Vol.67: e24220821, 2024
https://doi.org/10.1590/1678-4324-2024220821
ISSN 1678-4324 Online Edition

BABT
Brazilian Archives of Biology and Technology

Article - Human and Animal Health

Molecular Characterization of Abomasal-Related Bacteria in Sheep with *Haemonchus contortus* Infection

Adriane Holtz Tirabassi[1]
https://orcid.org/0000-0001-8992-5162

Rüdiger Daniel Ollhoff[1]
https://orcid.org/0000-0002-7972-9472

Nicoly Subtil de Oliveira[1,2]
https://orcid.org/0000-0002-5570-8036

Edvaldo Antonio Ribeiro Rosa[1,2]
https://orcid.org/0000-0001-6087-4365

Humberto Maciel França Madeira[3]
https://orcid.org/0000-0002-0567-8246

Cristina Santos Sotomaior[1*]
https://orcid.org/0000-0001-9281-3743

[1]Pontifícia Universidade Católica do Paraná, Escola de Medicina e Ciências da Vida, Programa de Pós-Graduação em Ciência Animal, Curitiba, Paraná, Brasil; [2]Pontifícia Universidade Católica do Paraná, Escola de Medicina e Ciências da Vida, Unidade de Pesquisa em Xenobióticos, Curitiba, Paraná, Brasil; [3]Pontifícia Universidade Católica do Paraná, Escola de Medicina e Ciências da Vida, Laboratório de Genoma, Curitiba, Paraná, Brasil.

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Paulo Vitor Farago

Received: 14-Oct-2022; Accepted: 26-Feb-2024

*Correspondence: cristina.sotomaior@pucpr.br; Tel.: +55-41-32712615 (C.S.S.).

HIGHLIGHTS

- Firmicutes and Bacteroidetes are predominant in abomasal load of sheep infected by *H. contortus.*
- Firmicutes and Proteobacteria are predominant in abomasal mucosa infected by *H. contortus.*
- Proteobacteria and Firmicutes are predominant in adult *H. contortus* infecting abomasum.

Abstract: *Haemonchus contortus* is an important parasite in sheep and the abuse of anti-helminthics has resulted in a rapid resistance. Little is known about the abomasal microbiota in sheep and the relation to local parasitism. This study aimed to identify bacteria in the abomasum of sheep, through the abomasal content, abomasal mucosa, and adult *H. contortus* parasites, in high and low parasitism. Eight sheep naturally infected with *H. contortus* were classified as high (n = 4) and low infection (n = 4). Samples of abomasal contents, abomasal mucosa, and adult *H. contortus* parasites were collected. Samples were pooled to form six groups, three in each level of parasitism: high infection/abomasal contents (HC), high infection/mucosa (HM), high infection/parasites (HP); low infection/abomasal contents (LC), low infection/mucosa (LM), and low infection/parasites (LP). Molecular identification of bacteria was performed by the amplification and sequencing of the 16S rRNA bacterial gene. A similar distribution of phyla was observed between pools HC/LC and HP/LP. For pools HM/LM, there was a significant difference (p = 0.01) in the proportion of phyla observed. The bacterial phyla predominant for libraries HC/LC were Firmicutes (82% and 62%) and Bacteroidetes (10.4% and 17.6%); for HM/LM, Firmicutes (76.9% and 56%) and Proteobacteria (10.2% and 38.4%); and for HP/LP, Proteobacteria (42.8% and 55%) and Firmicutes (31.6% and 40%). We observed differences about the samples analyzed, suggesting that there are various bacterial communities closely associated with the different materials analyzed, even from the close environment.

Keywords: Sheep; *Haemonchus contortus*; Abomasal microbiome; Parasitism.

INTRODUCTION

Haemonchus contortus is a gastrointestinal nematode (GIN) occurring in sheep and goats, with worldwide distribution [1]. Infection with *H. contortus* results in significant economic losses due to weight loss, reduced fertility, decreased yield, and growth retardation [2]. Treatment based on the use of anthelmintic drugs is quite limited and it requires frequent applications, resulting in nematode drug resistance [4-6]. Indeed, even resistance to the newest drug monepantel has been reported [7;8]. Therefore, the rapid spread of anthelmintic resistance makes it important to search for new control alternatives instead of the exclusive use of anthelmintics [9, 10].

Biological control may become a viable option for the control of GIN [11, 12] that includes the use of fungi [13, 14] and bacteria [14-17], as it has also been proposed for human and animal filarial nematodes [18].

However, little is known about the bacterial community found in the abomasum of sheep and the interactions between bacteria and the nematodes present in that environment. As far as it could be determined, studies on the association between GIN and bacteria are limited to a culture-based study of potential host pathogens carried by L3-stage larvae [19] and analyses of the bacterial profiles inhabiting adult worms, L3 larvae and eggs of *H. contortus* [20]. Thus, further studies are needed to establish the contribution of bacteria to parasitic infection in the abomasal environment.

As it may be hypothesized that the presence of specific bacterial populations associated with parasites affects the infectivity of the latter, this work aimed to identify bacteria collected from different sites in the abomasum of sheep (abomasal contents, abomasal mucosa and adult *H. contortus* parasites) by sampling animals with high and low parasitism.

MATERIAL AND METHODS

This study was approved by the institutional ethics committee for animal use under the protocols CEUA 558 and CEUA 747 and followed Brazilian federal regulation guidelines.

