OTUs in the abomasum content were dominated by bacterial genera: CPla-4 termite group (22%) followed by *Pirellula* (4%), *Lachnospiraceae* AC2044 group (3%), *Prevotellaceae* UGG003 (3%), *Prevotellaceae* UGG004 (3%), *Prevotella* (3%) and *Coprostanoligenes* group (3%). The most predominantly abundant unique OTUs associated with the adult male *H. contortus* were bacterial genera: *Corynebacterium* 1 (10%) followed by *Azospirillum* (7%), *Lysinibacillus* (4%), *Lactococcus* (4%), *Acetobacter* (4%), *Empedobacter* (3%) and *Paeniclostridium* (3%). The most predominantly abundant unique OTUs associated with the adult female *H. contortus* were bacterial genera: *Lactobacillus* (15%) followed by *Bradyrhizobium* (7%), *Pseudonocardia* (5%), *Prevotella* (5%), *Anaplasma* (4%), *Treponema*2 (4%). Furthermore, the free-living stages of *H. contortus*, such as L₁ (rhabditiform), and L₂ (rhabditiform) and L₃ filariform

infective larvae are environmental stages which interact with soil, pasture and manure microbiota. As results may be a vector or reservoir of environmental microbiota. These results support the suggestions by Aravindraja *et al.* (2013) who reported that the representative members from these microbiota are universal and are not limited to the particular environment. For example, *Lysinibacillus*, *Empedobacter* and *Bradyrhizobium* and *Acetobacter* are usually found in soil and plants (Sugawara *et al.*, 2017; Wang *et al.*, 2018) and they were observed in the abomasum content and adult *H. contortus* worms in the present study.

Among the aforementioned genera of adult *H. contortus*, *Corynebacterium*, *Lysinibacillus*, *Anaplasma* and *Paeniclostridium* are emerging opportunistic pathogens, which have been isolated from animals, soil, water sources and plants. The *Paeniclostridium* genera is mainly associated with trauma, toxic shock, soft tissue skin infections, and gynecologic infections (Kim *et al.*, 2017). *Anaplasma* genera causes tick-borne fever (TBF) in sheep (Stuen *et al.*, 2002; Yang *et al.*, 2016). *Corynebacterium* genera is an emerging pathogen of plants and animals (Salem *et al.*, 2015). Similarly, Lacharme-Lora, *et al.* (2009) reported the potential role of helminths as pathogen vectors, with potential to transmit pathogenic bacteria to their host. It can therefore be assumed that the *H. contortus* can transmit pathogenic bacteria to their host which might cause disease when the host immune system is compromised and can play a role in shaping the ovine immune response against pathogens. Interestingly, previous studies Lu *et al.* (2014); Gómez-Garzón *et al.* (2017) and Abdel-Salam *et al.* (2018) reported that bacterial strain, *Pseudoalteromonas marina* strain, *Vibrio atlantica* strain 5-16, *Pseudomonas aeruginosa* and *beteli*, and *Lysinibacillus* can produce nematicidal volatile compounds with activities against the root-knot nematode *Meloidogyne incognita* and Pine wood nematode. The bacterial genera that these bacterial strain follow under were observed in the present study as shown in Table 9 in the results section. As result of their nematicidal activity, these bacteria can be manipulated to target pathogenic GIN parasites in livestock such as *H. contortus* and become biological control for GIN parasites while reducing the use of chemical based treatment (Gómez-Garzón *et al.*, 2017; Abdel-Salam *et al.*, 2018). Moreover, the presence of these bacteria gives hope that it's possible to use symbiotic bacteria to control GIN parasites in livestock.

5.6. THE RELATIONSHIP BETWEEN ADULT *HAEMONCHUS CONTORTUS* MICROBIOTA AND ITS PREDILECTION SITE, THE ABOMASUM MICROBIOTA

The *H. contortus* microbiota are presumably transmitted vertically from the parents and horizontally from their respective environment and retain them during their development (El-Ashram & Suo, 2017; Sinnathamby *et al* 2018). The abomasum is one of the most, densely populated habitat in the sheep. As a result, *H. contortus* infective larval stage is exposed to huge number of variety of microbial communities when they infect the host and during their development into adult stage in the host (White *et al.*, 2018). To determine the interplay between *H. contortus* microbiota and its predilection site, the abomasum microbiota, we constructed Heatmap and Principal Coordinate Analysis (PCoA) using the result of UniFrac distance matrices. These results indicated that all abomasum content samples clustered together, suggesting that the sheep abomasum harbour similar microbiota. The adult male and female *H. contortus* samples clustered together in Figure 19 and 35, indicating that they harbour similar microbiota as well, this could be due to similar living environmental conditions and diet. El-Ashram & Suo, (2017) reported similar results which shows the *H. contortus* eggs and adult stage microbiota cluster together because they share similar environmental condition inside the host.

Furthermore, we compared the abomasum content microbiota with *H. contortus* microbiota, the results showed that the adult male and female *H. contortus* worms appeared to have a less bacterial diversity than the abomasum content. As shown in the results section with abundance depicted by color intensity on the heatmap, the abomasum content had higher diversity followed by adult male *H. contortus* while the adult female had the least diversity. This result shows that the abomasum content and adult male *H. contortus* microbiota are more related to each other and shared similar microbiota compared to adult female *H. contortus*. Hence, the adult worms microbiota does not simply reflect abomasum content microbiota. Previous study by Sinnathamby *et al.* (2018) reported that the parasite life-cycle stages microbiota are different from that of their predilection site. Moreover, El-Ashram & Suo, (2017); Sinnathamby *et al.* (2018) reported that during the *H. contortus* worms life cycle stages development, the egg stage, L₁ (rhabditiform), and L₂ (rhabditiform) and L₃ (filiariform) infective larvae interact with different microbiota from their respective environment, thus each stage might have distinct microbiota that they pick up from their environment and the mammalian host. Furthermore, detection of bacteria within the *H.*

contortus intestinal tract suggests a potential nutritional importance for these microbiota, either providing metabolites that the worm cannot make itself, or aiding the breakdown of ingested food.

5.7 CONCLUSION

In conclusion, the microbiota of the adult male and female *H. contortus* worms and their predilection site, the abomasum were characterised with the aid of the Illumina MiSeq sequencing technology. Our results demonstrated that the abomasum harbour more diverse and dense microbiota compared to the adult male and female *H. contortus* worms. These results indicated significant variations ($P=0.000636$; $P=0.035$) in microbiota observed between the proportions of the bacterial phyla and genus present in the adult *H. contortus* worms and their predilection site, the abomasum. Nevertheless, there was no variations in the most predominant bacterial phyla and genera between adult male and female *H. contortus*. The predominant bacterial phyla for the adult male and female *H. contortus* worms were *Proteobacteria*, *Firmicutes* and *Bacteroidetes*, and for the abomasum content were *Firmicutes*, *Bacteroidetes* and *Actinobacteria*. Predominant bacterial genera for the adult male and female *H. contortus* worms were (*Vibrio*, *Holomonas* and *Escheria-shigella*) and (*Escheria-shigella*, *Vibrio* and *Holomonas*) respectively and for the abomasum content were *Succiniclasticum*, *Rikenellaceae* RC9 gut group and *Candidatus Saccharimonas*. Our result showed bacterial genera that can produce nematicidal volatile compounds that are active against nematodes. It's possible that they control populations of GIN parasites within the sheep abomasum, hence, there are *Haemonchus* resistant Dohne Merino breeds. Furthermore, the occurrence of this bacteria brings about the possibility of using symbiotic bacteria as biocontrol for GIN parasites.

Moreover, to determine the exact nature of microbiome-*H. contortus* and host-*H. contortus* microbiome interaction, additional and complementary analyses are necessary to uncover specific molecular mechanisms underlying the nematode-microbiota as method of controlling nematodes. Moreover, microbiota of environmental stages of *H. contortus* (L1 – L3) needs to be characterized and there is abundant room for further studies in comparing microbiota of different parasitic helminths, which has, and will continue to offer an important parallel goal for the removal of a wide-variety of devastating animal and human diseases.

5.8. MAJOR IMPLICATIONS AND LIMITATIONS OF THE STUDY?

The current research has given an insight into the microbiota of the adult *H. contortus* and its predialtion site, the abomasum, which will be very crucial in the development of the biological control of GIT parasites in livestock in the near future. However, more research is necessary to refine and further elaborate the current findings. This research is necessary in the livestock farming, as it give insight into the microbes that causes diseases in small ruminants and the microbes that that are pathogenic to GIT parasites, which is important in removal of livestock diseases.

However, in the current research we could only research the adult *H. contortus* stage and its predilation site, the abomasum, because of the small sample size, limited funding and resources. The future studies are recommended in section 5.9.

5.9. RECOMMENDATIONS

Based on the observations of current study, the following future studies are recommended in order to obtain data that will ensure effective management interventions for biological control of gastrointestinal parasites in livestock:

Characterisation of environmental stages of *H. contortus* (L1 – L3) microbiota and in comparison to their environmental microbiota. These studies must include the microbiota of the manure and pasture that the larval stages inhabit.

Microbial community profiling of all gastrointestinal nematodes of livestock to determine microbial community that can be used as biocontrol for all the GIN parasites in order to remove wide-variety of devastating animal and human diseases. These studies must also include fungal community of GIN parasites and the host, as it has been shown that fungus can also be used to control nematodes.

Functional genomics of GIN parasites intestinal microbiota to determine their roles in GIN parasites biology and predict the microbial community which can produce nematicidal volatile compounds against pathogenic GIN parasites.

REFERENCES

- Aanen, D. K., & Eggleton, P. (2017). Symbiogenesis: Beyond the endosymbiosis theory? *Journal of Theoretical Biology*, 434, 99-103.
- Abdel-Salam, M. S., Amee, H. H. , Soliman, G. M, Elkelany U. S., Asar A. M. (2018). Improving the nematicidal potential of *Bacillus amyloliquefaciens* and *Lysinibacillus sphaericus* against the root-knot nematode *Meloidogyne incognita* using protoplast fusion technique. *Egyptian Journal of Biological Pest Control*. 2018.28:31.
- Adzitey, F., Huda, N., & Ali, G. R. (2012). Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks. 3 *Biotech*, 3(2), 97-107.
- Aitken, I. D. (2008). Diseases of Sheep, 4th ed. *The Canadian Veterinary Journal*, 49(7), 702.
- Alakomi, H. L., Skyttä, E., Saarela M., Mattila-Sandholm, T., Latva-Kala, K., Helander, I. M. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied Environmental Microbiology*, 66: 2001–2005.
- Alba-Hurtado, F., & Muñoz-Guzmán, M. A. (2012). Immune responses associated with resistance to haemonchosis in sheep. *BioMed research international*, 2013, 162158.
- Altaif, K. I., Al-Abbassy, S. N., Al-Saqr, I. M., Jawad, A. K. (1978). "Experimental studies on the suitability of aquatic snails as intermediate hosts for *Paramphistomum cervi* in Iraq". *Annals of Tropical Medicine and Parasitology*. 72(2): 151–155.
- Amarante, M. R. V., Santos, M. C., Bassetto, C. C., Amarante, A. F. T. (2017). PCR primers for straightforward differentiation of *Haemonchus contortus*, *Haemonchus placei* and their hybrids. *Journal of Helminthology*, 91(6):757-761.
- Anderson, N. L & Anderson, N. G. (1998). "Proteome and proteomics: new technologies, new concepts, and new words". *Electrophoresis*. 19(11): 1853–61.
- Anderson. R. C. (2000). Nematode parasites of vertebrates: their development and transmission. *Journal of the South African Veterinary Association*, 71(4): 239.

- Andronicos, N., Hunt, P., Windon, R. (2010). Expression of genes in gastrointestinal and lymphatic tissues during parasite infection in sheep genetically resistant or susceptible to *Trichostrongylus colubriformis* and *Haemonchus contortus*. *International Journal for Parasitology*, 40(4): 417–429.
- Ansari, M. A., Tirry, L., Moens, M. (2003). Entomopathogenic nematodes and their symbiotic bacteria for the biological control of *Hoplia philanthus* (Coleoptera: Scarabaeidae), *Biological Control*, 28(1): 111-117.
- Ansorge, W., Sproat, B.S., Stegemann, J., and Schwager, C. (1986). A non-radioactive automated method for DNA sequence determination. *Journal of Biochemical and Biophysical Methods*, 13: 315-323.
- Aravindraja, C., Viszwapriya, D., Karutha Pandian, S. (2013). Ultradeep 16S rRNA sequencing analysis of geographically similar but diverse unexplored marine samples reveal varied bacterial community composition. *PloS one*. 8:e76724.
- Arias, M. S., Sanchís, J., Francisco, I., Francisco, R., Piñeiro, P., Cazapal-Monteiro, C., Cortiñas, F. J., Suárez, J. L., Sánchez-Andrade, R., Paz-Silva, A. (2013). The efficacy of four anthelmintics against *Calicophoron daubneyi* in naturally infected dairy cattle. *Veterinary Parasitology*, 197(1-2):126-9.
- Arsenic, R., Treue, D., Lehmann, A., Hummel, M., Dietel, M., Denkert, C., Budczies, J. (2015). Comparison of targeted next-generation sequencing and Sanger sequencing for the detection of PIK3CA mutations in breast cancer. *BMC Clinical Pathology*, 15: 20.
- Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., Semenkovich, C. F., Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, 101(44): 15718-23.
- Bailey, R. (2017). Friend or Foe? *A Brief History of Bacteria*, 39-116.

