Research in Veterinary Science 196 (2025) 105916

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Gut microbiota alterations linked to classical scrapie in sheep

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ARTICLE INFO

Keywords:
Scrapie
Prion
Gut microbiota
16S rRNA
Diversity

ABSTRACT

There is growing evidence of the involvement of gut microbiota in neurodegenerative diseases. However, the relationship between gut microbiota and prion diseases is not yet well understood. Prion diseases are neurodegenerative disorders affecting humans and animals and caused by an infectious misfolded protein known as prion. Among prion diseases, scrapie is the one affecting sheep and goats, and its classical form is typically acquired. In the present work, a 16S rRNA-based microbiome profiling was performed in faecal samples from 10 sheep infected with classical scrapie and 10 control sheep. The phyla *Firmicutes* and *Bacteroidetes* appeared as the most abundant phyla in scrapie and control sheep. Moreover, both groups of samples showed similar richness and alpha diversity indices. The gut microbiota profile in terms of beta diversity was significantly different in scrapie-infected sheep compared to the control group. Additionally, similarly to other neurodegenerative diseases, scrapie animals displayed a significant increased abundance of *Cyanobacteria* and *Lentisphaerae* phyla. Our results point to an altered gut microbiota in sheep infected with classical scrapie, with a specific increase of two phyla that could be involved in scrapie disease pathogenesis, enabling conceptual advances in the understanding of prion diseases.

1. Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of neurodegenerative disorders that affect both humans and animals. These diseases are caused by a common aetiological agent, an infectious protein known as prion, which is the misfolded form (PrP^{Sc}) of the cellular prion protein (PrP^{C}). Prion diseases are chronic, fatal disorders characterised by long incubation periods and a pathology that primarily affects the central nervous system (CNS) (Johnson, 2005). In these diseases, PrP^{Sc} accumulates mainly in the CNS, leading to characteristic lesions such as spongiform vacuolization, gliosis and neurodegeneration (Budka, 2003).

Scrapie, a prion disease affecting sheep and goats, was the first TSE to be described (Jeffrey and González, 2007). Two forms of scrapie are recognised: classical and atypical scrapie. These forms differ in clinical signs, brain lesions in affected animals (Benestad et al., 2003), and the *PRNP* gene genotypes associated with disease susceptibility (Hunter, 1997; Moum et al., 2005). Additionally, classical scrapie is typically acquired, while atypical scrapie is considered a sporadic form of the disease (Acín et al., 2021).

Protein misfolding and aggregation are also features of other neurodegenerative diseases known as prion-like diseases. These include, among others, Parkinson's disease (PD), characterised by α-synuclein accumulation leading to dopamine neuron loss (Stefanis, 2012), and Alzheimer's disease (AD), characterised by extracellular deposition of amyloid-beta (Aβ) and accumulation of hyperphosphorylated tau proteins (Jaunmuktane and Brandner, 2020).

Recent research has highlighted a potential link between neurodegenerative diseases and the gut-brain axis. This bidirectional communication pathway, involving neural, endocrine, metabolic and

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https://doi.org/10.1016/j.rvsc.2025.105916
Received 5 November 2024; Received in revised form 18 August 2025; Accepted 22 September 2025
Available online 23 September 2025

Table 1
Characteristics of the animals used in the study.

Animal	Gender	Age (years)	Genotype	Breed	Originating location	Disease status
C1	Female	2	ARR/ARQ	Rasa Aragonesa	CEETE, Zaragoza	Control
C2	Female	2	ARQ/ARQ	Rasa Aragonesa	CEETE, Zaragoza	Control
C3	Female	4	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Control
C4	Female	3	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Control
C5	Female	1	ARR/ARQ	Crossbreed	CEETE, Zaragoza	Control
C6	Female	3	ARR/ARR	Crossbreed	Unknown	Control
C7	Female	5	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Control
C8	Female	4	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Control
C9	Female	5	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Control
C10	Female	4	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Control
Sc1	Female	4	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Clinical
Sc2	Female	3	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Clinical
Sc3	Female	4	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Clinical
Sc4	Female	4	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Clinical
Sc5	Female	5	ARQ/AHQ	Rasa Aragonesa	Villanueva de Gállego, Zaragoza	Clinical
Sc6	Female	5	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Clinical
Sc7	Female	5	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Clinical
Sc8	Female	4	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Clinical
Sc9	Female	2	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Clinical
Sc10	Female	4	ARQ/ARQ	Crossbreed	Tauste, Zaragoza	Clinical