There were chose twenty-six crossbred (Ile de France × Texel) male lambs from the breeding herd of the Pontifical Catholic University of Paraná (PUCPR), located at the University's Experimental Farm in the city of Fazenda Rio Grande, State of Paraná, Brazil.

After weaning, lambs were kept on contaminated pastures under the same environmental and management conditions. During the day, they were kept in paddocks formed by native pasture and Pensacola pasture (*Paspalum notatum*), with a stocking rate of 30–40 lambs/ha; at night, they remained in the pen. They received forage supplementation with hay and corn silage. The lambs were also supplemented with concentrate (16% of crude protein - CP) in a ratio of 1 to 1.5% of their live weight. Fecal egg counts (FEC) were performed on a regular basis, for 4 months, to monitor parasite infection [21]. Eight six-month-old lambs were slaughtered for subsequent sample collection and molecular analysis. Four with high egg per gram (EPG) loads and four with low EPG loads.

Immediately after slaughtering, the abomasa were tied at both ends and were transported in a disinfected polystyrene box to the laboratory. Samples were aseptically collected in a laminar flow hood. Following an external cleaning with 70% ethanol, incisions were made in the greater curvature of the abomasa to sample abomasal content, abomasal mucosa, and adult *H. contortus*. Samples of abomasal content were collected with sterile 10 mL syringes. Following complete removal of the remaining abomasal loads, the mucosae were sampled (4 samples/lamb) with tweezers and scissors, parallel to the mucosal grooves, in fragments of about 4 cm in length and 3 mm in width. An average of 30 adult parasites/abomasum were collected and washed three times with sterile water. Samples were stored in sterile tubes and kept at -20 °C until processing.

Abomasa and residual content were stored in Railliet-Henry's preservative solution for subsequent quantification of total adult *H. contortus*, either planktonic or adhered. Parasites were individually identified and counted, under a stereoscopic microscope at 40× of magnification, according to the method described by Ueno and Gonçalves [22].

Based on FEC and parasite quantification, 15% of the lambs were assigned as low infection (EPG ≤ 2,000; parasite counts ≤ 40) or high infection (EPG > 2,000; parasite counts ≥ 800).

Molecular assessment of bacteria was done after washing and differential centrifugation of cells. DNA extraction was performed by physical disruption using a BeadBeater (Biospec Products; Bartlesville, OK) in the presence of 0.1 % (w/v) sodium dodecyl sulfate (SDS) and 50 mg mL^{-1} lysozyme [23]. Final DNA concentrations were set to 20 ng mL^{-1}.

Samples were pooled to form six groups, three in each level of parasitism: low infection/abomasal content (LC), low infection/mucosa (LM), low infection/parasite (LP); high infection/abomasal content (HC), high infection/mucosa (HM), and high infection/parasite (HP).

16S rDNA gene was amplified by polymerase chain reaction (PCR) using 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) primers in a reaction volume of 50 µL (50 mM MgCl, 1.25 mM dNTPs, 20 pmol/µL of each primer, 5U Taq DNA polymerase, 20 ng/µL template DNA), according to Patel and coauthors [24]. Amplification conditions were as follows: one cycle at 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 55 °C to 57 °C for 30 s (the temperature here varied according to the amplified pool), and 72 °C for 2 min; and a final cycle at 72 °C for 7 min.

There were obtained 16S rRNA gene amplicons of about 1.4 kb for all pools (HA, LA, HM, LM, HL, and LL).

Clone libraries of 16S rDNA genes were constructed by ligating PCR amplified sequences to the pCR2.1 vector and transforming them into *Escherichia coli* TOP10 One Shot chemically competent cells (Invitrogen, Carlsbad, CA), following manufacturer's instructions. Plasmid DNA was extracted from 20 clones of each library using QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA), following manufacturer's instructions. The 16S rDNA genes were partially sequenced by a commercial laboratory (Macrogen Inc, Seoul, South Korea) using T7 promoter (5′-TAATACGACTCACTATAGGG-3′) and M13 (5′-CAGGAAACAGCTATGAC-3′) primers. The obtained 16S rRNA sequences were compared with those available in the Ribosomal Database Project (RDPII) Release 10, Update 32 (http://rdp.cme.msu.edu/) using the *CLASSIFIER* algorithm, with confidence of 80% or above. Each clone generated sequences with, on average, 1,500 pb. Taking out of the sequences obtained from the part referring to the vector coverage and ends with lesser quality, we aligned sequences of about 900 pb. The sequences were analyzed by similarity search using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The distribution of the identified phyla in the groups with high and low parasitism was compared using a chi-square test (5% significance level). Statistical analysis of the data was performed using the SPSS software (version 24.0. Armonk, NY: IBM).

RESULTS AND DISCUSSION

Out of eight lambs, four were classified with low parasitism and four with high parasitism (Table 1).

Table 1. Fecal egg count (FEC) and adult *H. contortus* load in lambs according to parasitism status

Group	**FEC**		**Adult *H. contortus* load**	
Parasitism status	Min-max	Avg	Min-max	Avg
Low (n = 4)	100-200	150	28-56	38
High (n = 4)	2,150-3,300	2,725	510-902	855

Sequencing data analysis

Through the pathway CLASSIFIER, samples were classified in different taxonomic levels, as class, order, family, genus, as well as "unclassified bacteria". This disparity in the distribution of samples in different taxonomic levels may be justified by a moderate inequality in the size and quality of the sequences analyzed. Larger and better sequences allowed the classification at a lower taxonomic level, with a higher reliability degree. Considering, 52% of bacteria were classified at genus level, while 2% were limited at class or order, 36% at family, and 8% were included as unclassified bacteria. The analysis of these sequences by similarity showed that about 95% of these sequences have a high identity and homogeneously aligns with sequences kept in the database.