- Baker, N. F., Cook, E. F., Douglas, J. R., Cornelius, C. E. (1959) The pathogenesis of *trichostrongyloid* parasites. III. Some physiological observations in lambs suffering from acute parasitic gastroenteritis. *Journal of Parasitology*. 45(6): 643-651.
- Baker, R. L., Audho, J. O., Adudo, E. O., Thorpe, W. (2001). Genetic resistance to gastrointestinal nematode parasites in Galla and Small East African goats in the sub-humid tropics. *Animal Science* 73: 61–70.
- Balic, A., Bowles, V. M., Meeusen, E. N. T. (2002). Mechanisms of immunity to *Haemonchus contortus* infection in sheep. *Parasite Immunology*, 24(1): 39–46.
- Bath, G. F., Malan, F. S., van Wyk, J. A. (1996). The "FAMACHA" ovine anaemia guide to assist with the control of haemonchosis. *Proceedings of the 7th Annual Congress of the Livestock Health and Production Group of the South African Veterinary Association*. Port Elizabeth, South Africa.
- Bayer, C., Heindl, N. R., Rinke, C., Lückner, S., Ott, J. A., Bulgheresi, S. (2009). Molecular characterization of the symbionts associated with marine nematodes of the genus *Robbea*. *Environmental Microbiology Reports*, 1(2): 136–144.
- Begum, K., Mannan, S. J., Rahman, M. M., Mitchell, A. A., Opoku, R. (2017). Identification of antibiotic producing bacteria from soil samples of Dhaka, Bangladesh. *Journal of Microbiology*, 4(6): 00134.
- Benavides, M. V., Sonstegard, T. S., Kemp, S., Mugambi, J. M., Gibson, J. P., Baker, R. L., Hanotte, O., Marshall, K., van Tassell, C. (2015). Identification of novel loci associated with gastrointestinal parasite resistance in a Red Maasai x Dorper backcross population. *PLoS ONE*, 10:e0122797.
- Benavides, M. V., Sonstegard, T. S., Tassell, C. V. (2016). Genomic regions associated with sheep resistance to gastrointestinal nematodes. *Trends in parasitology*, 32(6): 470-80.
- Bentley, D. R., Balasubramanian, S., Swerdlow, H. P., Smith, G. P., Milton, J., Brown, C. G., Hall, K. P., Evers, D. J., Barnes, C. L., Bignell, H. R., Boutell, J. M., Bryant, J., Carter, R. J., Keira Cheetham, R., Cox, A. J., Ellis, D. J., Flatbush, M. R., Gormley,

- N. A., Humphray, S. J., Irving, L. J., Karbelashvili, M. S., Kirk, S. M., Li, H., Liu, X., Maisinger, K. S., *et al.* (2008). Accurate whole human genome sequencing using reversible terminator chemistry. *Nature*, 456(7218): 53-9.
- Berg, M., Stenuit, B., Ho, J., Wang, A., Parke, C., Knight, M., Alvarez-Cohen, L., Shapira, M. (2016). Assembly of the *Caenorhabditis elegans* gut microbiota from diverse soil microbial environments. *The ISME journal*, 10(8), 1998-2009.
- Besier, B., Lyon, J., Michael, D., Newlands, G., Smith D. (2012). Towards a commercial vaccine against *Haemonchus contortus* - a field trial in Western Australia. *Proceedings of Australian Sheep Veterinarians Conference*. 14–18.
- Besier, R., Kahn, L., Sargison, N., van Wyk, J. (2016). Diagnosis, treatment and management of *Haemonchus contortus* in small ruminants. *Advances in Parasitology*. 93: 181–238.
- Bhuiyan, A. A., Li, J., Wu, Z., Ni, P., Adetula, A. A., Wang, H., Du, X. (2017). Exploring the genetic resistance to gastrointestinal nematodes infection in goat using RNA-sequencing. *International Journal of Molecular Sciences*, 18(4): 751.
- Bishop, S. (2012). Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. *Animal*, 6(5): 741-747.
- Bowman, D. D & Georgi, J. R. (2008). Georgis' parasitology for veterinarians (9 ed.). W.B. Saunders Company, 124.
- Bridgeman, B., Morgan-Richards, M., Wheeler, D., Trewick, S. A. (2018). First detection of *Wolbachia* in the New Zealand biota. *PLoS ONE*, 13(4): e0195517.
- Brown D. S. (2005). Freshwater snails of africa and their medical importance (2 ed.). *Taylor & Francis Ltd*, 366–370.
- Buermans, H. P. J & den dunnen, J. T. (2014). Next generation sequencing technology: Advances and applications. *Biochimica et Biophysica Acta (BBA). Molecular Basis of Disease*, 1842(10): 1932–1941.
- Buffie, C. G., Bucci, V., Stein, R. R., McKenney, P. T., Ling, L., Gobourne, A., No, D., Liu,

- H., Kinnebrew, M., Viale, A., Littmann, E., van den Brink, M. R., Jenq, R. R., Taur, Y., Sander, C., Cross, J. R., Toussaint, N. C., Xavier, J. B., Pamer, E. G. (2014). Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. *Nature*, 517(7533): 205-8.
- Burke, J. (2005). Management of barber pole worm in sheep and goats in the Southern U.S. USDA, ARS, *Dale Bumpers Small Farms Research Center*, Booneville, AR.
- Byomi, M., Samaha, H., Zidan, S. (2010). Epidemiological studies on some zoonotic enteric protozoa in different areas of Nile Delta. *Journal of the American Society of Nephrology*, 5:199–207.
- Can, T. (.2014). Introduction to bioinformatics methods. *Journal of Molecular Biology*, 1107: 51-71.
- Carpi, G., Cagnacci, F., Wittekindt, N. E., Zhao, F., Qi, J., Tomsho, L. P., Drautz, D. I., Rizzoli, A., Schuster, S. C. (2011). Metagenomic profile of the bacterial communities associated with *Ixodes ricinus* ticks. *PLoS ONE*, 6(10): e25604.
- Castro-Wallace, S. L., Chiu, C. Y., John, K. K., Stahl, S. E., Rubins, K. H., McIntyre A. B. R., Burton, A. S. (2017). Nanopore DNA sequencing and genome assembly on the international space station. *Scientific Reports*, 7: 18022.
- Chaucheyras-Durand, F & Ossa, F. (2014). Review: The rumen microbiome: composition, abundance, diversity, and new investigative tools. *Professional Animal Scientist*, 30: 1–12.
- Check Hayden, E. (2015). Pint-sized DNA sequencer impresses first users. *Nature*, 521(7550): 15-6.
- Chen, C. J., Wu, G. H., Kuo, R. L., Shih, S. R. (2017). Role of the intestinal microbiota in the immunomodulation of influenza virus infection, *Microbes and Infection*. *Microbes and infection*, 19(12): 570-579.
- Chow, J., Lee, S. M., Shen, Y., Khosravi, A., Mazmanian, S. K. (2010). Host–bacterial symbiosis in health and disease. *Advances in Immunology*, 107: 243–274.

- Ciccarelli, F. D., Doerks, T., Von Mering, C., Creevey, C. J., Snel, B., Bork, P. (2006). Toward automatic reconstruction of a highly resolved tree of life. *Science*. 311(5765): 12837.
- Claesson, M. J., Cusack, S., O'Sullivan, O., Greene-Diniz, R., de Weerd, H., Flannery, E., Marchesi, J. R., Falush, D., Dinan, T., Fitzgerald, G., Stanton, C., van Sinderen, D., O'Connor, M., Harnedy, N., O'Connor, K., Henry, C., O'Mahony, D., Fitzgerald, A. P., Shanahan, F., Twomey, C., Hill, C., Ross, R. P., ... O'Toole, P. W. (2010). Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proceedings of the National Academy of Sciences of the United States of America*, 108: 4586-91.
- Clardy, J., Fischbach, M., Currie, C. (2009). The natural history of antibiotics. *Current Biology : CB*, 19(11): R437–R441.
- Clarke, K. G & Gorley, R. N. (2006). PRIMER v6: user manual/tutorial. Plymouth: PRIMER-E.
- Cloete, S., Scholtz, A., Aucamp, B. (1998). Environmental effects, heritability estimates and genetic trends in a Western Cape Dohne Merino flock. *South African Journal of Animal Science*, 28: 185-195.
- Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Marsh, T., Garrity, G. M., Tiedje, J. M. (2008). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic acids research*, 37: 141-5.
- Cranston, L. M., Kenyon, P. R., Corner-Thomas, R. A., Morris, S. T. (2017). The potential interaction between ewe body condition score and nutrition during very late pregnancy and lactation on the performance of twin-bearing ewes and their lambs. *Asian-Australasian Journal of Animal Sciences*, 30(9): 1270–1277.
- Cunha, I. S., Barreto, C. C., Costa, O. Y., Bomfim, M. A., Castro, A. P., Kruger, R. H., Quirino, B. F. (2011). Bacteria and Archaea community structure in the rumen microbiome of goats (*Capra hircus*) from the semiarid region of Brazil. *Anaerobe*, 3: 118.124.

- Da Silva, M. E., Braga, F. R., de Gives, P. M., Uriostegui, M. A. M., Reyes, M., Soares, F.E. de, F., de Araújo, J. V. (2015). Efficacy of *Clonostachys rosea* and *Duddingtonia flagrans* in Reducing the *Haemonchus contortus* Infective Larvae. *BioMed Research International*, 2015: 474879.
- da Silva, M. E., Braga, F. R., de Gives, P. M., Uriostegui, M. A., Reyes, M., Soares, F. E., de Carvalho, L. M., Rodrigues, F. B., de Araújo, J. V. (2015). Efficacy of *Clonostachys rosea* and *Duddingtonia flagrans* in Reducing the *Haemonchus contortus* Infective Larvae. *BioMed research international*, 2015: 474879.
- De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G., Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, 107(33):14691-6.
- de la Chevrotière, C., Bambou, J.C., Arquet, R., Jacquet, P., Mandonnet, N. (2012). Genetic analysis of the potential role of IgA and IgE responses against *Haemonchus contortus* in parasite resistance of Creole goats. *Veterinary Parasitology*, 186(3-4): 337–343.
- De Mandal, S., Chatterjee, R., Kumar, N. S. (2017). Dominant bacterial phyla in caves and their predicted functional roles in C and N cycle. *BMC Microbiology*, 17: 90.
- De, S & Sanyal, P. K. (2009). Biological control of helminth parasites by predatory fungi. *Online veterinary journal*, 4: 1-8.
- Detman, A., Mielecki, D., Pleśniak, Ł., Bucha, M., Janiga, M., Matyasik, I., Chojnacka, A., Jędrysek, M. O., Błaszczak, M. K., ... Sikora, A. (2018). Methane-yielding microbial communities processing lactate-rich substrates: a piece of the anaerobic digestion puzzle. *Biotechnology for biofuels*, 11, 116.
- DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and environmental microbiology*, 72(7): 5069-72.

- Dimijian, G. G. (2000). Evolving together: the biology of symbiosis, part 1. *Proceedings of Baylor University Medical Center*, 13(3): 217–226.
- Dirksen, P., Marsh, S. A., Braker, I., Heitland, N., Wagner, S., Nakad, R., Mader, S., Petersen, C., Kowallik, V., Rosenstiel, P., Félix, M. A., Schulenburg, H. (2016). The native microbiome of the nematode *Caenorhabditis elegans*: gateway to a new host-microbiome model. *BMC biology*, 14: 38.
- Dodds, K. G & Gemmell, N. J. (2014). Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. *BMC Genomics*.15: 637.
- Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, 107: 11971-5.
- Donald, A. D., Southcott, W. H., Dineen, J. K. (1978). The epidemiology and control of gastrointestinal parasites of sheep in australia. *Commonwealth Scientific and Industrial Research Organisation*: Melbourne; 1978.
- Duarte, A. M., Jenkins, T. P., Latrofa, M. S., Giannelli, A., Papadopoulos, E., de Carvalho, L. M., Cantacessi, C. (2016). Helminth infections and gut microbiota – a feline perspective. *Parasites & Vectors*, 9: 625.
- Eckbur, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E., Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*. 308: 1635-8.
- Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E., ... Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science (New York, N.Y.)*, 308(5728): 1635-8.
- El-Ashram, S & Suo, X. (2017). - Exploring the microbial community (microflora) associated with ovine *Haemonchus contortus* (macroflora) field strains. *Scientific Reports*, 7: 70.

- Elhady, A., Giné, A., Topalovic, O., Jacquiod, S., Sørensen, S. J., Sorribas, F. J., Heuer, H. (2017). Microbiomes associated with infective stages of root-knot and lesion nematodes in soil. *PLoS ONE*, 12(5): e0177145.
- Engström, M. (2016). Understanding the bioactivity of plant tannins: developments in analysis methods and structure-activity studies. Thesis, university of turku, finland.
- Feng, Y, Xiao, L. (2011). Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Journal of Clinical Microbiology*, 24: 110– 40.
- Fernández, L., Langa, S., Martín, V., Maldonado, A., Jiménez, E., Martín, R., Rodríguez, J.M. (2013). The human milk microbiota: origin and potential roles in health and disease. *Pharmacological Research*. 69(1):1-10.
- Fischer, M. A., Güllert, S., Neulinger, S. C., Streit, W. R., Schmitz, R. A. (2016). Evaluation of 16S rRNA gene primer pairs for monitoring microbial community structures showed high reproducibility within and low comparability between datasets generated with multiple archaeal and bacterial primer pairs. *Frontiers in microbiology*, 7: 1297.
- Fisher, R. M., Henry, L. M., Cornwallis, C. K., Kiers, E. T., West, S. A. (2017). The evolution of host-symbiont dependence. *Nature Communications*, 8: 15973.
- Fransen, F., van Beek, A. A., Borghuis, T., Meijer, B., Hugenholtz, F., van der Gaast-de Jongh, C., Savelkoul, H. F., de Jonge, M. I., Faas, M. M., Boekschoten, M. V., Smidt, H., El Aidy, S., de Vos, P. (2017). The Impact of Gut Microbiota on gender-specific differences in immunity. *Frontiers in immunology*, 8: 754.
- Fricke, W. F., Song, Y., Wang, A. J., Smith, A., Grinchuk, V., Mongodin, E., Pei, C., Ma, B., Lu, N., Urban, J. F., Shea-Donohue, T., Zhao, A. (2015). Type 2 immunity-dependent reduction of segmented filamentous bacteria in mice infected with the helminthic parasite *Nippostrongylus brasiliensis*. *Microbiome*, 3: 40.
- Firdich, E., & Gaynor, E. C. (2013). Peptidoglycan hydrolases, bacterial shape, and pathogenesis. *Current Opinion in Microbiology*, 16(6): 767-778.

- Fukruksa, C., Yimthin, T., Suwannaroj, M., Muangpat, P., Tandhavanant, S., Thanwisai, A., Vitta, A. (2017). Isolation and identification of *Xenorhabdus* and *Photorhabdus* bacteria associated with entomopathogenic nematodes and their larvicidal activity against *Aedes aegypti*. *Parasites & Vectors*, 10: 440.
- Gause, W. C., & Maizels, R. M. (2016). Macrobiora - helminths as active participants and partners of the microbiota in host intestinal homeostasis. *Current opinion in microbiology*, 32: 14-18.
- Ghasemikhah, R., Mirhendi, H., Kia, E., Mowlavi, G., Sarmadian, H., Meshgi, B., Mobedi, I. (2011). Morphological and morphometrical description of *Trichostrongylus* Species Isolated from domestic ruminants in Khuzestan Province, Southwest Iran. *Iranian Journal of Parasitology*, 6(3): 82–88.
- Gholam, S., Babamahmoodi, F., Abedian, R., Sharif, M., Shahbazi, A., Pagheh, A., Fakhar, M. (2015). *Trichostrongylus colubriformis*: possible most common cause of human infection in Mazandaran Province, North of Iran. *Iranian Journal of Parasitology*. 10(1): 110 – 115.
- Gibbons, L. M. (1979): Revision of the genus *Haemonchus* Cobb 1898 (Nematoda: *Trichostrongylidae*). *Systematic Parasitology*, 1: 3 – 24.
- Gill, H. S., Altmann, K., Cross, M. L., Husband, A. J. (2000). Induction of T helper 1- and T helper 2-type immune responses during *Haemonchus contortus* infection in sheep. *Immunology*, 99(3), 458-63.
- Gilleard, J. (2006). Understanding anthelmintic resistance: the need for genomics and genetics. *International Journal of Parasitology*, 36(12):1227–1239. 4.
- Glendinning, L., Nausch, N., Free, A., Taylor, D. W., Mutapi, F. (2014). The microbiota and helminths: sharing the same niche in the human host. *Parasitology*, 141: 1255-1271.
- Gómez-Garzón, C., Hernández-Santana, A., Dussán, J. (2017) A genome-scale metabolic reconstruction of *Lysinibacillus sphaericus* unveils unexploited biotechnological potentials. *PLoS ONE*, 12(6): e0179666.
- Gong, J & Yang, C. (2012). Advances in the methods for studying gut microbiota and their

relevance to the research of dietary fiber functions, Food Research International. Elsevier B.V., 48(2): 916–929.

Gordon, D. K., Roberts, L. C. P. Lean, N., Zadoks, R. N., Sargison, N. D., Skuce, P. J. (2013). Identification of the rumen fluke, *Calicophoron daubneyi*, in GB livestock: possible implications for liver fluke diagnosis. *Veterinary parasitology*, 0304-4017.

Gordon, D., Zadoks, R., Skuce, P., Sargison, N. (2012) Confirmation of *triclabendazole* resistance in liver fluke in the UK. *Veterinary Record*, 171: 159-160.

Grada, A & Weinbrecht, K. (2013). Next-generation sequencing: methodology and application. *Journal Of Investigative Dermatology*, 133: e11.

Grenni, P., Ancona, V., Caracciolo, A. B. (2018). Ecological effects of antibiotics on natural ecosystems: a review. *Microchemical Journal*, 136: 25-39.

Guarner, F. (2006). Enteric flora in health and disease. *Digestion*, 73: 5–12.