immunological signals, connects the CNS with the gut and its microbiota (Carabotti et al., 2015). This gut-brain axis includes the gut microbiota, the gut itself, the peripheral nervous system, the autonomic nervous system and the CNS. The CNS influences the gut via the sympathetic and parasympathetic nervous systems, particularly the vagus nerve, which controls peristalsis and secretion, thereby affecting gut microbiota nutrition. Conversely, gut microbiota metabolites, such as short-chain fatty acids (SCFA), can influence the brain by affecting microglial maturation and function (Erny et al., 2015). Neurotransmitters produced by gut bacteria, such as gamma-aminobutyric acid (GABA) and dopamine, also play a role in gut-brain communication (Chen et al., 2021). Additionally, humoral pathways involving cytokines, hormones of the hypothalamic-pituitary-adrenal axis, and gastrointestinal hormones like ghrelin further mediate this communication (Rusch et al., 2023).

The gut microbiota is involved in various neural processes related to stress hormone signalling, neuronal function and neuroprotection, as well as in development, myelination, neurogenesis, and microglial activation (Margolis et al., 2021). Dysregulation of the gut microbiota can compromise the immune system, which has been linked to the pathophysiology and progression of neurodegenerative diseases (Margolis et al., 2021). Given the close interaction between gut bacteria and the brain, gut microbiota dysbiosis has emerged as a potential area of interest for investigating new diagnostic and therapeutic approaches in neurodegenerative diseases, including prion and prion-like diseases.

While several studies have demonstrated changes in the gut microbiota in prion-like diseases such as PD, which could serve as sources of biomarkers for early diagnosis or as targets for therapeutic development (Varesi et al., 2022), research on prion diseases is limited. Studies in mouse models have explored the effect of commensal gut microbiota on prion disease pathogenesis, but results have been conflicting (Trichka and Zou, 2021). Early studies suggested a delay in symptom onset in infected germ-free mice compared to controls (Lev et al., 1971), but later studies indicated that this delay might vary depending on the infection route (Wade et al., 1986). The most recent study found that the absence of commensal microbiota did not affect disease duration or the severity of typical disease lesions, regardless of the infection route (Bradford et al., 2017).

Despite these inconsistencies, recent studies have shown alterations in the gut microbiota in scrapie-inoculated mice (Losa et al., 2024; Yang et al., 2020b), in Creutzfeldt-Jakob disease (CJD) patients, the most common form of human prion diseases (Guo et al., 2022; Kong et al., 2023; Mahbub et al., 2024), and in chronic wasting disease (CWD), a naturally occurring prion disease in cervids that is rapidly spreading in Noth America (Didier et al., 2024; Minich et al., 2021). These microbiota changes have been proposed as tools for diagnosing and monitoring CWD. To our knowledge, no similar studies have been conducted in sheep with scrapie. As in deer, identifying potential differences in the gut microbiota could be useful for early diagnosis and could involve the characterisation of a large animal model for use in preclinical trials of microbiota-based therapies.

Using a 16S rRNA amplicon sequencing approach, we characterised the microbial composition of the gut of sheep naturally infected with scrapie and compared it to that of control sheep. We identified a dysbiosis associated with scrapie infection, contributing to our understanding of the disease and opening new research lines for identifying potential diagnostic biomarkers or evaluating microbiota-related therapies.