Diversity of bacterial communities

The sequences obtained were arranged into five phyla and into "no identifiable bacteria" (NIB) distributed in different libraries in a variable proportion (Figure 1). Relative abundance of phyla varied from 29.4% to 81.2% for Firmicutes (HC, LC, HM, LM, HP, and LP); 11.1% to 64.7% for Proteobacteria (LC, HM, LM, HP, and LP); 9.1% to 16.7% for Bacteroidetes (HC, LC, HM, and HP); 2.6% to 5.6% for Verrucomicrobia (HC, LC, and HM); 31.9% for Actinobacteria (LM, only); and 5.4% to 20.7% for NIB (HC, LC, HM, HP, and LP).

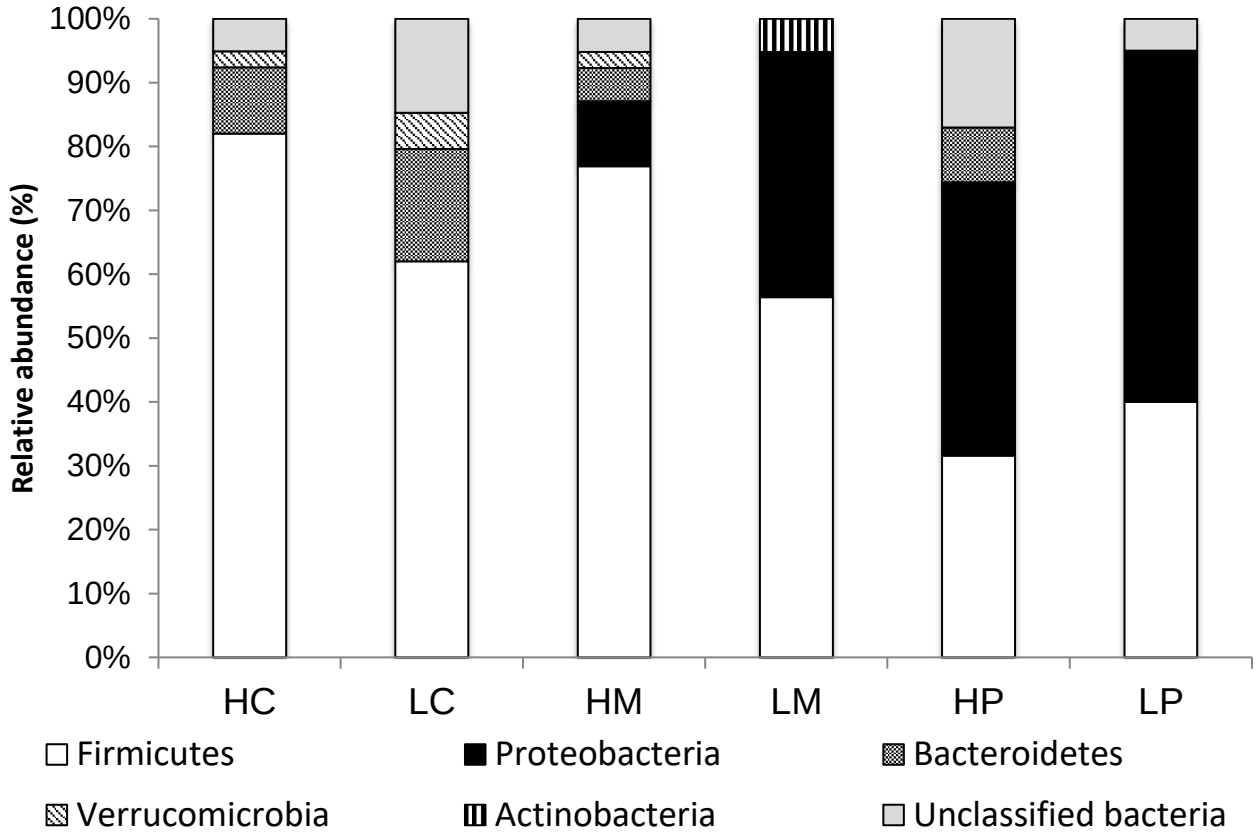

Figure 1. Distribution of predominant bacterial phyla obtained through sequencing of clones from the libraries of 16S rRNA gene. HC = high infection/abomasal contents; LC = low infection/abomasal contents; HM = high infection /abomasal mucosa; LM = low infection/abomasal mucosa; HP = high infection /parasites; LP = low infection/parasites. Data obtained from the Ribosomal Database Project (RDP) with reliability ≥ 80% for the deposited sequences.

When analyzing abomasal content, it is important to consider that as the digestive system in ruminants is a continuous system and that ruminal bacteria can migrate and persist in abomasal content [25, 26], sometimes becoming residents [27]. Here, abomasal content showed variable distribution of different phyla; however, no significant differences in the proportion of phyla could be assessed for the groups HC and LC ($p = 0.26$). It can let us to infer that the infection status is not responsible for microbial shifts in abomasal bacterial contents.