Guernier, V., Brennan, B., Yakob, L., Milinovich, G., Clements, A. C. A., Soares Magalhaes, R. J. (2017). Gut microbiota disturbance during helminth infection: Can it affect cognition and behaviour of children?. *BMC Infectious Diseases*, 17: 58.

Guo, Z., González, J. F., Hernandez, J. N., McNeilly, T. N., Corripio-Miyar, Y., Frew, D., Li, R. W. (2016). Possible mechanisms of host resistance to *Haemonchus contortus* infection in sheep breeds native to the Canary Islands. *Scientific Reports*, 6: 26200.

Hale, M. (2006). Managing internal parasites in sheep and goats. *Attra*, 1 – 8.

Hansen, J & Perry, B . (2018). Post-mortem differential parasite counts. [ONLINE] Available at: <http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e06.htm>. [Accessed 13 November 2018].

Haque, S. Z., & Haque, M. (2017). The ecological community of commensal, symbiotic, and pathogenic gastrointestinal microorganisms – an appraisal. *Clinical and Experimental Gastroenterology*, 10: 91–103.

- Haro, C., Rangel-Zúñiga, O. A., Alcalá-Díaz, J. F., Gómez-Delgado, F., Pérez-Martínez, P., Delgado-Lista, J., Quintana-Navarro, G. M., Landa, B. B., Navas-Cortés, J. A., Tena-Sempere, M., Clemente, J. C., López-Miranda, J., Pérez-Jiménez, F., ... Camargo, A. (2016). Intestinal Microbiota Is Influenced by gender and body mass index. *PloS one*, 11(5): e0154090.
- Hart, S. P & Dawson, L. J. (2010). Using FAMACHA and alternative dewormers to manage gastrointestinal nematodes in a dairy goat herd. *Journal of Animal Science*, 88(2): 580.
- Hasegawa, M., Osaka, T., Tawaratsumida, K., Yamazaki, T., Tada, H., Chen, G. Y., Tsuneda, S., Núñez, G., Inohara, N. (2009). Transitions in oral and intestinal microflora composition and innate immune receptor-dependent stimulation during mouse development. *Infection and immunity*, 78(2), 639-50.
- Hayes, K. S., Bancroft, A. J., Goldrick, M., Portsmouth, C., Roberts, I. S., Grencis, R. K. (2010). Exploitation of the intestinal microflora by the parasitic nematode *Trichuris muris*. *Science*, 328: 1391–1394.
- Heckendorn, F., Bieber, A., Werne, S., Saratsis, A., Maurer, V., Stricker, C. (2017). The genetic basis for the selection of dairy goats with enhanced resistance to gastrointestinal nematodes. *Parasite*, 24: 32.
- Hesper, B & Hogeweg, P. (1970). "Bioinformatica: een werkconcept". 1 (6). *Kameleon*, 28–29.
- Hogeweg, P. (2011). Searls, David B., ed. "The roots of bioinformatics in theoretical biology". *PLoS Computational Biology*, 7 (3): e1002021.
- Holm, J. B., Sorobetea, D., Kiillerich, P., Ramayo-Caldas, Y., Estellé, J., Ma, T., Svensson-Frej, M. (2015). Chronic *Trichuris muris* infection decreases diversity of the intestinal microbiota and concomitantly increases the abundance of *Lactobacilli*. *PLoS ONE*, 10(5): e0125495.
- Holscher H. D. (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut microbes*, 8(2), 172-184.

- Howell, S. B., Burke, J. M., Miller, J. E., Terrill, T. H., Valencia, E., Williams, M. J., Williamson L. H., Zajac A. M., Kaplan, R. M. (2008). Prevalence of anthelmintic resistance on sheep and goat farms in the south eastern United States. *Journal of the American Veterinary Medical Association*, 233: 1913–1919.
- Hsiao, A., Ahmed, A. M., Subramanian, S., Griffin, N. W., Drewry, L. L., Petri, W. A., Haque, R., Ahmed, T., Gordon, J. I. (2014). Members of the human gut microbiota involved in recovery from *Vibrio cholerae* infection. *Nature*, 515(7527): 423-6.
- Hsu, S., Johansson, K., Donahue, M. (1986). The Bacterial Flora of the Intestine of *Ascaris suum* and 5-Hydroxytryptamine Production. *The Journal of Parasitology*, 72(4): 545-549.
- Huang, J., Li, Y., Lu, y. (2016): Bacterial community in the rumen of Tibetan sheep and Gansu alpine fine-wool sheep grazing on the Qinghai-Tibetan Plateau, China. *Journal of General and Applied Microbiology*, 08: 003.
- Hutchinson, G. W. (2009). Nematode parasites of small ruminants, camelids and cattle: diagnosis with emphasis on anthelmintic efficacy and resistance testing.
- Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, 486: 207–214.
- Huws, S. A., Kim, E. J., Lee, M. R. F., Scott, M. B., Tweed, J. K. S., Pinloche, E., Wallace, R. J., Scollan, N. D. (2011). As yet uncultured bacteria phylogenetically classified as *Prevotella*, *Lachnospiraceae* incertae sedis and unclassified *Bacteroidales*, *Clostridiales* and *Ruminococcaceae* may play a predominant role in ruminal biohydrogenation. *Environmental Microbiology*, 13: 1500–1512.
- Itani, T., Ayoub Moubareck, C., Melki, I., Rousseau, C., Mangin, I., Butel, M. J., Karam Sarkis, D. (2017). Establishment and development of the intestinal microbiota of preterm infants in a Lebanese tertiary hospital. *Anaerobe*, 43: 4–14
- Jacobs, H. J., Ashman, K., Meeusen, E. N. T. (1995). Humoral and cellular responses following local immunization with a surface antigen of the gastrointestinal parasite *Haemonchus contortus*. *Veterinary Immunology and Immunopathology*, 48(3-4):

- Jacquiet, P, Cabaret, J, Cheikh, D, Thiam, E. (1996). Identification of *Haemonchus* species in domestic ruminants based on morphometrics of spicules. *Parasitology Research*, 83(1): 82-86.
- Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., Nageshwar Reddy, D. (2015). Role of the normal gut microbiota. *World journal of gastroenterology*, 21(29): 8787-803.
- Jiménez, E., Marín, M. L., Martín, R., Odriozola, J. M., Olivares, M., Xaus J., Fernández, L., Rodríguez, J. M. (2008). Is meconium from healthy newborns actually sterile? *Research in Microbiology*, 159: 187 – 193.
- Jones, R. A., Brophy, P. M., Mitchell, E. S., Williams, H. W. (2017). Rumen fluke (*Calicophoron daubneyi*) on Welsh farms: prevalence, risk factors and observations on co-infection with *Fasciola hepatica*. *Parasitology*, 144(2): 237–247.
- Junquera, P. (2016). "*Haemonchus* Spp, parasitic roundworm of cattle, sheep and goats. biology, prevention and control. *Haemonchus contortus*, *Haemonchus placei*. Haemonchosis.". *Parasitipedia.net*. N.p., 2017. Web. 1 June 2017.
- Junquera, P. (2016). *Trichostrongylus* spp, parasitic roundworms of cattle, sheep, goats, pigs and horses. Biology, prevention and control. *Trichostrongylus colubriformis*, *Trichostrongylus axe*. *Parasitipedia.net*. N.p., 2017. Web. 1 June 2017.
- Kamagata, Y & Narihiro T. (2016). Symbiosis studies in microbial ecology. *Microbes and Environments*, 31(3): 201–203.
- Kanfra, X., Liu, B., Beerhues, L., Sørensen, S. J., & Heuer, H. (2018). Free-Living Nematodes Together With Associated Microbes Play an Essential Role in Apple Replant Disease. *Frontiers in plant science*, 9, 1666.
- Kaplan, R. M. (2004). Drug resistance in nematodes of veterinary importance: a status report. *Trends in Parasitology*, 20(10): 477–481.

- Karlsson, F. H., Tremaroli, V., Nookaew, I., Bergström, G., Behre, C. J., Fagerberg, B., Nielsen, J., Bäckhed, F. (2013). Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*, 498: 99–103.
- Kay, G. L., Millard, A., Sergeant, M. J., Midzi, N., Gwisai, R., Mduluzi, T., Ivens, A., Nausch, N., Mutapi, F., Pallen, M. (2015). Differences in the faecal microbiome in *Schistosoma haematobium* Infected Children vs. Uninfected Children. *PLoS neglected tropical diseases*, 9(6): e0003861.
- Kelly, G. A., Kahn, L. P., Walkden-Brown, S. W. (2013). Measurement of phenotypic resilience to gastro-intestinal nematodes in Merino sheep and association with resistance and production variables. *Veterinary Parasitology*, 193: 111-117.
- Kerkhof, L. J., Dillon, K. P., Häggblom, M. M., McGuinness, L. R. (2017). Profiling bacterial communities by MinION sequencing of ribosomal operons. *Microbiome*, 5: 116.
- Kerra, C. L., Brighta, A., Smitha, B., Armstrongb, D. R., Highama, L. E. (2017). A monitoring study to explore gastrointestinal helminth burdens of ewes of different fecundities in the periparturient period and effects on lamb growth rates. *Small Ruminant Research*, 151: 98–103.
- Khan, A. R & Fallon, P. G. (2013). Helminth therapies: translating the unknown unknowns to known knowns. *International Journal of Parasitology*, 43: 293–299.
- Kilani, M., Chermette, R., Guillot, J., Polack, B., Duncan, J. L., Cabaret, J. (2010). Gastrointestinal helminthoses: amphistomosis, in Infectious and parasitic diseases of livestock, *Lavoisier*, 1589–1601.
- Kim, J. Y., Kim, Y. B., Song, H. S., Chung, W. H., Lee, C., Ahn, S. W., Lee, S. H., Jung, M. Y., Kim, T. W., Nam, Y. D., ... Roh, S. W. (2017). Genomic analysis of a pathogenic bacterium, *Paeniclostridium sordellii* CBA7122 containing the highest number of rRNA operons, isolated from a human stool sample. *Frontiers in pharmacology*, 8: 840.
- Kinross, J. M., Darzi, A. W., Nicholson, J. K. (2011). Gut microbiome-host interactions in health and disease. *Genome Medicine*, 3(3):14.

- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, 41(1): e1.
- Kotze, A. C., O Grady, J., Gough, J. M., Pearson, R., Bagnall, N. H., Kemp, D. H. Akhurst, R. J. (2005). Toxicity of *Bacillus thuringiensis* to parasitic and free-living life-stages of economically important nematode parasites of livestock. *International Journal of Parasitology*, 35: 1013-1022.
- Kozek, W. J & Marroquin, H. F. (1977). Intracytoplasmic bacteria in *Onchocerca volvulus*. *The American Journal of Tropical Medicine and Hygiene*, 26: 663–678.
- Kraneburg, W. (1977). Contribution to the biology and pathogenicity of domestic omasal leech *Paramphistomum cervi*. 1. Developmental stages in the free state and in the intermediary host. *Berliner und Münchener Tierärztliche Wochenschrift*, 90(16): 316–320.
- Krawczyk, A & Słota E. (2009). Genetic markers to gastrointestinal nematode resistance in sheep: a review. *Helminthologia*, 46(1): 3 – 8.
- Krebs, Robert E, Carolyn A. (2003). Groundbreaking scientific experiments, inventions & discoveries of the ancient world. Westport, CT: *Greenwood Press*. ISBN 0-313-31342-3.
- Kreisinger, J., Bastien, G., Hauffe, H. C., Marchesi, J., Perkins, S. E. (2015). Interactions between multiple helminths and the gut microbiota in wild rodents. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 370(1675): 20140295.
- Kuczynski, J., Stombaugh, J., Walters, W. A., González, A., Caporaso, J. G., Knight, R. (2012). Using QIIME to analyze 16S rRNA gene sequences from microbial communities. *Current Protocols in Microbiology*, 01: 1E.5.

- Kumar, N., Rao, T. K. S., Varghese, A., Rathor, V. S. (2013). Internal parasite management in grazing livestock. *Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology*, 37(2): 151–157.
- Kumar, V. (1998). *Trematode infections and diseases of man and animals* (1 ed.). Springer, 275–321.
- Lacharme-Lora, L., Salisbury, V., Humphrey, T. J., Stafford, K., Perkins, S. E. (2009). Bacteria isolated from parasitic nematodes—a potential novel vector of pathogens? *Environmental health: a global access science source*, 8(1): 17.
- Lavelle, E. C., Murphy, C., O'Neill, L. A., & Creagh, E. M. (2009). The role of TLRs, NLRs, and RLRs in mucosal innate immunity and homeostasis. *Mucosal immunology*, 3(1): 17-28.
- Lee, J., Choe, J., Kim, J., Oh, S., Park, S., Kim, S., Kim, Y. (2015). Heat-killed *Lactobacillus* spp. cells enhance survivals of *Caenorhabditis elegans* against *Salmonella* and *Yersinia* infections. *Letters in Applied Microbiology*. 61: 523–530.
- Levine, N. D. (1968). Nematode parasites of domestic animals and of man. Minneapolis: Burgess Publishing Company.
- Levitt-Barmats, Y & Shenkar, N. (2018). Observations on the symbiotic relationship between the caridean shrimp *Odontonia sibogae* (Bruce, 1972) and its ascidian host *Herdmania momus* (Savigny, 1816). *PLoS ONE*, 13(2): e0192045.
- Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R.R., Bircher, J. S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R. Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science*. 320: 1647-51.
- Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R., ... Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science*, 320(5883): 1647-51.

- Li, R. W., Connor, E. E., Li, C., Baldwin, V. I., Ransom, L., Sparks, M. E. (2012). Characterization of the rumen microbiota of pre-ruminant calves using metagenomic tools. *Environmental Microbiology*, 1: 129–139.
- Li, R. W., Li, W., Sun, J., Yu, P., Baldwin, R. L., & Urban, J. F. (2016). The effect of helminth infection on the microbial composition and structure of the caprine abomasal microbiome. *Scientific reports*, 6: 20606.
- Li, R. W., Wu, S., Li, W., Navarro, K., Couch, R. D., Hill, D., Urban, J. F. (2012). Alterations in the porcine colon microbiota induced by the gastrointestinal nematode *Trichuris suis*. *Infection and immunity*, 80(6): 2150-7.
- Lichtenfels, J. R., Pilit, P. A., Le jambre, L. F. (1986): Cuticular ridge patterns of *Haemonchus contortus* and *Haemonchus placei* (Nematoda: Trichostrongyloidea). *Proceedings of the Helminthological Society of Washington*, 53(1): 94 – 101.
- Lichtenfels, J. R., Pilit, P. A., Le jambre, L. F. (1988): Spicule lengths of the ruminant stomach nematodes *Haemonchus contortus*, *Haemonchus placei*, and their hybrids. *Proceedings of the Helminthological Society of Washington*, 55(1): 97 – 100.
- Lichtenfels, J. R., Pilit, P. A., Hoberg, E. P. (1994): New morphological characters for identifying individual specimens of *Haemonchus* spp. (Nematoda: Trichostrongyloidea) and a key to species in ruminants of North America. *Journal of Parasitology*, 80(1): 107 – 119.
- Lloyd, J. (2007). Stomach fluke (*paramphistomes*) in ruminants. dpi.nsw.gov.au. retrieved 1 June 2017.
- Lu, D., Macchietto, M., Chang, D., Barros, M. M., Baldwin, J., Mortazavi, A., Dillman, A. R. (2017). Activated entomopathogenic nematode infective juveniles release lethal venom proteins. *PLoS Pathogens*, 13(4): e1006302.
- Lu, H., Wang, X., Zhang, K., Xu, Y., Zhou, L., Li, G. (2014). Identification and nematicidal activity of bacteria isolated from cow dung. *Annals of Microbiology*, 64(1): 407–411.
- Mackie, R. I., Krecek, R. C., Els, H. J., van Niekerk, J. P., Kirschner, L. M., Baecker A. A. (1989). Characterization of the microbial community colonizing the anal and vulvar

pores of helminths from the hindgut of zebras. *Applied and Environmental Microbiology*, 55(5): 1178–1186.