2. Materials and methods

2.1. Animals and sample collection

A total of 20 sheep, consisting in 10 control healthy animals (C) and 10 sheep infected with classical scrapie (Sc), were selected for this study. All sheep were adult females from 1 to 5 years of age and carried different genotypes for the *PRNP* gene (Table 1). The *PRNP* genotyping was performed using standard PCR amplification and sequencing protocols as previously described (Serrano et al., 2007). Both control and scrapie sheep were collected from different herds depending on the availability of the farmers to be kept afterwards in a flock from the Centre of Encephalopathies and Emerging Transmissible Diseases (CEETE; University of Zaragoza), maintained for research purposes. The naturally infected scrapie sheep were selected from different herds by observation of disease-related symptoms after confirmation by the regional health authorities of a disease outbreak in these herds. After culling, the disease status was confirmed using two rapid diagnostic tests (Prionics-Check Western blotting; ThermoFisher Scientific and Idexx HerdChek; IDEXX, Westbrook, ME, USA) and by immunohistochemical examination of CNS tissue as previously described (Bolea et al., 2005). All animals were fed with the same type of diet, which included commercial feed and ad libitum straw.

Faecal samples from both groups of animals were collected from the rectal ampoule with sterile gloves under the same conditions (same day and time of day) to ensure consistency and comparability between groups and immediately frozen at −80 °C to stop bacterial growth and preserve DNA content.

All procedures were performed under Project License PI17/21

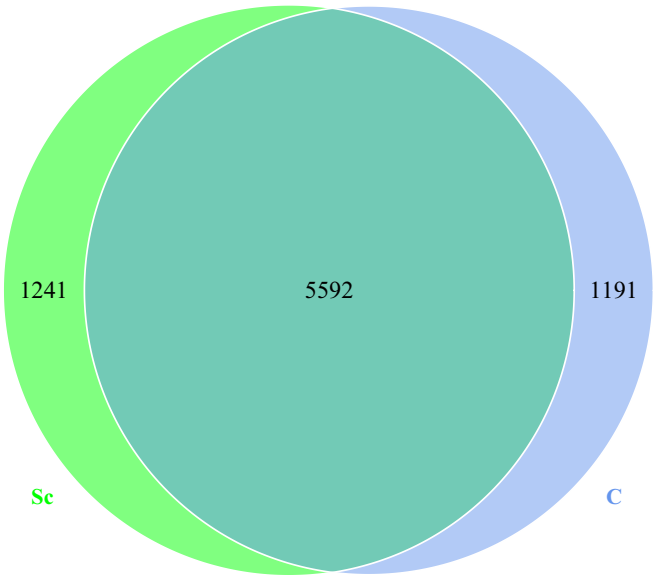

Fig. 1. Venn diagram showing the common and uniquely identified OTUs in scrapie (Sc) and control (C) groups.

approved by the Ethic Committee for Animal Experiments from the University of Zaragoza. The care and use of sheep were conducted accordingly to the Spanish Policy for Animal Protection RD53/2013 which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

2.2. Bacterial DNA extraction

Bacterial DNA was extracted from faecal samples using the NZY Soil gDNA Isolation kit (NZYTech, Lisboa, Portugal) following the manufacturer's instructions. Faecal samples were mixed with 700 µL NSL1 buffer in NZYSpin Soil Bead Tubes and processed by using the Precellys® 24 homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) for 2 cycles of 30 s at 6500 rpm and 10 s delay between cycles. After elution of the DNA, the concentration of the DNA was measured with a Qubit® 4.0 fluorometer (Invitrogen, Thermo Fisher Scientific, MA, USA). DNA purity was assessed with a NanoDrop® ND-1000 Spectrophotometer V3.0.1 (Thermo Scientific, MA, USA) and monitored on 1 % agarose gels.

2.3. Library preparation and sequencing of bacterial 16S rRNA gene

Depending on the concentration, DNA was diluted to 1 ng/µL using sterile water. The V4 region of the 16S rRNA gene, which is a widely used and validated target for microbial community profiling (Hockney et al., 2022; Tang et al., 2017; Thompson et al., 2017; Walters et al.,

Table 2
Alpha diversity indices of the control and scrapie group. Results are shown as mean ± SD (standard deviation) along with the statistical p-value between the two groups.