Regarding microbiota composition for ruminants, our results do not diverge from other published elsewhere [28, 29] revealing that the most common phyla found are (in decreasing order of occurrence) Firmicutes, Bacteroidetes, and Proteobacteria, although some minor disparities have been reported [30-33]. The relative low presence of Verrucomicrobia may be attributed to their ability to adhere to solid fractions of transient ruminal content [31, 32] with low dispersion.

There is a great diversity of microorganisms that colonize mucosal surfaces of vertebrate and invertebrate species [34], and this is the predominant site for microbial interaction, as it provides an environment that facilitates the establishment of microbiota, maintaining an exchange interface [35]. In this study, Firmicutes, followed by Proteobacteria, Bacteroidetes, Verrucomicrobia, Actinobacteria and unclassified bacteria were the phyla found in HM and LM pools.

This distribution of phyla between HM and LM differed statistically ($p = 0.01$). However, the prevalence of Firmicutes did not differ ($p = 0.085$) between the two set of animals. This suggests again that bacteria from such phylum are not involved with *H. contortus* parasitism. On the other hand, Proteobacteria, not observed in HC and LC, comprise the second largest phylum in HM and LM, suggesting an intimate relationship between this phylum and the mucosa. In grain-finished bison, it was found more Proteobacteria colonizing abomasum [36], what is not a good prognostic. *Per se,* such relative elevation in mucosal colonization by Proteobacteria (as well as Firmicutes) can be considered dysbiosis [37-39]. In addition, it is perceptible the higher proportion of Actinobacteria compounding the mucosal microbiota of low infected animals. Their participation in such microcosmos is uncertain. It is known that they act as decomposer of organic matter [40]. We hypothesize here that their presence is a consequence of a “near-to-healthy” state, not displaying any determinacy role.

Bacterial communities of adult *H. contortus* present stronger similarities than those found in abomasal content [20]. However, in this study, no differences in the colonization patterns were assessed for abomasal loads ($p = 0.260$). Proteobacteria were present in abomasal content samples (only in LC) and in mucosal samples (HM and LM) at lower levels than in parasites; such fact suggests an increased affinity of that phylum to parasites than to the other two sites.

Bacteria can live in a wide range of environments and have developed close relations to protozoa, invertebrates, vertebrates, and plants [41, 42]. By their turn, nematodes also are colonized by a myriad of bacteria. These bacteria may colonize the intestine of nematodes and contribute to host's nutritional requirements, as well as play a key role in parasite's biology, depending on the parasite so that the nematode can successfully complete its life cycle [20]. Indeed, bacteria can be found even inside eggs of *H. contortus* [43]. Here, the phyla detected in association to adult nematodes were (for HP and LP, respectively) Proteobacteria, 34.5% and 64.7%; Firmicutes, 34.5% and 29.4%; unclassified bacteria, 20.7% and 5.9%; and Bacteroidetes, 10.3% (only in HP); Despite any preliminary expectations, there was no significant difference in the proportion of phyla ($p = 0.071$) for HP and LP.

Associations between nematodes and prokaryotes vary regarding the type of parasitism [42]. An accurate definition of these associations is somehow difficult since the boundaries between them are unclear [41]. There are few reports assigned to the identification of bacteria naturally associated with any GIN in sheep [19, 20].

Regarding classes, the identification of sequences at class level for all six libraries of 16S rRNA is represented by Figures 2 and 3. The distribution of sequences within the phylum Firmicutes comprises the classes Clostridia, Negativicutes, Bacilli, and unclassified Firmicutes. The class Clostridia is prevalent in all libraries, followed by Negativicutes (absent only in HM). A third class is the Bacilli, observed only in HP. The class Bacilli is strongly associated with all stages of the *H. contortus* [20]. Unclassified bacteria were named "unclassified Firmicutes". According to Kim and coauthors [29], out of the ruminal sequences available in the database RDP, about 90.6% of the bacterial sequences of the phylum Firmicutes were assigned to the class Clostridia, the rest were Bacilli, Erysipelotrichi, and unclassified Firmicutes.

The families observed within the phylum Firmicutes were Acidaminococcaceae, Veillonellaceae, Ruminococcaceae, Selenomonadaceae, Lachnospiraceae, Ruminococcaceae, Unclassified Clostridiales, Clostridiales incertae sedis XIII, and NI Bacillales. Confirmed genera observed were *Succiniclasticum, Megasphaera*, *Shwartzia*, *Butyrivibrio, Pseudobutyrivibrio, Saccharofermentans, Ruminococcus, Anaerovorax,* and several non-identified (NI) genera. In a study conducted by Kim and coauthors [29], the larger families found for ruminal samples were Lachnospiraceae and Ruminococcaceae, followed by Veillonellaceae. The predominant genera included *Butyrivibrio*, *Acetivibrio*, *Ruminococcus*, *Succiniclasticum*, *Pseudobutyrivibrio*, and *Mogibacterium*. According to the same authors, many sequences (about 60%) of Firmicutes were not ranked in any family, order or genus within the Clostridia class ("unclassified Clostridiales"), and the largest group of reported sequences remains classified in the order Clostridiales. The second largest group of unclassified sequences was found within the group Ruminococcaceae. Despite minor variations, our study and the Kim's group do not diverge significantly, showing the tendency of conservation of taxonomic groups in the ruminal microenvironment.