Mackinnon, K. M., Zajac, A. M., Kooyman, F. N., Notter D. R. (2010). Differences in immune parameters are associated with resistance to *Haemonchus contortus* in Caribbean hair sheep. *Parasite immunology*, 32(7): 484-493.

Magwira, C. A., Kullin, B., Lewandowski, S., Rodgers, A., Reid, S. J., Abratt, V. R. (2012). Diversity of faecal oxalate-degrading bacteria in black and white South African study groups: Insights into understanding the rarity of urolithiasis in the black group, *Journal of Applied Microbiology*, 113(2): 418–428.

Maizels, R.M. (2005). Infections and allergy – helminths, hygiene and host immune regulation. *Current Opinion Immunology*, 17: 656–661.

Malmuthuge, N., Griebel, P. J., and Guan, L. L. (2015). The gut microbiome and its potential role in the development and function of newborn calf gastrointestinal tract. *Frontiers in Veterinary Science* 2: 36.

Maltby, R., Leatham-Jensen, M. P., Gibson, T., Cohen, P. S., Conway, T. (2013). Nutritional basis for colonization resistance by human commensal *Escherichia coli* strains HS and Nissle 1917 against *E. coli* O157:H7 in the mouse intestine. *PloS one*, 8(1): e53957.

Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L., Nalin, R., Jarrin, C., Chardon, P., Marteau, P., Roca, J., Dore, J. (2006). Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut*, 55(2): 205-11.

Mansfield, L. S., Gauthier, D. T., Abner, S. R., Jones, K. M., Wilder, S. R., Urban, J. F. (2003). Enhancement of disease and pathology by synergy of *Trichuris suis* and *Campylobacter jejuni* in the colon of immunologically naive swine. *The American journal of tropical medicine and hygiene*, 68(3): 70–80.

Mardis, E. (2008). Next generation DNA sequencing methods. *Annual Review of Genomics and Human Genetics*, 9: 387-402.

- Maree, C & Casey, N. H. (1993). Livestock Production Systems Principle and Practices. *AGRI Development Foundation*, 361–373.
- Martinez-Ibeas, A. M., Gonzalez-Warleta, M., Martinez-Valladares, M., Castro-Hermida, J. A., Gonzalez-Lanza, C., Minambres, B., Ferreras, C., Mezo, M., Manga-Gonzalez, M. Y. (2013). Development and validation of a mtDNA multiplex PCR for identification and discrimination of *Calicophoron daubneyi* and *Fasciola hepatica* in the *Galba truncatula* snail. *Veterinary Parasitology*, 195: 57–64.
- Martínez-Valladares, M., Vara-Del Río, M. P., Cruz-Rojo, M. A., Rojo-Vázquez, F. A. (2005). Genetic resistance to *Teladorsagia circumcincta*: IgA and parameters at slaughter in Churra sheep. *Parasite Immunology*, 27(6): 213–218.
- Mashruwala, D. (2013). "Ectoparasites and endoparasites". prezi.com. N.p., 2017. Web. 1 June 2017.
- Mavenyengwa, M., Mukaratirwa, S., Obwolo, M., Monrad, J. (2005). "A macro- and light microscopical study of the pathology of *Calicophoron microbothrium* infection in experimentally infected cattle". *The Onderstepoort Journal of Veterinary Research*. 72(4): 321–32.
- McMaster, J. C & Kotze, J. J. J. (1991). Dohne Merino Breed Society of South Africa, *Souvenir Brochure*, 25th Annual council report, 2 - 4.
- McMurdie, P. J & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. 8(4): e61217–e61217.
- McRae, K. M., Stear, M. J., Good, B., Keane, O. M. (2015). The host immune response to gastrointestinal nematode infection in sheep. *Parasite Immunology*, 37(12): 605–613.
- Meeusen, E. N. T., Balic, A. (2000). "Do eosinophils have a role in the killing of helminth parasites?" *Parasitology Today*, 16(3): 95–101.
- Meeusen, E. N. T., Balic, A., Bowles, V. (2005). Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. *Veterinary*

Immunology and Immunopathology, 108(1-2): 121–125.

- Mickael, R., Fabrice, G., Le Vern, Y., Kerboeuf, D. (2003). Modulation of multidrug resistance (MDR) system the nematode *Haemonchus contortus* by changing cholesterol content: effects on resistance to anthelmintics. *Journal of Antimicrobial Chemotherapy*. 52: 180-187.
- Midha, A., Schlosser, J., Hartmann, S. (2017). Reciprocal Interactions between nematodes and their microbial environments. *Frontiers in cellular and infection microbiology*, 7: 144.
- Mikheyev, A. S., Tin, M. M. Y. (2014). "A first look at the Oxford Nanopore MinION sequencer". *Molecular Ecology Resources*. 14(6): 1097–102.
- Molyneux, D. H., Bradley, M., Hoerauf, A., Kyelem, D., Taylor, M. J. (2003) Mass drug treatment for lymphatic filariasis and onchocerciasis. *Trends in Parasitology*, 19: 516–522.
- Morimoto, J., Simpson, S. J., Ponton, F. (2017). Direct and trans-generational effects of male and female gut microbiota in *Drosophila melanogaster*. *Biology letters*, 13(7): 20160966.
- Mosites, E., Sammons, M., Otiang, E., Eng, A., Noecker, C., Manor, O. (2017). Microbiome sharing between children, livestock and household surfaces in western Kenya. *PLoS ONE*, 12(2): e0171017.
- Moya, A., Gil R., Latorre, A. (2009). The evolutionary history of symbiotic associations among bacteria and their animal hosts: A model. *Clinical Microbiology and Infection*, 15: 11-13.
- Moya, A., Pereto, J., Gil, R., Latorre, A. (2008) Learning how to live together: genomic insights into prokaryote- animal symbioses. *Nature Reviews Genetics*, 9: 218–229.
- Muñoz-Guzmán, M. A., Cuéllar-Ordaz, J. A., Valdivia-Anda, A. G., Buendía-Jiménez, J. A., Alba-Hurtado, F. (2006). Correlation of parasitological and immunological parameters in sheep with high and low resistance to haemonchosis. *Canadian*

Journal of Animal Science, 86(3): 363–371.

- Murfin, K. E., Dillman, A. R., Foster, J. M., Bulgheresi, S., Slatko, B. E., Sternberg, P. W., Goodrich-Blair, H. (2012). Nematode-bacterium symbioses - cooperation and conflict Revealed in the “omics” age. *The Biological Bulletin*, 223(1): 85–102.
- Murthy, G. S. S & Rao, P. V. (2014). Prevalence of gastro intestinal parasites in ruminants and poultry in Telangana region of Andhra Pradesh. *Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology*, 38(2): 190–192.
- Naidoo, P., Olivier, J. J., Morris, J., Cloete S. W. P. (2016). Does selecting for ner wool result in higher incidence of creeping belly in the South African Dohne Merino sheep breed? *Elsenburg journal*, 13: 2.
- Nalin, D. R & McLaughlin, J. (1976). Colonization of *ascaris lumbricoides* by *V. cholerae*. *Journal of Parasitology*, 62: 839–841.
- Nelson, T. M., Rogers, T. L., Brown, M. V. (2013). The gut bacterial community of mammals from marine and terrestrial habitats. *PLoS ONE*, 8(12): e83655.
- Nicola, S., Boernigen, D., Tickle, T. L., Morgan, X. C., Garrett, W. S., Huttenhower, C. (2013). "Computational meta'omics for microbial community studies". *Molecular Systems Biology*, 9(666): 666.
- Nikoopour, E., Singh, B. (2014). "Reciprocity in microbiome and immune system interactions and its implications in disease and health". *Inflammation Allergy Drug Targets*, 13(2): 94–104.
- Nyrén, P. (2007). The history of pyrosequencing. *Methods molecular Biology*. 373:1-14.
- Oksanen, J., Kindt, R., Legendre, P. (2008). The vegan package. 10(01).
- Olsen, O. W. (1974). Animal parasites: their life cycles and ecology (3 ed.). *Dover Publications, Inc.*, New York/University Park Press, Baltimore, US. 273–274.
- Otranto, D & Traversa, D. (2003). Dicrocoeliosis of ruminants: a little known fluke disease. *Trends in Parasitology*. 19: 12–15.

- Oulas, A., Pavludi, C., Polymenakou, P., Pavlopoulos, G. A., Papanikolaou, N., Kotoulas, G., Iliopoulos, I. (2015). Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. *Bioinformatics and Biology Insights*, 9: 75–88.
- Pace, N. R. (2009). Mapping the tree of life: progress and prospects. *Microbiology and molecular biology reviews*, 73(4): 565-76.
- Paerewijck, O., Johnny Vlaminc, R. W., Li R. W., Urban, J., Zaiss, M. M., Harris, N. L., Taminiau, B., Geldhof, P. (2015). "The influence of an infection with *Ascaris Suum* on the intestinal microbiota of the host." In *IPVS Belgian Branch Studiedag*, Abstracts. *International Pig Veterinary Society (IPVS)*. Belgian Branch.
- Palma, L., Muñoz, D., Berry, C., Murillo, J., Caballero, P. (2014). *Bacillus thuringiensis* toxins: an overview of their biocidal activity. *Toxins*, 6(12): 3296–3325.
- Pavlopoulos, G. A., Oulas, A., Iacucci, E. (2013). Unraveling genomic variation from next generation sequencing data. *BioData Mining*, 6(1): 13.
- Peachey, L. E., Jenkins, T. P., Cantacessi, C. (2017). This Gut Ain't Big Enough for Both of Us. Or Is It? Helminth-Microbiota Interactions in Veterinary Species. *Trends in Parasitology*, 33(8):619-632
- Periasamy, K., Pichler, R., Poli, M., Cristel, S., Cetrá, B., Medus, D. (2014). Candidate gene approach for parasite resistance in sheep – variation in immune pathway genes and association with fecal egg count. *PLoS ONE*, 9: e88337.
- Perkel, J. (2011). "Making contact with sequencing's fourth generation". *Biotechniques*, 50(2): 93-5.
- Pernthaner, A., Cole, S. A., Morrison, L., Hein W. R. (2005). Increased expression of interleukin-5 (IL-5), IL-13, and tumor necrosis factor alpha genes in intestinal lymph cells of sheep selected for enhanced resistance to nematodes during infection with *Trichostrongylus colubriformis*. *Infection and Immunity*, 73(4): 2175–2183.
- Pernthaner, A., Shaw, R. J., McNeill, M. M., Morrison, L., Hein, W. R. (2005). Total and

nematode-specific IgE responses in intestinal lymph of genetically resistant and susceptible sheep during infection with *Trichostrongylus colubriformis*. *Veterinary Immunology and Immunopathology*, 104(1-2): 69–80.

Petrosino, J. F., Highlander, S., Luna, R. A., Gibbs, R. A., & Versalovic, J. (2009). Metagenomic pyrosequencing and microbial identification. *Clinical chemistry*, 55(5): 856-66.

Pevsner, J. (2009). *Bioinformatics and functional genomics* (2nd ed.). Hoboken, N.J: Wiley-Blackwell. ISBN 9780470085851.

Plummer, E., Twin, J., Bulach, D. M., Garland, S. M., Tabrizi, S. N. (2015). A comparison of three bioinformatics pipelines for the analysis of preterm gut microbiota using 16S rRNA gene sequencing data. *Journal of Proteomics and Bioinformatics*. 8: 283-291.

Prevaes, S. M., van Wamel, W. J., de Vogel, C. P., Veenhoven, R. H., van Gils, E. J., van Belkum, A., Sanders, E. A., Bogaert, D. (2012). Nasopharyngeal colonization elicits antibody responses to *staphylococcal* and *pneumococcal* proteins that are not associated with a reduced risk of subsequent carriage. *Infection and immunity*, 80(6): 2186-93.

Pringle, E. G & Moreau, C. S. (2017). Community analysis of microbial sharing and specialization in a costa rican ant–plant– hemipteran symbiosis. *Proceedings of the Royal Society of London Biological*, 284: 20162770.

Qamar, M. F., Maqbool, A., Khan, S. M., Ahmad, N., Muneer, M. A. (2009). Epidemiology of Haemonchosis in sheep and goats under differential managemental conditions. *Veterinary World*. 2: 413 – 417.

Quast, C., Priesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, 41: D590-6.

Quesada-Moraga, E., Garcia-Tovar, E., Vaverde-Garcia, P., Santiago-Alvareiz, C. (2004). Isolation, geographical diversity and insecticidal activity of *Bacillus thuringiensis* from soils in Spain. *Microbiology Research*, 159: 59-71.

- Rahman, W. A & Abd Hamid, S. (2007). Morphological characterization of *Haemonchus contortus* in goats (*Capra hircus*) and sheep (*Ovis aries*) in Penang, Malaysia. *Tropical Biomedecine*, 24(1): 23-7.
- Rangel-Ruíz LJ., Albores-Brahms ST., Gamboa-Aguilar J. (2003). Seasonal trends of *Paramphistomum cervi* in Tabasco, Mexico. *Veterinary Parasitology*, 116: 217–222.
- Rashid, A., Khattak, M. N. K., Khan, M. F., Ayaz, S., Rehman, A. U. (2016). Gastrointestinal helminthoses: prevalence and associated risk factors in small ruminants of district kohat, pakistan. *The Journal of Animal & Plant Sciences*, 26(4): 956-962.
- Rausch, S., Held, J., Fischer, A., Heimesaat, M. M., Kühl, A. A., Bereswill, S., & Hartmann, S. (2013). Small intestinal nematode infection of mice is associated with increased enterobacterial loads alongside the intestinal tract. *PloS one*, 8(9): e74026.
- Regassa, F., Teshale, S., Reta, D., Yosef, K. (2006). Epidemiology of gastrointestinal parasites of ruminants in Western Oromia, The International Journal of Applied Research in Veterinary Medicine, 4(1): 51–57.
- Reinhardt, S., Scott, I., Simpson, H. V. (2011). Neutrophil and eosinophil chemotactic factors in the excretory/secretory products of sheep abomasal nematode parasites—NCF and ECF in abomasal nematodes. *Parasitology Research*, 109(3): 627–635.
- Reynolds, L. A., Finlay, B. B., Maizels, R. M. (2015). Cohabitation in the Intestine: Interactions among Helminth Parasites, bacterial microbiota, and host immunity. *The Journal of Immunology*, 195(9): 4059–4066.
- Reynolds, L. A., Smith, K. A., Filbey, K. J., Hargus, Y., Hewitson, J. P., Redpath, S. A., Valdez, Y., Yebra, M. J., Finlay, B. B., Maizels, R. M. (2014). Commensal-pathogen interactions in the intestinal tract: *Lactobacilli* promote infection with, and are promoted by, helminth parasites. *Gut microbes*, 5(4): 522-32.
- Rodríguez, A. V., Goldberg, V., Viotti, H., Ciappesoni, G. (2015). Early detection of *Haemonchus contortus* infection in sheep using three different faecal occult blood tests. *Open Veterinary Journal*, 5(2): 90–97.

- Rodríguez, J. M., Murphy, K., Stanton, C., Ross, R. P., Kober, O. I., Juge, N., Avershina, E., Rudi, K., Narbad, A., Jenmalm, M. C., Marchesi, J. R., Collado, M. C. (2015). The composition of the gut microbiota throughout life, with an emphasis on early life. *Microbial ecology in health and disease*, 26: 26050.
- Rodríguez, J. M., Murphy, K., Stanton, C., Ross, R. P., Kober, O. I., Juge, N., Avershina, E., Rudi, K., Narbad, A., Jenmalm, M. C., Marchesi, J. R., Collado, M. C. (2015). The composition of the gut microbiota throughout life, with an emphasis on early life. *Microbial ecology in health and disease*, 26: 26050.
- Roeber, F., Jex, A. R., Gasser, R. B. (2013). Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance an Australian perspective. *Parasites & Vectors*, 6: 153.
- Roeber, F., Morrison, A., Casaert, S., Smith, L., Claerebout, E., Skuce, P. (2017). Multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep: an European validation study. *Parasites & Vectors*, 10: 226.
- Rojo-Vázquez, F. A., Meana, A., Valcárcel, F., Martínez-Valladares, M. (2012). Update on trematode infections in sheep. *Veterinary Parasitology*, 189(1): 15-38.
- Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. (2017). Gut microbiota functions: metabolism of nutrients and other food components. *European journal of nutrition*, 57(1): 1-24.
- Rusk, N. (2011). Torrents of sequence. *Nature Methods*, 8(1): 44-44.
- Rutayisire, E., Huang, K., Liu, Y., Tao, F. (2016). The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life. *a systematic review BMC Gastroenterol*, 16: 86.
- Ryss, A. Y., Kulinich, O. A., Turitsin, V. S. (2011). Mazurin. *Entmology E. S*, 91: 908.
- Saccareau, M., Sallé, G., Robert-Granié, C., Duchemin, T., Jacquet, P., Blanchard, A., Moreno, C. R. (2017). Meta-analysis of the parasitic phase traits of *Haemonchus contortus* infection in sheep. *Parasites & Vectors*, 10: 201.

- Sahoo, A & Khan, F.A. (2016). Nutritional and biological control synergism against gastrointestinal nematodes in small ruminants. *Journal of Veterinary Science and Animal Husbandry*, 4(1): 104.
- Salem, N., Salem, L., Saber, S., Ismail, G., Bluth, M. H. (2015). *Corynebacterium urealyticum*: a comprehensive review of an understated organism. *Infection and drug resistance*, 8: 129-45.
- Schoenian, S. (2010). "Guirra", Sheep Breeds G–I, *Sheep101.info*, retrieved, 2010-07-27.
- Schöttner, S., Hoffmann, F., Cárdenas, P., Rapp, H. T., Boetius, A., Ramette, A. (2013). Relationships between host phylogeny, host type and bacterial community diversity in cold-water coral reef sponges. *PLoS ONE*, 8(2): e55505.
- Scott A. I., Ghedin E., Nutman T. B., McCreynolds L. A., Poole C. B., Slatko B. E., Foster J. M. (2012). Filarial and *Wolbachia* genomics. *Parasite Immunology*, 34(2-3): 121–129.
- Shahkolahi, A. M & Donahue, M. J. (1993). Bacterial flora. a possible source of serotonin in intestines of adult female *Ascaris suum*. *The journal of parasitology*. 79: 17-22.
- Sinnathamby, G., Henderson, G., Umair, S., Janssen, P., Bland, R., Simpson, H. (2018). The bacterial community associated with the sheep gastrointestinal nematode parasite *Haemonchus contortus*. *PLoS ONE*, 13(2): e0192164.
- Slatko, B. E., Taylor, M. J., Foster, J. M. (2010). The *Wolbachia* endosymbiont as an anti-filarial nematode target. *Symbiosis (Philadelphia, Pa.)*, 51(1): 55–65.
- Slavova, P., Dimova, N., Ivanova, I., Peeva, Z. H., Laleva, S., Popova, Y., Vasilev, V. (2010). Relation between fertility and body condition score and wool production in sheep from Trakia Merino breed. 20 th Anniversary International Scientific Conference, 3-4 June, Stara Zagora, Bulgaria. Volume I, *Agricultural science, Animal studies & Veterinary medicine*, 68-75.
- Soulsby, E. J. L. (1965). Textbook of veterinary clinical parasitology volume 1. Helminths. *Blackwell Scientific Publications*, 1120.

- Stear, M. J., Doligalska, M., Donskow-Schmelter, K. (2007). Alternatives to anthelmintics for the control of nematodes in livestock. *Parasitology*. 134: 139-151.
- Stear, M. J., Fitton, L., Innocent, G. T., Murphy, L., Rennie, K., & Matthews, L. (2007). The dynamic influence of genetic variation on the susceptibility of sheep to gastrointestinal nematode infection. *Journal of the Royal Society, Interface*, 4(16): 767-76.
- Stubbendieck, R. M., Vargn-Bautista, C., Straight, P. D. (2016). Bacterial communities' interactions to scale. *Frontiers in Microbiology*, 7: 1234.
- Stuen, S., Van De Pol, I., Bergström, K., Schouls, L. M. (2002). Identification of *Anaplasma phagocytophila* (formerly *Ehrlichia phagocytophila*) variants in blood from sheep in Norway. *Journal of clinical microbiology*, 40(9): 3192-7.
- Su, X. L., Tian, Q., Zhang, j., Yuan, X. Z., Shi, X. S., Guo, R. B., Qiu, Y. L. (2014). *Acetobacteroides hydrogenigenes* gen. nov., sp. nov., an anaerobic hydrogen-producing bacterium in the family *Rikenellaceae* isolated from a reed swamp. *International Journal of Systematics and Evolutionary Microbiology*, 64: 2986-91.
- Sugawara, M., Tsukui, T., Kaneko, T., Ohtsubo, Y., Sato, S., Nagata, Y., Tsuda, M., Mitsui, H., Minamisawa, K. (2017). Complete genome sequence of *Bradyrhizobium diazoefficiens* USDA 122, a nitrogen-fixing soybean symbiont. *Genome announcements*, 5(9): e01743-16.
- Summers, L., Clingerman, K. J., Yang, X. (2012). Validation of a body condition scoring system in *Rhesus Macaques* (*Macaca mulatta*): assessment of body composition by using dual-energy X-ray Absorptiometry. *Journal of the American Association for Laboratory Animal Science : JAALAS*, 51(1): 88–93.
- Tan, T. K., Panchadcharam, C., Low, V. L., Lee, S. C., Ngui, R., Sharma, R. S., Lim, Y. A. (2014). Co-infection of *Haemonchus contortus* and *Trichostrongylus* spp. among livestock in Malaysia as revealed by amplification and sequencing of the internal transcribed spacer II DNA region. *BMC Veterinary Research*, 10: 38.

- Tanca, A., Fraumene, C., Manghina, V., Palomba, A., Abbondio, M., Deligios, M., Pagnozzi, D., Addis, M. F., Uzzau, S. (2017). Diversity and functions of the sheep faecal microbiota: a multi-omic characterization. *Microbial biotechnology*, 10(3): 541-554.
- Taylor, M. J., Bandi, C., Hoerauf, A. (2005). *Wolbachia* bacterial endosymbionts of filarial nematodes. *Advances in Parasitology*, 60: 245–284.
- Taylor, M. J., Voronin, D., Johnston, K. L., Ford, L. (2013). *Wolbachia* filarial interactions. *Cell Microbiology*. 15(4): 520-6.
- Team, R. D. C. (2008). R: a language and environment for statistical computing. Vienna, Austria: *R Foundation for Statistical Computing*. p. I.
- Thijs, S., Op De Beeck, M., Beckers, B., Tuyens, S., Stevens, V., Van Hamme, J. D., Weyens, N., Vangronsveld, J. (2017). Comparative evaluation of four bacteria-specific primer pairs for 16S rRNA gene surveys. *Frontiers in microbiology*, 8: 494.
- Thomas, T., Gilbert, J., Meyer, F. (2012). Metagenomics - a guide from sampling to data analysis. *Microbial Informatics and Experimentation*, 2: 3.
- Thompson J. M & Meyer, H. (1994). Body condition scoring of sheep. <http://extension.oregonstate.edu/catalog/pdf/ec/ec1433.pdf>.
- Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal*, 474(11): 1823–1836.
- Timmerman, H. M., Rutten, N. B. M. M., Boekhorst, J., Saulnier, D. M., Kortman, G. A. M., Contractor, N., Rijkers, G. T. (2017). Intestinal colonisation patterns in breastfed and formula-fed infants during the first 12 weeks of life reveal sequential microbiota signatures. *Scientific Reports*, 7: 8327.
- Titi, A., Mekroud, A., Chibat, M. H., Boucheikhchoukh, M., Zein-Eddine, R., Djuikwo-Teukeng, F. F., Dreyfuss, G. (2014). Ruminant *paramphistomosis* in cattle from northeastern Algeria: prevalence, parasite burdens and species identification. *Parasite*, 21: 50.
- Tshikhudo, P., Nnzeru, R., Ntushelo, K., Mudau, F. (2013). Bacterial species identification

getting easier. *African Journal of Biotechnology*, 12(41): 5975-5982.

Tsotetsi, A. M & Mbatia, P. A. (2003). Parasitic helminths of veterinary importance in cattle, sheep and goats on communal farms in the North Eastern Free State, South Africa. *Journal of the South African Veterinary Association*. 74(2): 45–48.

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., Gordon, J. I. (2007). The human microbiome project. *Nature*, 449: 804-810.

Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., Solovyev, V. V., Rubin, E. M., Rokhsar, D. S., Banfield, J. F. (2004). "Insights into community structure and metabolism by reconstruction of microbial genomes from the environment". *Nature*, 233: 455-464

Ubeda, C., Djukovic, A., Isaac, S. (2017). Roles of the intestinal microbiota in pathogen protection, clinical & translational immunology. *Nature Publishing Group*, 6(2): p. e128.

Ulahannan, D., Kovac, M. B., Mulholland, P. J., Cazier, J. B., Tomlinson, I. (2013). Technical and implementation issues in using next-generation sequencing of cancers in clinical practice. *British journal of cancer*, 109(4): 827-35.

Valentini, M., Piermattei, A., Di Sante, G., Migliara, G., Delogu, G., Ria, F. (2014). Immunomodulation by gut microbiota: role of toll-like receptor expressed by T cells. *Journal of immunology research*, 2014: 586939.

Van Teeseling, M. C. F., de Pedro, M. A., Cava, F. (2017). Determinants of bacterial morphology: from fundamentals to possibilities for antimicrobial targeting. *Frontiers in Microbiology*, 8: 1264.

Van Wyk, J. A & Bath, G. F. (2002). The FAMACHA© system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Veterinary Research*, 33: 509–529.

Van Wyk, J. A & Van Wyk, L. (2002). Freezing of sheep faeces invalidates *Haemonchus contortus* faecal egg counts by the McMaster technique. *Onderstepoort Journal of Veterinary Research*, 69(4): 299-304.

- Van Wyk, J., Swanepoel, J., Cloete, S., Olivier, J., Delpont, G. (2008). Across ock genetic parameter estimation for yearling body weight and eece traits in the South African Dohne Merino population. *South African Journal of Animal Science*, 38: 31-37.
- Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., Wu, D., Paulsen, I., Nelson, K. E., Nelson, W., Fouts, D. E., Levy, S., Knap, A. H., Lomas, M. W., Nealson, K., White, O., Peterson, J., Hoffman, J., Parsons, R., Baden-Tillson, H., Pfannkoch, C., Rogers, Y. H., Smith, H. O. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science*, 304: 66–74.
- Voelkerding, K. V., Dames, S., Durtschi, J. D. (2010). Next generation sequencing for clinical diagnostics-principles and application to targeted resequencing for hypertrophic cardiomyopathy: a paper from the 2009 William Beaumont Hospital Symposium on Molecular Pathology. *The Journal of Molecular Diagnostics: JMD*, 12(5): 539–551.
- Walk, S. T., Blum, A. M., Ewing, S. A. S., Weinstock, J. V., Young, V. B. (2010). Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus* inflammation. *Inflammatory Bowel Diseases*, 16: 1841–1849.
- Wang, J., Fan, H., Han, Y., Zhao, J., Zhou, Z. (2016). Characterization of the microbial communities along the gastrointestinal tract of sheep by 454 pyrosequencing analysis. *Asian-Australasian journal of animal sciences*, 30(1): 100-110.
- Wang, J., Fan, H., Han, Y., Zhao, J., Zhou, Z. (2017). Characterization of the microbial communities along the gastrointestinal tract of sheep by 454 pyrosequencing analysis. *Asian-Australasian Journal of Animal Sciences*, 30(1): 100–110.
- Wang, J., Zhong, Q., Yang, Y., Li, H., Wang, L., Tong, Y., Fang, X., Liao, Z. (2018). Comparison of bacterial diversity between two traditional starters and the round-koji-maker starter for traditional cantonese chi-flavor liquor brewing. *Frontiers in microbiology*, 9: 1053.

- Wang, W. L., Xu, S. Y., Ren, Z. G., Tao, L., Jiang, J. W., Zheng, S. S. (2015). Application of metagenomics in the human gut microbiome. *World Journal of Gastroenterology : WJG*, 21(3): 803–814.
- Waterfield, N., Wren, B. W., French-Constant, R. H. (2004): Opinion: Invertebrates as a source of emerging human pathogens. *Nature Reviews Microbiology*, 2: 833-84.
- White, E. C. (2016). Infection by the gastrointestinal parasite *Trichuris muris*: defining the microbiota of the parasite and the host. University of Manchester for the degree of Doctor of Philosophy in Microbiology in the Faculty of Biology, Medicine and Health.
- White, E. C., Houlden, A., Bancroft, A. J., Hayes, K. S., Goldrick, M., Grencis, R. K., Roberts, I. S. (2018). Manipulation of host and parasite microbiotas: survival strategies during chronic nematode infection. *Science Advances*, 4(3): eaap7399.
- Whittington, R. J., Lloyd, J. B., Reddacliff, L. A. (2001). Recovery of *Mycobacterium avium* subsp. Paratuberculosis from nematode larvae cultured from the faeces of sheep with Johne's disease. *Veterinary Microbiology*. 81: 273–279.
- Wittekindt, N. E., Padhi, A., Schuster, S. C., Qi, J., Zhao, F., Tomsho, L. P., Kasson, L. R., Packard, M., Cross, P., Poss, M. (2010). Nodeomics: pathogen detection in vertebrate lymph nodes using meta-transcriptomics. *PloS one*, 5(10): e13432.
- Wu, D., Wu, M., Halpern, A., Rusch, D. B., Yooseph, S., Frazier, M., Venter, J. C., Eisen, J. A. (2011). Stalking the fourth domain in metagenomic data: searching for, discovering, and interpreting novel, deep branches in marker gene phylogenetic trees. *PloS one*, 6(3): e18011.
- Wu, S., Li, R. W., Li, W., Beshah, E., Dawson, H. D., Urban, J. F Jr. (2012) Worm burden-dependent disruption of the porcine colon microbiota by *Trichuris suis* infection. *PLoS One*, 7(4): e35470.
- Wu, S., Wang, G., Angert, E. R., Wang, W., Li, W., Zou, H. (2012). Composition, diversity, and origin of the bacterial community in Grass Carp intestine. *PLoS ONE*, 7(2): e30440.