The results here obtained for HP/LP agree with the data presented by Sinnathamby [20] regarding the presence of the families Veillonellaceae and Lachnospiraceae (with the genera *Butyrivibrio* and *Pseudobutyrivibrio*). Attempts to cultivate potentially pathogenic bacteria through larvae L3 obtained by culture of feces from animals infected with *H. contortus* detected *Sphingobacterium multivorum* and *Streptococcus macacae* [43].

The distribution of sequences within the phylum Proteobacteria comprised the classes Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. The families represented within the Alphaproteobacteria were Sphingomonadaceae (for LM), Acetobacteriacea (for HM), Phillobacteriaceae (HP), and NI Rhodospirillales (for LM). None of they reach a classification at the genus level. The presence of Phillobacteriaceae for HC agrees with the results of Sinnathamby [20], where that is the predominant family for adult *H. contortus* and L3 larvae. According to Kim and coauthors [29], the phylum Alphaproteobacteria was fully represented by a small number of sequences retrieved from non-cultivated bacteria.

Figure 2. Taxonomic organization of predominant bacteria obtained through sequencing of clones from the libraries of 16S rRNA gene

The families represented within the class Betaproteobacteria were Burkholderiaceae (only in LM) and Comamonadaceae (for LM), reaching the genus level *Curvibacter* (for LP). According to Sinnathamby [20], the Burkholderiaceae family has not been identified for any stage of *H. contortus*. The Burkholderiales order includes strict aerobic and facultative aerobic chemoorganotrophs, nitrogen fixing organisms, as well as pathogens of plants, animals, and humans [44]. Within the Betaproteobacteria, 17 genera are known in the census of ruminal bacteria, many of which are represented by few sequences [29]. The Comamonadaceae family was identified for the genera *Acidovorax*, *Comamonas*, and *Delfia* [20], and the genus *Curvibacter* was not identified. It is unlikely that there is a vertical transmission of those bacteria, as they were identified only in adult and larvae L3 parasites, and their absence in in vitro cultures and eggs extracted from feces [20]. There is a greater probability of acquiring those bacteria from the environment, where they are omnipresent [45]. However, this phylum has not been identified for HC/LC, and only a few representatives of those families are observed in HM/LM.

Gammaproteobacteria were identified only in HP. Only Enterobacteriaceae family was identified in large numbers, and its presence was also reported for eggs extracted from feces and in vitro cultivation of the parasite [20]. According to Kim and coauthors [29], Gammaproteobacteria is the most prevalent class of ruminal bacteria, representing up to 73% of all sequences of Proteobacteria. However, the results presented by the authors did not agree with the results obtained here, as that was the smallest group identified within the Proteobacteria.

The distribution of classes within the phylum Bacteroidetes is restricted to the class Bacteroidales, the Prevotellaceae family (HP), the genus *Hallella* (for LC). The rest were unclassified Bacteroidales and Prevotellaceae. According to Kim and coauthors [29], 88.5% of the bacteria from RPD were assigned to the classes unclassified Bacteroidetes and Bacteroidia. The presence of the Prevotellaceae family for HP agrees with prior results published elsewhere [20].

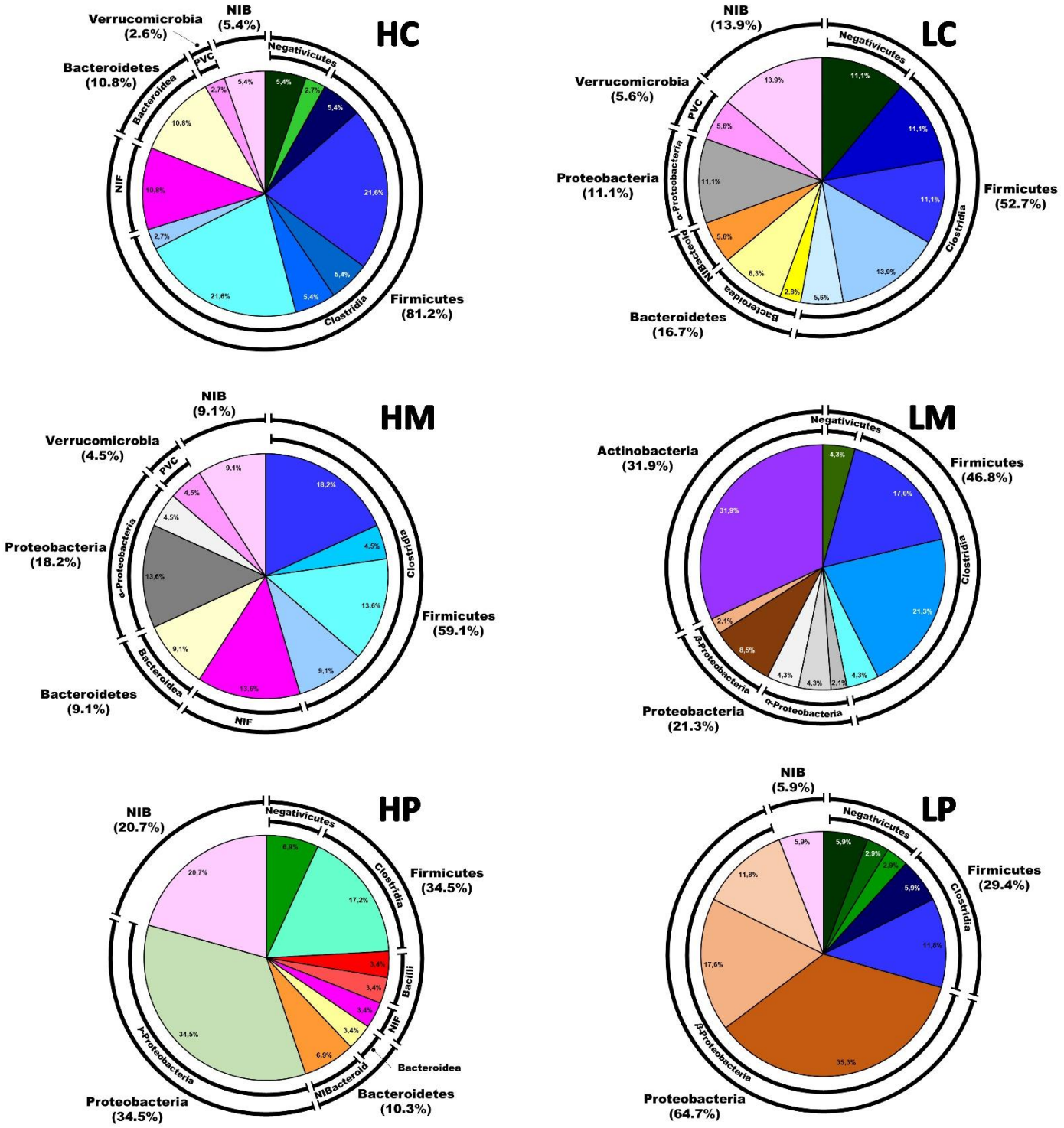

Figure 3. Taxonomic distribution of predominant bacteria obtained through sequencing of clones from the libraries of 16S rRNA gene. HC = high infection/abomasal contents; LC = low infection/abomasal contents; HM = high infection /abomasal mucosa; LM = low infection/abomasal mucosa; HP = high infection /parasites; LP = low infection/parasites. Data obtained from the Ribosomal Database Project (RDP) with reliability ≥ 80% for the deposited sequences.

Independent culture methods were used for identifying microorganisms, but 99% of the bacterial species are not cultivable by conventional methods [46]. Molecular techniques and phylogenetic analyses are the methods of choice, which have been successfully used in the exploration of the composition of microbial community and the identification of species previously not detected by cultivation methods [47]. Among molecular techniques, the analysis of amplicons generated by the PCR of 16S ribosomal rRNA genes is regarded as an effective method, due to the presence of preserved and variable domains and its reliability for inferring phylogenetic relationships [48].

A relationship between a greater or lesser presence of parasitic and microbial diversity, between pairs HC/LC, HM/LM, and HP/LP is still something difficult to assert; however, it is possible to observe a different distribution of bacterial communities between high and low parasitism. In a study carried out by Li and coauthors [33], immune animals and control group (without prior exposure to the parasite) were infected with *O. ostergtagi* and their abomasal contents were analyzed. The infection apparently generated a minimal impact on the abomasal microbial diversity at genus level for immune and control animals leading to a thought that immune animals can develop skills to keep a proportional stability on the ecosystem of abomasal microbiota.

Regardless of belonging to the same environment, load, mucosa, and abomasal parasites, and the existence of a closeness to the diversity of bacteria observed in the samples, there are differences in the distribution of taxonomic groups. This allows us to conclude that there are communities related to a pool of samples, not observed in others, regardless of the small number of samples analyzed. High proportions of the ribosomal 16S rRNA gene for a certain group in a community may indicate that this group is numerically dominant or that this group has the capacity to grow faster when compared to the other ones in this community [49]. In this study, 20 clones were analyzed, and the representativeness of libraries was decreased. The relative abundance of phylogenetic groups may considerably differ between various libraries generated from the same sample due to a small number of clones analyzed or factors affecting the amplification by PCR [50]. Besides, tendencies due to preferential amplification of a primer [51, 28], the lack of amplification of certain templates and the variation in amplification effectiveness in different environmental samples [52] are usual. The pair of primers 27F/1492F may be amplified preferentially in certain phyla of different samples or different effectiveness in three materials analyzed in this study (abomasal load, abomasal mucosa, and abomasal parasites). Moreover, there is a need for considering the individual viability of pools (samples).

Another methodological issue to be considered is the material collection and DNA extraction. At the collection time, material may be more solid in presence of larger amounts of food particles, or pasty, depending on water consumption by the animal. Besides, there may be differences regarding the processing of material, prior to DNA extraction, due to its individual characteristics. According to Cunha and coauthors [32], rather liquid or solid fractions of samples analyzed may affect the results, as well as methods used for DNA extraction.

Despite the small number of samples and the recognized limitations of the techniques used, sequencing allowed an overview of libraries, consistent with data from studies with samples of the rumen and abomasum in a representative way. Sequencing of clones allowed the validation of libraries and provided a preliminary analysis of this diversity. Despite the results regarding populations are not precisely quantitative, we attempted to identify qualitative differences in the populations, with a view on future control strategies aimed at specific microorganisms.