- Yang, J., Liu, Z., Niu, Q., Liu, J., Han, R., Guan, G., Li, Y., Liu, G., Luo, J., Yin, H. (2016). *Anaplasma phagocytophilum* in sheep and goats in central and Southeastern China. *Parasites & vectors*, 9(1): 593.
- Yang, S. H., Lim, J. S., Khan, M. A., Kim, B. S., Choi, D. Y., Lee, E. Y., Ahn, H. K. (2015). High-throughput nucleotide sequence analysis of diverse bacterial communities in leachates of decomposing pig carcasses. *Genetics and Molecular Biology*, 38(3): 373-380.
- Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., Hsiao, E. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, 161(2): 264–276.
- Yu, Z., Xiong J., Zhou, Q., Luo, H., Hu, S., Xia, L., Sun, M., Li, L., Yu, Z. (2015). The diverse nematicidal properties and biocontrol efficacy of *Bacillus thuringiensis* Cry6A against the root-knot nematode. *Meloidogyne hapla*. *Journal of Invertebrate Pathology*, 125: 73–80.
- Zaiss, M. M & Harris, N. L. (2016). Interactions between the intestinal microbiome and helminth parasites. *Parasite Immunology*, 38(1): 5–11.
- Zeng, Y., Zeng, D., Ni, X., Zhu, H., Jian, P., Zhou, Y., Xu, S., Lin, Y., Li, Y., Yin, Z., Pan, K., Jing, B. (2017). Microbial community compositions in the gastrointestinal tract of Chinese Mongolian sheep using Illumina MiSeq sequencing revealed high microbial diversity. *AMB Express*, 7(1): 75.
- Zeng, Y., Zeng, D., Zhang, Y., Ni, X., Tang, Y., Zhu, H., Wang, H., Yin, Z., Pan, K., Jing. B. (2015). Characterization of the cellulolytic bacteria communities along the gastrointestinal tract of Chinese Mongolian sheep by using PCR-DGGE and real-time PCR analysis. *Journal of Microbiology Biotechnology*, 31: 1103–13.
- Zeryehun, T. (2012). Helminthosis of sheep and goats in and around Haramaya, Southeastern Ethiopia. *Journal Veterinary Medecine. Animal Health*, 4(3): 48-55.

- Zhang, F., Berg, M., Dierking, K., Félix, M. A., Shapira, M., Samuel, B. S., Schulenburg, H. (2017). *Caenorhabditis elegans* as a model for microbiome research. *Frontiers in Microbiology*, 8: 485.
- Zhang, H., Shao, M., Huang, H., Wang, S., Ma, L., Wang, H., Hu, L., Wei, K., Zhu, R. (2018). The dynamic distribution of small-tail han sheep microbiota across different intestinal segments. *Frontiers in microbiology*, 9: 32.
- Zhoua, J., Hea, Z., Yangb, Y., Denga, Y., Tringee, S. G., Alvarez-Cohenc, L. (2015). High-throughput metagenomic technologies for complex microbial community analysis: *open and closed formats*. *mBio*, 6(1): e02288-14.
- Zoetendal, E. G., Collier, C. T., Koike, S., Mackie, R. I., Gaskins, H. R. (2004). Molecular Ecological Analysis of the Gastrointestinal Microbiota: A Review, *The Journal of Nutrition*, 134(2): 465–472.
- Zvinorovaa, P. I., Halimanic, T. E., Muchadeyid, F. C., Matikae, O., Riggioe, V., Dzamaaa, K. (2016). Prevalence and risk factors of gastrointestinal parasitic infections in goats in low-input low-output farming systems in Zimbabwe. *Small Ruminant Research*, 143: 75 – 83.

APPENDIX

APPENDIX I: Bacterial OTUs counts of the microbiota shared between abomasum, adult male and female *H. contortus*.

Aboma sum	Male	Female							
Hits	Hits	Hits	Kingdom	Phylum	Class	Order	Family	Genus	Species
98	71	50	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	--	--
487	40	42	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	F082	Bacteroidales bacterium Bact_22	Bacteroidales bacterium Bact_22
4583	39	11	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	U29-B03	uncultured rumen bacterium
926	117	12	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus 1	uncultured rumen bacterium
2368	175	29	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio 2	--
25395	56	42	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	p-1088-a5 gut group	uncultured rumen bacterium
23645	732	298	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	Candidatus Saccharimonas	uncultured rumen bacterium
47	9	8	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	hoa5-07d05 gut group	uncultured rumen bacterium
3717	201	24	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	--
2605	126	15	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R- 7 group	bacterium AC2043
1762	63	13	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Solobacterium	uncultured rumen bacterium

1840	261	13	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Syntrophococcus	uncultured rumen bacterium
1611	28	27	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales	BS11 uncultured rumen bacterium	uncultured rumen bacterium
559	469	149	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae YAB2003 group	uncultured rumen bacterium
2158	236	24	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Anaerovorax	uncultured rumen bacterium
35107	2184	802	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	--	--
2657	27	21	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	uncultured Spirochaetaceae bacterium
4262	48	19	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	uncultured bacterium
716	86	5	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Pseudoramibacter	uncultured bacterium
202	39	24	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae UCG-004	uncultured rumen bacterium
2607	324	36	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Family XIII AD3011 group	uncultured organism
64457	6868	1162	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	--	--
12034	1067	30	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae NK3A20 group	
469	95	17	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	Prevotella sp. BP1-56
594	55	16	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales	BS11 --	--
							gut group		

4017	149	66	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	uncultured	uncultured	rumen bacterium
3187	10	10	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	uncultured	uncultured	rumen bacterium
512	28	11	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Sphaerochaeta	uncultured	Spirochaeta sp.
347	54	46	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio 2	Butyrivibrio	fibrisolvens
1494	37	75	Bacteria	Patescibacteria	Gracilibacteria	Absconditabacteriales (SR1)	uncultured bacterium	uncultured bacterium	rumen	uncultured rumen bacterium
731	15	9	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	uncultured	--	--	--
9	1375	205	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	--	--
2056	322	55	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R-7 group	uncultured	prokaryote
2588	47	56	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Flexilinea	uncultured	rumen bacterium
5337	1072	58	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium stricto 1	sensu	Clostridium sp. ND2
503	243	18	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] ruminantium group	uncultured	rumen bacterium
4633	475	115	Bacteria	Firmicutes						
81	75	15	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	uncultured	Lachnospiraceae bacterium
892	10	13	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	Atopobium	--	--

12	830	1440	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	--
232	69	8	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacteriales	Campylobacteraceae	Campylobacter	--
2142	452	85	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas 1	
6290	675	45	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	--
1406	337	41	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-005	metagenome
47971	3231	510	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	uncultured Veillonellaceae bacterium
242	167	3	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium 12	uncultured rumen bacterium
24250	3758	360	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-011	uncultured rumen bacterium
1357	221	86	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas 1	Selenomonas ruminantium AB3002
4292	56	19	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae RC9 gut group	uncultured Rikenella sp.
10622	1517	26	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus] gauvreauii group	uncultured rumen bacterium
36	272	119	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	--
7056	224	36	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Eggerthellaceae	DNF00809	uncultured bacterium
5556	1216	117	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	Treponema bryantii

2287	439	55	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Kandleria	Kandleria vitulina
1201	72	20	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	uncultured rumen bacterium
16676	355	173	Bacteria	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	--	--	--
1711	134	24	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R-7 group	uncultured Clostridia bacterium
10365	419	297	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 9	uncultured rumen bacterium
49071	2686	858	Bacteria	Bacteroidetes	Bacteroidia	--	--	--	--
8622	432	51	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonellaceae UCG-001	uncultured bacterium
506	57	6	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-002	--
79	11	10	Bacteria	Tenericutes	Mollicutes	Mollicutes RF39	uncultured Erysipelotrichaceae bacterium	uncultured Erysipelotrichaceae bacterium	uncultured Erysipelotrichaceae bacterium
1517	393	54	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-005	--
3581	61	61	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales RF16 group	uncultured rumen bacterium	uncultured rumen bacterium
13	39	30	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacteriales	Caulobacteraceae	Brevundimonas	--
747	125	6	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Anaerofustis	uncultured bacterium

5593	37	28	Bacteria	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	unidentified bacterium RFP12	rumen unidentified bacterium RFP12	rumen unidentified bacterium RFP12	unidentified rumen bacterium RFP12
71	108	20	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	--	--
1078	29	31	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable genus 10	--	--
8	669	440	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Stenotrophomonas rhizophila	Stenotrophomonas rhizophila
1048	1043	52	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Mogibacterium	--	--
25365	1556	331	Bacteria	--	--	--	--	--	--	--
726	291	17	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae NK4A214 group	metagenome	metagenome
1790	3020	844	Bacteria	Cyanobacteria	Oxyphotobacteria	Chloroplast	Ammopiptanthus mongolicus	Ammopiptanthus mongolicus	Ammopiptanthus mongolicus	Ammopiptanthus mongolicus
2842	479	17	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] ruminantium group	--	--
528	30	19	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-014	uncultured Ruminococcaceae bacterium	uncultured Ruminococcaceae bacterium
543	32	32	Bacteria	Firmicutes	Clostridia	Clostridiales	Defluviitaleaceae	Defluviitaleaceae UCG-011	uncultured rumen bacterium	uncultured rumen bacterium
9064	344	132	Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	uncultured euryarchaeote	uncultured euryarchaeote
9581	1290	276	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	Clostridiales bacterium Firm_14	Clostridiales bacterium Firm_14
606	26	7	Bacteria	Actinobacteria	--	--	--	--	--	--
920	38	12	Bacteria	Tenericutes	Mollicutes	Mollicutes RF39	--	--	--	--

2234	306	17	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] group	hallii	uncultured rumen bacterium
26990	4357	642	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	--	--	--
12057	1303	336	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae NK4A214 group	--	--
937	35	34	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales UCG-001	uncultured bacterium	rumen	uncultured rumen bacterium
6223	198	16	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	uncultured	uncultured bacterium	rumen	uncultured rumen bacterium
141	36	32	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae UCG-006	--	uncultured rumen bacterium
16340	1355	223	Bacteria	Firmicutes	Clostridia	Clostridiales	--	--	--	--
222	159	61	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	--	Prevotella bryantii
31356	2462	464	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	--	--	--	--
12	44	32	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	--	--	--
1857	37	28	Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	--	uncultured rumen bacterium
14468	1261	214	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1	--	--
1074	212	47	Bacteria	Actinobacteria	Actinobacteria	--	--	--	--	--
1197	78	33	Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	--	Fibrobacter sp.
2826	148	41	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	uncultured	--	uncultured rumen bacterium
2360	86	4	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	--	uncultured rumen bacterium
3048	201	27	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	--	--

1054	152	14	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] cellulosolvens group	--
3812	360	66	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] ventriosum group	uncultured rumen bacterium
719	117	91	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-010	--
36	15	6	Bacteria	Proteobacteria	Gammaproteobacteria	--	--	--	--
1507	246	22	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	uncultured rumen bacterium
8112	477	114	Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	--
8317	898	57	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Family XIII AD3011 group	uncultured rumen bacterium
95	47	4	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1	rumen bacterium NK4B29
13	91	19	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea	--
9065	334	79	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium] coprostanoligenes group	--
4189	889	26	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Family XIII UCG-001	uncultured rumen bacterium
1663	396	61	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1	Ruminococcus albus
7854	1426	524	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	--	--
1111	50	10	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae UCG-009	uncultured rumen bacterium

7516	820	40	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	--	--
501	12	8	Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	--	--	--
12	330	115	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	--
3508	259	31	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae	uncultured
								UCG-004	rumen bacterium
1553	277	98	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	--
369	148	19	Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	uncultured
									rumen bacterium
390	650	209	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium- Neorhizobium- Pararhizobium- Rhizobium	--
6466	615	43	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	--	--
481	17	20	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae	UCG- 001
									bacterium P3
1081	104	57	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] oxidoreducens group	uncultured rumen bacterium
613	124	7	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] ruminantium group	uncultured Lachnospiraceae bacterium
3030	28	25	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Sediminispirochaeta	--
1009	1399	1528	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	--
16787	3037	236	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	--
536	215	16	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	
465	13	27	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	--
17	365	380	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Ochrobactrum	--
2821	7	56	Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured bacterium	--

18	2133	1718	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	--
9096	1718	610	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	Prevotella ruminicola
182	19	19	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	uncultured alpha proteobacterium
5343	706	111	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 2	--
2166	33	10	Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	--
1133	8	14	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales RF16 group	--	--
251	40	15	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Quinella	--
436	5	15	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured	--	--
1262	99	23	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrvibrio	--
3503	286	17	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] hallii group	--
4935	176	131	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-014	--
28729	389	198	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae RC9 gut group	--
4864	213	19	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	uncultured microorganism
267	949	1098	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	--	--
2977	569	49	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	uncultured rumen bacterium
2182	142	34	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	

1382	108	70	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae	--
								XPB1014 group	
2785	69	37	Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	uncultured rumen bacterium
9458	216	17	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	Candidatus Saccharimonas	--
306	378	226	Bacteria	Cyanobacteria	Oxyphotobacteria	Chloroplast	--	--	--
15149	1384	272	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	--
2200	18	47	Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Elusimicrobium	--
12914	923	48	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R- 7 group	
833	6	48	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrionaceae UCG-002	uncultured rumen bacterium
111	1693 6	100113	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella	unidentified
1278	3	26	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG- 001	--
5	3	16	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosia	--
890	2666 2	149550	Bacteria	Proteobacteria	Alphaproteobacteria	--	--	--	--
3203	37	52	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	--	--
6524	341	13	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	Olsenella	uncultured rumen bacterium
6	1601 7	19847	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	--

APPENDIX I: Total of 660 OTUs were detected in the abomasum, adult male and female *H. contortus*.

Hits	Kingdom	Phylum	Class	Order	Family	Genus	Species
32	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pirellula	uncultured Planctomycetales bacterium
138	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pantoea	
24	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	uncultured	uncultured rumen bacterium
16	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Nocardia	
219	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae		
177	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-014	gut metagenome
19	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	p-2534-18B5 gut group	uncultured bacterium	uncultured bacterium
41	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae		
729	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriales Incertae Sedis	Raoultibacter	uncultured bacterium
15	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae		
50	Bacteria	Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Mycoplasma	uncultured rumen bacterium
71	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	Finegoldia	Finegoldia magna
677	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes BD2-2	uncultured rumen bacterium	uncultured rumen bacterium
11	Bacteria	Acidobacteria	Acidobacteriia	Subgroup 2			
202	Bacteria	Proteobacteria	Deltaproteobacteria	Bradymonadales	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium
569	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	F082	Bacteroidales bacterium	Bacteroidales bacterium
						Bact_22	Bact_22
8	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerocolumna	
4633	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	U29-B03	uncultured rumen bacterium
55	Bacteria	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae		
5	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 7	

13	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae	Idiomarina	
101	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-010	uncultured rumen bacterium 4C0d-9
1055	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus 1	uncultured rumen bacterium
2572	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio 2	
18	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales vadinBB60 group	uncultured Firmicutes bacterium	uncultured Firmicutes bacterium
461	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	Treponema porcinum
10	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	uncultured Dysgonomonas sp.
25493	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	p-1088-a5 gut group	uncultured rumen bacterium
76	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	unidentified rumen bacterium 12-12
111	Bacteria	Firmicutes	Bacilli	Bacillales			
210	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae		
592	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	uncultured Lachnospiraceae bacterium
24675	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	Candidatus Saccharimonas	uncultured rumen bacterium
2933	Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	
31	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 3	Clostridiisalibacter	
9	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-009	uncultured Firmicutes bacterium
39	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Myroides	Myroides odoratimimus
469	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	
579	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae UCG-002	
118	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinifilaceae	uncultured	uncultured rumen bacterium
1082	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	p-251-o5	uncultured rumen bacterium	uncultured rumen bacterium

64	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	hoa5-07d05 gut group	uncultured rumen bacterium
15	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Staphylococcus aureus
3942	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	
290	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae NK4A214	uncultured organism group
11	Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae		
2746	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R-7	bacterium AC2043 group
33	Bacteria	Armatimonadetes	Armatimonadia	Armatimonadales			
132	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	p-251-o5	uncultured Bacteroidales bacterium	uncultured Bacteroidales bacterium
726	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-004	uncultured rumen bacterium 4C0d-8
646	Bacteria	Elusimicrobia	Endomicrobia	Endomicrobiales	Endomicrobiaceae	Candidatus Endomicrobium	uncultured rumen bacterium
1838	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Solobacterium	uncultured rumen bacterium
71	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	
2114	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Syntrophococcus	uncultured rumen bacterium
847	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	
1666	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales BS11 gut group	uncultured rumen bacterium	uncultured rumen bacterium
1509	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Cutibacterium	
165	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	bacterium XPB4001
3	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Dialister	unidentified
97	Bacteria	Actinobacteria	Coriobacteriia				
69275	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	
380	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	F082	bacterium P201	bacterium P201
11	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Altererythrobacter	