CONCLUSION

By taxonomic identification of clone sequences for the 16S rRNA gene, a similar distribution of phyla was observed for the pools HC/LC and HP/LP. For the pools HM/LM, there was a significant difference in the distribution of phyla ($p = 0.01$). The predominant bacterial phyla for the libraries HC/LC were Firmicutes (82% and 62%) and Bacteroidetes (10.4% and 17.6%); for HM/LM they were Firmicutes (76.9% and 56%) and Proteobacteria (10.2% and 38.4%); and for HP and LP they were Proteobacteria (42.8% and 55%) and Firmicutes (31.6% and 40%). There were differences regarding materials analyzed (abomasal load, mucosa, and parasite), suggesting that there are different bacterial communities closely related to various niches in the same environment. Furthermore, in the presence of a greater or lesser parasitism, the bacteria found were distributed into different proportions, perhaps suggesting differences in the community diversity of local bacteria.

Funding: This research was funded in part by Coordenação de Aperfeiçoamente de Pessoal de Nível Superior (CAPES) – Finance Code 001.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Waller PJ, Chandrawathani P. *Haemonchus contortus*: parasite problem no. 1 from tropics to polar circle. Problems and prospects for control based on epidemiology. Trop Biomed. 2005; 22:131–7.
2. Holmes PH, Coop RL. Pathophysiology of gastrointestinal parasites. Vet Parasitol. 1994; 54:299–304.

3. Wolstenholme AJ, Fairweather I, Prichard R, Samson-Himmelstjerna JV, Sangster NC. Drug resistance in veterinary helminths. Trends Parasitol. 2004; 20(10):469–76.
4. Papadopoulos E (2008). Anthelmintic resistance in sheep nematodes. Small Rumin Res. 2008; 76(1-2):99-103.
5. Torres-Acosta JF, Mendoza-de-Gives P, Aguilar-Caballero AJ, Cuéllar-Ordaz JA. Anthelmintic resistance in sheep farms: update of the situation in the American continent. Vet Parasitol. 2012; 189(1):89-96.
6. Rose H, Rinaldi L, Bosco A, Mavrot F, de Waal T, Skuce P, et al. Widespread anthelmintic resistance in European farmed ruminants: a systematic review. Vet Rec. 2015; 176(21):546.
7. Scott I, Pomroy WE, Kenyon PR, Smith G, Adlington B, Moss A. Lack of efficacy of monepantel against *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Vet Parasitol. 2013; 198(1-2):166-171.
8. Van den Brom R, Moll L, Kappert C, Vellema P. *Haemonchus contortus* resistance to monepantel in sheep. Vet Parasitol. 2015; 209(3-4):278-280.
9. Jackson F, Miller J. Alternative approaches to control-Quo vadit? Vet Parasitol. 2006; 139:371–384.
10. Woodgate RG, Love S. WormKill to WormBoss - Can we sell sustainable sheep worm control? Vet Parasitol. 2012; 186:51-57.
11. Meyer SLF. United States Department of Agriculture - Agricultural Research Service research programs on microbes for management of plantparasitic nematodes. Pest Manag Sci. 2003; 59: 665–670.
12. Larsen M. Biological control of nematode parasites in sheep. J Anim Sci. 2006; 84;133-139.
13. Larsen M, Wolstrup J, Henriksen SA, Dackman C, Grønvold J, Nansen P. In vitro stress selection of nematophagous fungi biocontrol of parasitic nematodes in ruminants. J Helminthol. 1999; 65:193-200.
14. Aguilar-Marcelino L, Quintero-Martínez MT, Mendoza de Gives P, López-Arellano ME, Liébano-Hernández E, Torres-Hernández G, et al. Evaluation of predation of the mite *Lasioseius penicilliger* (Aracnida: Mesostigmata) on *Haemonchus contortus* and bacteria-feeding nematodes. J Helminthol. 2014; 88(1):20-23.
15. Kotze AC, O'Grady J, Gough JM, Pearson R, Bagnall NH, Kemp DH, et al. Toxicity of *Bacillus thuringiensis* to parasitic and free-living life-stages of nematode parasites of livestock. Int J Parasitol. 2005; 37:577.
16. O'Grady J, Akhurst RJ, Kotze AC. The requirement for early exposure of *Haemonchus contortus* larvae to *Bacillus thuringiensis* for effective inhibition of larval development. Vet Parasitol. 2007; 150:97–103.
17. Byoung-Joo S, Rehish Kumar VJ, Irfan Ahmad R, Byung-Chun K, Park W, Se-Eun K, et al. Bacterial mixture from greenhouse soil as a biocontrol agent against root-knot nematode, *Meloidogyne incognita* on oriental melon. J Microbiol Biotechnol. 2012; 22:114–117.
18. Hoerauf A, Specht S, Büttner M, Pfarr K, Mand S, Fimmers R, et al. *Wolbachia endobacteria* depletion by doxycycline as antifilarial therapy has macrofilaricidal activity in onchocerciasis: a randomized placebo-controlled study. Med Microbiol Immunol. 2008; 197(3):295-311.
19. Lacharme-Lora L, Salisbury V, Humphrey TJ, Stafford K, Perkins SE. Bacteria isolated from parasitic nematodes - a potential novel vector of pathogens? Environ Health. 2009; 8:S17.
20. Sinnathamby G. Bacteria associated with *Haemonchus contortus*. PhD Thesis, Massey University. 2012. Available at: https://mro.massey.ac.nz/items/ec9fa344-a89d-47a7-b30a-9ff74529f56f.
21. Gordon HML, Whitlock HV. A new technique for counting nematode eggs in sheep faeces. J Council Sci Ind Res. 1939; 12:50.
22. Ueno H, Gonçalves PC. [Handbook for diagnosis of helminthiasis of ruminants]. Japan International Cooperation Agency, Tokyo, pp. 72-76. 1998.
23. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press. 1989.
24. Patel JKM, Jhala MK, Soni P, Josi CG. Molecular characterization and diversity of rumen bacterial flora. Online Vet J. 2011; 6(1):1-9.
25. Simcock DC, Joblin KN, Scott I, Burgess D, Rogers CW, Ponroy WE, et al. Hypergastrinaemia, abomasal bacterial population densities and pH in sheep infected with *Ostertagia circuncincta*. Int J Parasitol. 1999; 29:1053-63.
26. Simcock DC, Lawton DEB, Scott I, Simpson HV. Abomasal bacteria produce an inhibitor of gastrin secretion in vitro. Res Vet Sci. 2006; 81:152-7.
27. Laporte-Uribe J, Gibbs SJ. Differential passage of rumen bacterial populations to the abomasum in sheep. Proc New Zealand Soc Anim Prod. 2009; 69:242-3.
28. Edwards JE, McEwan NR, Travis AJ, Wallace RJ. 16S rDNA library-based analysis of ruminal bacterial diversity. Antonie van Leeuwenhoek. 2004; 86:263-281.
29. Kim M, Morrison M, Yu Z. Status of the phylogenetic diversity census of ruminal microbiomes. FEMS Microbiol Ecol. 2011; 76:49–63.
30. Ozutsumi Y, Tajima K, Takenaka A, Itabashi H. The effect of protozoa on the composition of rumen bacteria in cattle using 16S RNAr gene clone libraries. Biosci Biotechnol Biochem. 2005; 69:499–506.