39	Bacteria	Cyanobacteria	Oxyphotobacteria	Chloroplast	Medicago truncatula (barrel medic)	Medicago truncatula (barrel medic)	Medicago truncatula (barrel medic)
1177	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae YAB2003 group	uncultured rumen bacterium
6	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Proteiniclasticum	uncultured Clostridium sp.
2418	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Anaerovorax	uncultured rumen bacterium
31	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sandaracinobacter	uncultured bacterium
11	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Paenarthrobacter	
274	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium] coprostanoligenes group	gut metagenome
6	Bacteria	Actinobacteria	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	uncultured organism
4	Bacteria	Proteobacteria	Alphaproteobacteria	Tistrellales	Geminicoccaceae	Geminicoccus	uncultured bacterium
38093	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae		
1679	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Acetitumaculum	uncultured rumen bacterium
373	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbus	uncultured rumen bacterium
2705	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	uncultured Spirochaetaceae bacterium
3	Bacteria	Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae	Neochlamydia	metagenome
4329	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	uncultured bacterium
807	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Pseudoramibacter	uncultured bacterium
57	Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	
265	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae UCG-004	uncultured rumen bacterium
26	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae		
1129	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	
9	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] oxidoreducens group	Eubacterium oxidoreducens
2967	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Family XIII AD3011 group	uncultured organism

792	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium] coprostanoligenes group	uncultured Clostridia bacterium
72487	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
11	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylogellaceae	uncultured	
4	Bacteria	Tenericutes	Mollicutes	Izimaplasmatales	Izimaplasmataceae	unidentified rumen bacterium	unidentified rumen bacterium
						RF3	RF3
8	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Patulibacter	uncultured bacterium
92	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	Peptoniphilus	uncultured organism
11	Eukaryota	Opisthokonta	Holozoa	Metazoa (Animalia)	D_7Chromadorea	D_8Rhabditida	D_9Haemonchus contortus (barber pole worm)
949	Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	Z20	uncultured rumen bacterium
162	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	
407	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-002	gut metagenome
20	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae		
13131	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae NK3A20 group	
38	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	
581	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	Prevotella sp. BP1-56
73	Bacteria	Planctomycetes	Phycisphaerae	Tepidisphaerales	WD2101 soil group		
665	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales BS11 gut group		
19	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Salinimicrobium	uncultured Salegentibacter sp.
634	Bacteria	Proteobacteria	Deltaproteobacteria	Oligoflexales	0319-6G20	uncultured rumen bacterium	uncultured rumen bacterium
16	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	uncultured		
16	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	Sphingobacterium sp.
22	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae RC9 gut group	uncultured Bacteroidales bacterium

72	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Sharpea	uncultured bacterium
147	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Acetitomaculum	
4232	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Marinilabiliaceae	uncultured	uncultured rumen bacterium
1218	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-013	uncultured rumen bacterium
8	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Alloiococcus	uncultured bacterium
3207	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	uncultured	uncultured rumen bacterium
2300	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured	uncultured Clostridium sp.
10	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	Akkermansia	uncultured Verrucomicrobia bacterium
710	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Mailhella	
551	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Sphaerochaeta	uncultured Spirochaeta sp.
447	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio 2	Butyrivibrio fibrisolvens
6	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Hypnocyclicus	uncultured bacterium
1995	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae		
23	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	Sphingobacterium spiritivorum
2023	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium] coprostanoligenes group	uncultured Ruminococcaceae bacterium
1606	Bacteria	Patescibacteria	Gracilibacteria	Absconditabacteriales (SR1)	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium
1013	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pirellula	uncultured rumen bacterium
9	Bacteria	Tenericutes	Mollicutes	Izimaplasmatales			
86	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae		
5	Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	uncultured bacterium
7	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Adhaeribacter	uncultured Bacteroidetes bacterium
54	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Papillibacter	
755	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	uncultured		

30	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina	
2895	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Family XIII AD3011 group	
26	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-007	uncultured rumen bacterium
2127	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae ND3007 group	uncultured Lachnospiraceae bacterium
42	Bacteria	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Pseudonocardia	
12	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Dermabacteraceae	Brachybacterium	Brachybacterium paraconglomeratum
45	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae		
1589	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	
9	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	Anaerococcus	
2433	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R-7 group	uncultured prokaryote
2691	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Flexilinea	uncultured rumen bacterium
7	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Dietziaceae	Dietzia	Dietzia maris
79	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Mesonina	
1990	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Papillibacter	uncultured rumen bacterium
45	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured	uncultured rumen bacterium 4C0d-17
6467	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 1	Clostridium sp. ND2
764	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] ruminantium group	uncultured rumen bacterium
157	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae ND3007 group	
135	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Pseudochrobactrum	Pseudochrobactrum saccharolyticum
5223	Bacteria	Firmicutes					

171	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	uncultured Lachnospiraceae bacterium
76	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardiodaceae	Nocardioides	
38	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	
915	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	Atopobium	
140	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium 1	uncultured bacterium
58	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae RC9 gut group	unidentified rumen bacterium RF36
2282	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	
309	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	
317	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae FE2018 group	
33	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriales Incertae Sedis	uncultured	
88	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	CAG-352	uncultured rumen bacterium
15	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1	uncultured organism
2679	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas 1	
7010	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	
22	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	uncultured	uncultured rumen bacterium
1784	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-005	metagenome
16	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Rothia	
121	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriales Incertae Sedis	Phoenicibacter	uncultured bacterium
51712	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	uncultured Veillonellaceae bacterium
412	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium 12	uncultured rumen bacterium
44	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	uncultured	uncultured bacterium

143	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae	Aliidiomarina	Aliidiomarina sp.
112	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	
61	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XI	Anaerococcus	uncultured Anaerococcus sp.
318	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium 1	
105	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales BS11 gut group	uncultured bacterium	uncultured bacterium
4	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae		
8	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Catonella	
49	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	uncultured	uncultured Coriobacteriaceae bacterium	uncultured Coriobacteriaceae bacterium
12	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Eggerthellaceae	Slackia	unidentified
28	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Lautropia	metagenome
28368	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-011	uncultured rumen bacterium
14	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Streptococcus parauberis
459	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae		
4	Bacteria	Actinobacteria	MB-A2-108	metagenome	metagenome	metagenome	metagenome
40	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	Chlamydia trachomatis
24	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	uncultured	uncultured Actinomycetales bacterium	uncultured Actinomycetales bacterium
29	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacteriales	Arcobacteraceae	Arcobacter	
9	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Turicella	
8	Archaea	Euryarchaeota	Thermoplasmata	Methanomassiliicoccales	Methanomethylophilaceae	Candidatus Methanomethylophilus	
24	Bacteria	Firmicutes	Bacilli	Bacillales	Family X	Thermicanus	
1962	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	uncultured rumen bacterium 5C0d-4

89	Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	Z20	
1664	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas 1	Selenomonas ruminantium AB3002
23	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	Fusobacterium necrophorum subsp. necrophorum
12	Bacteria	Firmicutes	Bacilli	Lactobacillales	uncultured	uncultured bacterium	uncultured bacterium
4367	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae RC9 gut group	uncultured Rikenella sp.
223	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium 9	uncultured Clostridia bacterium
163	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Eggerthellaceae	Parvibacter	uncultured bacterium
139	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae ND3007 group	unidentified rumen bacterium RC20
58	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	CAG-352	uncultured bacterium
27	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Rheinheimera	
12165	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus] gauvreauii group	uncultured rumen bacterium
427	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	
4	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Cloacibacterium	
303	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae		
1002	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	F082		
21	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	
1414	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium	
30	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable genus 10	
6003	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Alishewanella	uncultured bacterium
208	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	[Eubacterium] nodatum group	
353	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae UCG-009	

353	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio 2	uncultured Lachnospiraceae bacterium
26	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Kocuria	
7	Bacteria	Chloroflexi	KD4-96	uncultured Chloroflexi bacterium	uncultured Chloroflexi bacterium	uncultured Chloroflexi bacterium	uncultured Chloroflexi bacterium
43	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	Oscillospira guilliermondii
305	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	uncultured rumen bacterium
7316	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Eggerthellaceae	DNF00809	uncultured bacterium
13	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Sediminispirochaeta	Spirochaeta sp. canine oral taxon 314
45	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae UCG-009	
18	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	uncultured	
6889	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	Treponema bryantii
2781	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Kandleria	Kandleria vitulina
1293	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	SP3-e08	uncultured rumen bacterium
300	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriales Incertae Sedis	uncultured	uncultured rumen bacterium
23	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	uncultured	uncultured Clostridium sp.
17204	Bacteria	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41			
17	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae		
29	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 1	
1869	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R-7 group	uncultured Clostridia bacterium
9	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Caproiciproducens	
92	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-012	uncultured rumen bacterium
4	Bacteria	Patescibacteria	Gracilibacteria	JGI 0000069-P22			

101	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae ND3007 group	bacterium V9D2002
25	Bacteria	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	
114	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	M2PB4-65 termite group	uncultured Bacteroidetes bacterium	uncultured Bacteroidetes bacterium
13	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	Treponema sp. S
55	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
11081	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 9	uncultured rumen bacterium
281	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-003	unidentified rumen bacterium RFN64
111	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acetobacter	
3686	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-003	uncultured Bacteroidales bacterium
179	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Undibacterium	
215	Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	uncultured	uncultured rumen bacterium
17	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Elizabethkingia	Elizabethkingia meningoseptica
35	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	FD2005	uncultured bacterium
658	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae		
152	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Aeriscardovia	uncultured bacterium
48	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodopirillaceae	Defluviicoccus	
232	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-001	uncultured bacterium
215	Bacteria	Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Azospirillum	
52615	Bacteria	Bacteroidetes	Bacteroidia				
57	Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium
30	Bacteria	Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	uncultured	
158	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-014	unidentified

9105	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonellaceae UCG-001	uncultured bacterium
282	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured	uncultured rumen bacterium	uncultured rumen bacterium
3	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	Prevotella brevis
569	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-002	
101	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-013	
17	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	Lentimicrobium	
100	Bacteria	Tenericutes	Mollicutes	Mollicutes RF39	uncultured	uncultured	uncultured Erysipelotrichaceae
					Erysipelotrichaceae	Erysipelotrichaceae	bacterium
					bacterium	bacterium	
1964	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-005	
3703	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales RF16 group	uncultured rumen bacterium	uncultured rumen bacterium
9	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Roseimaritima	uncultured bacterium
31	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	M2PT2-76 termite group	uncultured rumen bacterium
5	Archaea	Euryarchaeota	Thermoplasmata	Methanomassiliicoccales	Methanomethylophilaceae	Candidatus Methanomethylophilus	uncultured archaeon
310	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium 5	uncultured rumen bacterium
82	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacteriales	Caulobacteraceae	Brevundimonas	
4	Bacteria	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Mannheimia	Mannheimia haemolytica
468	Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	gut metagenome	gut metagenome	gut metagenome
878	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Anaerofustis	uncultured bacterium
418	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Catenisphaera	uncultured rumen bacterium
39	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales vadinBB60 group	uncultured rumen bacterium	uncultured rumen bacterium
					group	4C28d-15	4C28d-15
14	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae UCG-004	uncultured Erysipelotrichaceae bacterium
122	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	

5658	Bacteria	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	unidentified rumen bacterium RFP12	unidentified rumen bacterium RFP12	unidentified rumen bacterium RFP12
497	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Lawsonella	
11	Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	uncultured methanogenic archaeon
44	Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	Fibrobacter succinogenes subsp. succinogenes
199	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	
28	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales RF16 group	uncultured Porphyromonadaceae bacterium	uncultured Porphyromonadaceae bacterium
1138	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	probable genus 10	uncultured bacterium
1117	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Stenotrophomonas rhizophila
39	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Pelagibacterium	uncultured alpha proteobacterium
897	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae AC2044 group	
1503	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae UCG-002	uncultured bacterium
11	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	uncultured	
2143	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Mogibacterium	
8	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Aliicoccus	uncultured bacterium
568	Bacteria	Epsilonbacteraeota	Campylobacteria	Campylobacteriales	Campylobacteraceae	Campylobacter	uncultured rumen bacterium
25	Bacteria	Firmicutes	Clostridia				
72	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	uncultured Spirochaeta sp.
253	Bacteria	Tenericutes	Mollicutes	Mollicutes RF39	gut metagenome	gut metagenome	gut metagenome
27252	Bacteria						

1034	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae NK4A214	metagenome group
80	Bacteria	Patescibacteria	Gracilibacteria	Absconditabacteriales (SR1)			
186	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium	uncultured rumen bacterium
5654	Bacteria	Cyanobacteria	Oxyphotobacteria	Chloroplast	Ammopiptanthus mongolicus	Ammopiptanthus mongolicus	Ammopiptanthus mongolicus
13	Bacteria	Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	Fimbriiglobus	uncultured Gemmata sp.
26	Bacteria	Cyanobacteria	Oxyphotobacteria	Oxyphotobacteria Incertae Sedis	Unknown Family	uncultured	uncultured cyanobacterium
8	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Methylotenera	
3338	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] ruminantium	group
29	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	UBA1819	
577	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-014	uncultured Ruminococcaceae bacterium
607	Bacteria	Firmicutes	Clostridia	Clostridiales	Defluviitaleaceae	Defluviitaleaceae UCG-011	uncultured rumen bacterium
9540	Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	uncultured euryarchaeote
11147	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	Clostridiales bacterium Firm_14
58	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Veillonella	
156	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1	uncultured Ruminococcus sp.
7	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Hymenobacter	
26	Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacteriales	Ktedonobacteraceae		
193	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriales Incertae Sedis	Raoultibacter	
9	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzereella 3	

362	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae ND3007	uncultured rumen bacterium
10	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14		
132	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae UCG-002	uncultured rumen bacterium
639	Bacteria	Actinobacteria					
29	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium 5	
184	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae NK3A20	bacterium ND2018
						group	
9	Bacteria	Tenericutes	Mollicutes	Mollicutes RF39	unidentified rumen bacterium RF39	unidentified rumen bacterium RF39	unidentified rumen bacterium RF39
13	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ethanoligenens	uncultured bacterium
757	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	uncultured	uncultured rumen bacterium
				s			
43	Bacteria	Tenericutes	Mollicutes	Mollicutes RF39	Acholeplasmatales	Acholeplasmatales bacterium	Acholeplasmatales bacterium
					bacterium canine oral taxon 316	canine oral taxon 316	canine oral taxon 316
					316		
4	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Haoranjiana	
33	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	Treponema sp. AC3
23	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium 5	
88	Bacteria	Thermotogae	Thermotogae	Petrotogales	Petrotogaceae	Oceanotoga	
910	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales			
970	Bacteria	Tenericutes	Mollicutes	Mollicutes RF39			
3	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1		
36	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella	
529	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae UCG-010	uncultured rumen bacterium
22	Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	uncultured bacterium
1240	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Paraprevotella	uncultured rumen bacterium

175	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae NK3A20 group	uncultured Clostridium sp.
742	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Salinivibrio	
52	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Romboutsia	
10	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Aeromicrobium	
2	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
55	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	unidentified rumen bacterium RFN19
178	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Desulfuromonadaceae		
43	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinimonas	uncultured rumen bacterium
2557	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] hallii group	uncultured rumen bacterium
31989	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae		
198	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria	uncultured rumen bacterium	uncultured rumen bacterium
442	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae UCG-008	uncultured Lachnospiraceae bacterium
13696	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae NK4A214 group	
408	Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	Victivallaceae	uncultured rumen bacterium	uncultured rumen bacterium
1006	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales UCG-001	uncultured rumen bacterium	uncultured rumen bacterium
42	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-001	
6437	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	uncultured	uncultured rumen bacterium	uncultured rumen bacterium
10	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae NK4A214 group	unidentified rumen bacterium RF26
259	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	uncultured bacterium	uncultured bacterium
5359	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	
38	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus 1	
209	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae UCG-006	uncultured rumen bacterium

17918	Bacteria	Firmicutes	Clostridia	Clostridiales			
3010	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae NK3B31 group	uncultured rumen bacterium
144	Bacteria	WPS-2	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium
65	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45		
6	Bacteria	Chlamydiae	Chlamydiae	Chlamydiales	Parachlamydiaceae		
442	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	Prevotella bryantii
34282	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales			
88	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae		
31	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Sediminispirochaeta	
41	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Kocuria	Kocuria palustris
44	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	uncultured Porphyromonas sp.
1922	Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	uncultured rumen bacterium
8	Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales	uncultured cyanobacterium	uncultured cyanobacterium	uncultured cyanobacterium
14	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Eubacterium uniforme	Eubacterium uniforme
48	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales RF16 group	unidentified rumen bacterium RFN46	unidentified rumen bacterium RFN46
12	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 1	Clostridium beijerinckii
561	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R-7 group	gut metagenome
627	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-003	unidentified rumen bacterium 12-129
133	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Asteroleplasma	uncultured Anaeroplasmataceae bacterium
15943	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1	
18	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pirellula	

4	Bacteria	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured rumen bacterium	uncultured rumen bacterium
96	Bacteria	Proteobacteria	Alphaproteobacteria	Paracaedibacterales	Paracaedibacteraceae	uncultured	uncultured rumen bacterium
82	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	uncultured	bacterium AC2042
646	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	uncultured	uncultured bacterium	uncultured bacterium
7	Bacteria	Cyanobacteria	Oxyphotobacteria	Nostocales	Chroococcidiopsaceae	Aliterella CENA595	uncultured cyanobacterium
51	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae		
525	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Alloprevotella	uncultured rumen bacterium
23	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales			
22	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	Treponema sp. oral taxon 271
279	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Comamonas	uncultured rumen bacterium
454	Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Fretibacterium	uncultured bacterium
1333	Bacteria	Actinobacteria	Actinobacteria				
51	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Massilia	
6	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	Iamiaceae	Iamia	
1308	Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	Fibrobacter sp.
113	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Family XIII AD3011 group	uncultured rumen bacterium 4C28d-20
90	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Anaerovorax	
29	Archaea	Euryarchaeota	Thermoplasmata	Methanomassiliicoccales	Methanomethylophilaceae	uncultured	uncultured Methanobacteriales archaeon
10	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pirellula	uncultured Pasteuria sp.
105	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	
68	Bacteria	Spirochaetes	MVP-15	Firmicutes bacterium canine oral taxon 309	Firmicutes bacterium canine oral taxon 309	Firmicutes bacterium canine oral taxon 309	Firmicutes bacterium canine oral taxon 309

54	Bacteria	Cyanobacteria	Oxyphotobacteria	Chloroplast	Chlorophyta symbiont of Lubomirskia sp.	Chlorophyta symbiont of Lubomirskia sp.	Chlorophyta symbiont of Lubomirskia sp.
24	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae		
11826	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	
69	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales vadinBB60 group	uncultured rumen bacterium	uncultured rumen bacterium
189	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae NK3A20 group	bacterium AC3007
1290	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Acetitomaculum	uncultured rumen bacterium 6C3d-13
723	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Marvinbryantia	uncultured rumen bacterium
404	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Desulfobulbus	
41	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae NK3B31 group	rumen bacterium NK3B31
51	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	unidentified rumen bacterium 12-130
7	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	uncultured		
286	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae		
4	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-005	uncultured prokaryote
3015	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	uncultured	uncultured rumen bacterium
2292	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	Lelliottia amnigena
2450	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	uncultured rumen bacterium
635	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] hallii group	unidentified rumen bacterium RF24
3276	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	
524	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	[Anaerorhabdus] furcosa group	uncultured rumen bacterium

67	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae		
9	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leucobacter	
236	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae RC9 gut group	unidentified rumen bacterium
						RF14	
898	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	[Eubacterium] nodatum group	Eubacterium sp. AB3007
1220	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] cellulosolvens	
						group	
13	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	Brevundimonas olei
4	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aminobacter	uncultured bacterium
79	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Achromobacter	
				s			
4238	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] ventriosum	uncultured rumen bacterium
						group	
15	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	unidentified
451	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Schwartzia	
927	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-010	
57	Bacteria	Proteobacteria	Gammaproteobacteria				
2534	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	Atopobium	uncultured rumen bacterium
834	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Ruminobacter	uncultured rumen bacterium
50	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-001	uncultured rumen bacterium
25	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	uncultured soil bacterium	uncultured soil bacterium
1775	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	uncultured rumen bacterium
11	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Intestinimonas	uncultured Firmicutes
							bacterium
8703	Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	
8	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae		

328	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] xylanophilum group	uncultured rumen bacterium
42	Bacteria	Spirochaetes	MVP-15	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium
25	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	
9272	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Family XIII AD3011 group	uncultured rumen bacterium
146	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1	rumen bacterium NK4B29
123	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea	
761	Bacteria	Firmicutes	Bacilli	Lactobacillales			
13	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Eggerthellaceae	Denitrobacterium	Denitrobacterium detoxificans
17	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Mesorhizobium	
10	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	uncultured	
481	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 2	uncultured organism
9478	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	[Eubacterium] coprostanoligenes group	
755	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1	Ruminococcus flavefaciens
65	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	
13	Bacteria	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae		
184	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium 9	uncultured rumen bacterium
481	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Quinella	uncultured rumen bacterium
4	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	uncultured Candidatus Saccharibacteria bacterium	uncultured Candidatus Saccharibacteria bacterium
5	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae		
760	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured	gut metagenome	gut metagenome
114	Bacteria	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium
5104	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Family XIII UCG-001	uncultured rumen bacterium

32	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae FCS020 group	
11	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella	
2120	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1	Ruminococcus albus
9804	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae		
131	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	
11	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae	Aliidiomarina	
70	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Streptococcus salivarius subsp. salivarius
544	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	
4	Bacteria	Firmicutes	Bacilli				
43	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Herbaspirillum	
80	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae		
18	Bacteria	Proteobacteria	Gammaproteobacteria	Diplorickettsiales	Diplorickettsiaceae	Aquicella	
1171	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae UCG-009	uncultured rumen bacterium
15	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	unidentified rumen bacterium RFN93
8376	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		
419	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae NK3A20 group	bacterium WCD3001
521	Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales			
457	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	
182	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Micrococcus	
3798	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-004	uncultured rumen bacterium
1928	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
341	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pectobacterium	

358	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Sutterella	gut metagenome
536	Bacteria	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	uncultured rumen bacterium
1249	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	
7124	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII		
13	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aureimonas	
558	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae RC9 gut group	gut metagenome
813	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Howardella	uncultured rumen bacterium
19	Bacteria	Patescibacteria	Gracilibacteria	JGI 0000069-P22	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium
181	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae FCS020 group	uncultured rumen bacterium
10	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	uncultured rumen bacterium
76	Archaea	Euryarchaeota	Thermoplasmata	Methanomassiliicoccales	Methanomethylophilaceae	Candidatus Methanomethylophilus	uncultured methanogenic archaeon
14	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Schwartzia	uncultured bacterium
3992	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	Candidatus Saccharimonas	uncultured Candidatus Saccharibacteria bacterium
12	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto 13	
240	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae		
11	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	Moraxella catarrhalis
518	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-001	bacterium P3
190	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae		
1242	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] oxidoreducens group	uncultured rumen bacterium
129	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae		
651	Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	horsej-a03	

59	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas	
744	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] ruminantium group	uncultured Lachnospiraceae bacterium
67	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzereella 3	uncultured rumen bacterium
103	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinimonas	
7	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Cupriavidus	
125	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	[Eubacterium] nodatum group	uncultured rumen bacterium
3083	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Sediminispirochaeta	uncultured rumen bacterium
282	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Quinella	uncultured bacterium
119	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Anoxybacillus	
3936	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
104	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Paeniclostridium	
6	Bacteria	Patescibacteria	ABY1	Candidatus	uncultured Parcubacteria	uncultured Parcubacteria group bacterium	uncultured Parcubacteria group bacterium
20060	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	
767	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	
6	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Agathobacter	uncultured rumen bacterium
23	Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacteriales	Ktedonobacteraceae	HSB OF53-F07	
22	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus 2	uncultured rumen bacterium
84	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Elizabethkingia	
505	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	
942	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium 1	uncultured rumen bacterium
633	Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema 2	bacterium WCE3006
762	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Ochrobactrum	
58	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-010	uncultured organism
825	Bacteria	Proteobacteria					

30	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	uncultured	
28	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Methylophilus	uncultured Methylophilus sp.
735	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-004	uncultured rumen bacterium
19	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	uncultured gamma proteobacterium	uncultured gamma proteobacterium	uncultured gamma proteobacterium
2884	Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured bacterium	uncultured bacterium
15	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	uncultured	uncultured Planctomyces sp.	uncultured Planctomyces sp.
1508	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Eggerthellaceae		
28	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae		
16	Bacteria	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	
3869	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	
20	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Turcibacter	uncultured organism
8	Bacteria	Patescibacteria	WS6 (Dojkabacteria)	uncultured anaerobic bacterium	uncultured anaerobic bacterium	uncultured anaerobic bacterium	uncultured anaerobic bacterium
1785	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Acinetobacter indicus
11424	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	Prevotella ruminicola
220	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	uncultured alpha proteobacterium
10	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae V9D2013 group	
19	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Glutamicibacter	
6160	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 2	
2209	Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	
59	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	Geodermatophilus	
79	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	uncultured	uncultured rumen bacterium

126	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	uncultured Firmicutes bacterium
1155	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales RF16 group		
19	Bacteria	Planctomycetes	Planctomycetacia	Isosphaerales	Isosphaeraceae	Singulisphaera	uncultured Planctomycetaceae bacterium
60	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae NK3A20 group	uncultured Lachnospiraceae bacterium
13	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	uncultured Leptotrichia sp.
306	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Quinella	
15	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae NK3A20 group	uncultured Firmicutes bacterium
898	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Serratia	Serratia nematodiphila
456	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	uncultured		
102	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	
1384	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrvibrio	
51	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Tepidimonas	
77	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Roseomonas	
44	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Sporobacter	
36	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Anaplasmataceae	Anaplasma	Anaplasma marginale
224	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae UCG-008	uncultured rumen bacterium
577	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Muribaculaceae		
198	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Mitochondria		
569	Bacteria	Lentisphaerae	Lentisphaeria	Victivallales	vadinBE97	uncultured rumen bacterium	uncultured rumen bacterium
3806	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] hallii group	
19	Bacteria	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae		

159	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae V9D2013 group	uncultured rumen bacterium
48	Bacteria	Tenericutes	Mollicutes	NED5E9	uncultured rumen bacterium	uncultured rumen bacterium	uncultured rumen bacterium
43	Bacteria	Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Cardiobacteriaceae	Suttonella	
7	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 2	unidentified
38	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae NK4A136 group	bacterium XPB1013
7	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae NK3A20 group	Lachnospiraceae bacterium KH1T2
5934	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	CPla-4 termite group	uncultured rumen bacterium
8	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Tannerellaceae	Parabacteroides	
5242	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-014	
556	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	unidentified rumen bacterium RC17
32	Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae		
50	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	hoa5-07d05 gut group	uncultured Bacteroidales bacterium
29316	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae RC9 gut group	
5096	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	uncultured microorganism
6	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	Mogibacterium	uncultured rumen bacterium 3C0d-4
68	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Gimesiaceae	uncultured	uncultured Planctomyces sp.
3	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Dysgonomonas	
16	Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae		
1538	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae Ga6A1 group	uncultured rumen bacterium
37	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	unidentified rumen bacterium RFN92

2314	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae		
19	Bacteria	Tenericutes	Mollicutes	Mollicutes RF39	metagenome	metagenome	metagenome
138	Archaea	Euryarchaeota	Thermoplasmata	Methanomassiliicoccales	Methanomethylophilaceae	uncultured	
3595	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	uncultured rumen bacterium
278	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	bacterium YAD2002
2358	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	uncultured rumen bacterium
1560	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae XPB1014 group	
2891	Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	uncultured rumen bacterium
5	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	uncultured	
41	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Blvii28 wastewater-sludge group	
10	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Paludibacteraceae	uncultured	uncultured rumen bacterium
225	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-010	gut metagenome
87	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Empedobacter	uncultured bacterium
8	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Rhizorhapis	
11	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia	uncultured rumen bacterium
9691	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	Candidatus Saccharimonas	
910	Bacteria	Cyanobacteria	Oxyphotobacteria	Chloroplast			
209	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-001	uncultured Prevotella sp.
13	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales vadinBB60 group		
42	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Dysgonomonadaceae		
16805	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 1	
2265	Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales	Elusimicrobiaceae	Elusimicrobium	uncultured bacterium

59	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	uncultured	uncultured rumen bacterium
13885	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R-7 group	
887	Bacteria	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrionaceae UCG-002	uncultured rumen bacterium
117160	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella	unidentified
132	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	Olsenella	uncultured Olsenella sp.
13	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerovibrio	
11	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Sutterella	uncultured rumen bacterium
24	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Nitrincolaceae	Nitrincola	uncultured bacterium
30	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae		
636	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-003	
28	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Propioniciclava	metagenome
1307	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae UCG-001	
30	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Eubacterium] ruminantium group	bacterium YSB2008
2	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Eggerthellaceae	DNF00809	
21	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	
1475	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	U29-B03	
11	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae UCG-005	uncultured organism
12	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae		
490	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales			
190	Bacteria	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Flexilinea	
855	Bacteria	Firmicutes	Clostridia	Clostridiales	Family XIII	[Eubacterium] saphenum group	uncultured bacterium

87	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Succiniclasticum	
113	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	
424	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Neisseriaceae	uncultured	
28	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae NK3B31 group	
370	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus 1	uncultured Ruminococcaceae bacterium
11	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R-7 group	uncultured rumen bacterium 4C0d-14
1360	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae R-7 group	uncultured rumen bacterium 4C0d-15
24	Archaea	Euryarchaeota	Thermoplasmata	Methanomassiliicoccales	Methanomethylophilaceae	uncultured	uncultured rumen methanogen
346	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Delftia	
259	Bacteria	Synergistetes	Synergistia	Synergistales	Synergistaceae	Fretibacterium	
24	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosia	
17710	Bacteria	Proteobacteria	Alphaproteobacteria				
2							
27	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnobacterium	Lachnobacterium bovis
383	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium 6	uncultured rumen bacterium
23	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Ornithinimicrobium	uncultured bacterium
65	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella 7	Prevotella melaninogenica
3292	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae		
9	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Cloacibacterium	uncultured Cloacibacterium sp.
78	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	
696	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Selenomonas	

5	Bacteria	Lentisphaerae	Oligosphaeria	Oligosphaerales	Oligosphaeraceae	Z20	uncultured Lentisphaerae
765	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium 10	uncultured rumen bacterium
65	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	XBB1006	uncultured rumen bacterium
12	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Acidipropionibacterium	
6878	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Atopobiaceae	Olsenella	uncultured rumen bacterium
35870	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	
8	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae RC9 gut group	Eubacterium sp. F1