31. Brulc JM, Antonopoulos DA, Miller MEB. Genecentric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. Proc Natl Acad of Sci USA. 2009; 106:1948–53.
32. Cunha IS, Barreto CC, Costa OYA, Bomfim MA, Castro AP, Kruger RH, et al. Bacteria and Archaea community structure in the rumen microbiome of goats (*Capra hircus*) from the semiarid region of Brazil. Anaerobe. 2011; 17(3):118–24.
33. Li RW, Wu S, Li W, Huang W, Gasbarre LC. Metagenome plasticity of the bovine abomasal microbiota in immune animals in response to *Ostertagia ostertagi* infection. PLoS ONE. 2011; 6(9):24417.
34. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science. 2010; 24:1768-73.
35. Taschuk R, Griebel FJ. Commensal microbiome effects on mucosal immune system development in the ruminant gastrointestinal tract. Anim Health Res Rev. 2012; 13(1):129–41.
36. Bergmann GT. Microbial community composition along the digestive tract in forage- and grain-fed bison. BMC Vet Res. 2017; 13(1):253.
37. Krause KM, Oetzel GR. Understanding and preventing subacute ruminal acidosis in dairy herds: a review. Anim Feed Sci Technol. 2006; 126(3–4):215–326.
38. Nagaraja, TG, Titgemeyer EC. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. J Dairy Sci. 2007; 90 Suppl 1(7):E17–38.
39. Plaizier JC, Krause DO, Gozho GN, McBride BW. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. Vet J. 1997; 176(1):21–31.
40. Rappé MS, Gordon DA, Vergin KL, Giovannoni SJ. Phylogeny of actinobacteria small subunit (SSU) rRNA gene clones recovered from marine bacterioplankton. Syst Appl Microbiol. 1999; 22(1):106–112.
41. Dale C, Moran NA. Molecular interactions between bacterial symbionts and their hosts. Cell. 2006; 126(3):453–465.
42. Goodrich-Blair H, Clarke DJ. Mutualism and pathogenesis in *Xenorhabdus* and *Photorhabdus*: two roads to the same destination. Mol Microbiol. 2007; 64(2):260–268.
43. Sinnathamby G, Henderson G, Umair S, Janssen P, Bland R, Simpson H. The bacterial community associated with the sheep gastrointestinal nematode parasite *Haemonchus contortus*. PLoS One. 2018; 13(2):e0192164.
44. Garrity GM, Bell JA, Lilburn T. Order I. Burkholderiales. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (ed) Bergey's Manual of Systematic Bacteriology. Springer, New York. 2005.
45. Kersters K, De Vos P, Gillis M, Swings J, Vandamme P, Stackebrandt E. Introduction to the Proteobacteria. The Prokaryotes. Springer, New York, NY. pp. 3-37. 2006.
46. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol Rev. 1995; 59(1):143-69.
47. Hugenholtz P, Pace NR. Identifying microbial diversity in the natural environment: a molecular phylogenetic approach. Trends Biotechnol. 1996; 14(6):190-7.
48. Woese CR. Bacterial evolution. Microbiol Rev. 1987; 51(2):221–271.
49. Buckley DH, Schmidt TM. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environ Microbiol. 2003; 5(6):441–452.
50. Leser TD, Amenuvor JZ, Jensen TK, Lindecrona RH, Boye M, Møller K. Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. Appl Environ Microbiol. 2002; 68(2):673-690.
51. Polz MF, Cavanaugh CM. Bias in template-to-product ratios in multitemplate PCR. Appl Environ Microbiol. 1998; 64(10):3724-3730
52. Skillman LC, Evans PN, Strömpl C, Joblin KN. 16S rDNA directed PCR primers and detection of methanogens in the bovine rumen. Lett Appl Microbiol. 2006; 42(3):222–228.