Control group			Scrapie group		
Index	Mean	SD	Mean	SD	p-value
Observed species	2795.7	546.4	2911.5	450.3	0.612
Shannon	9.019	0.625	9.407	0.202	0.089
Simpson	0.992	0.008	0.996	0.001	0.138
Chao1	3468.4	615.4	3528.9	736.4	0.844

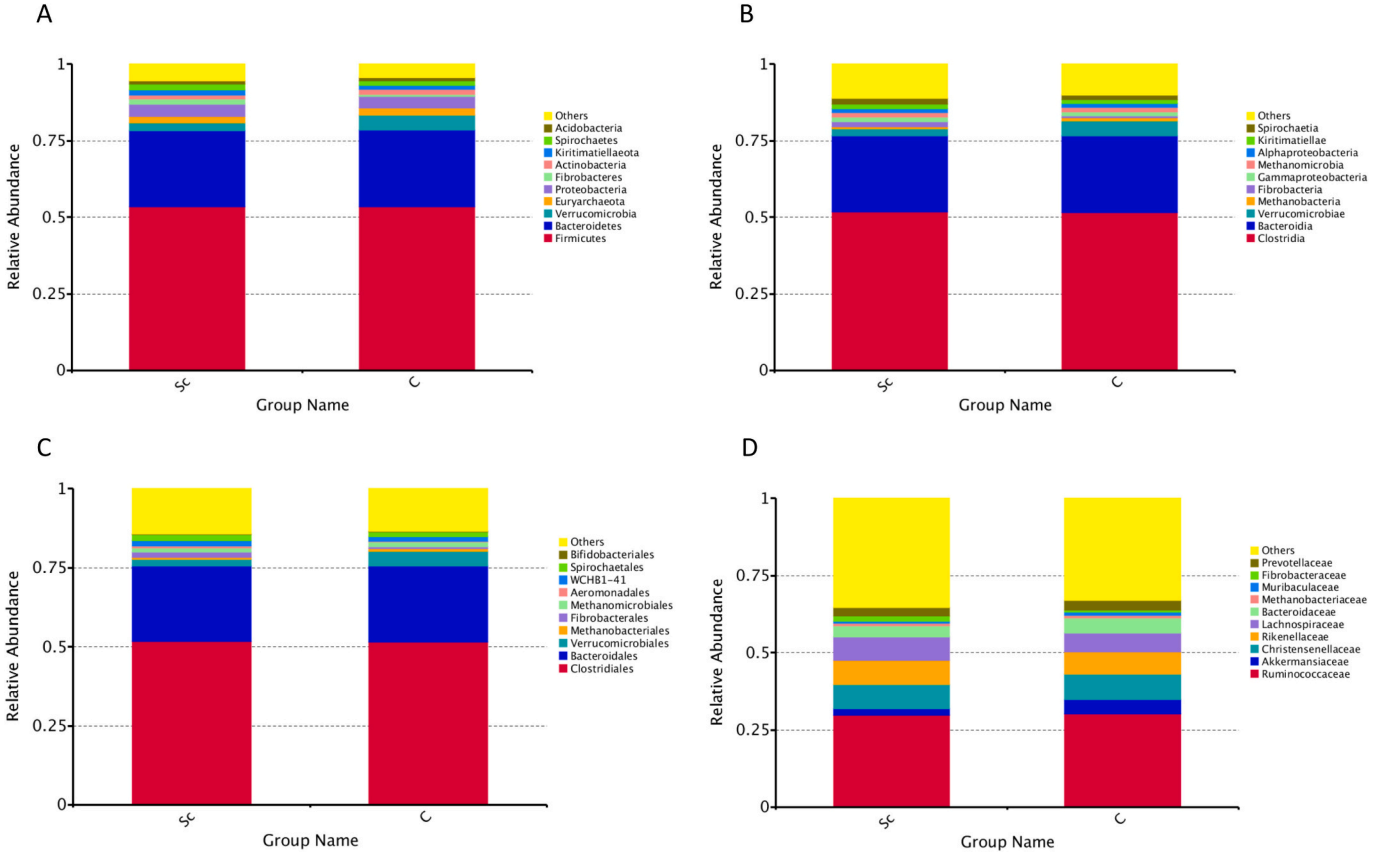

Fig. 2. Relative abundance of the ten most abundant phyla (A), classes (B), orders (C), families (D), and genera (E) in the scrapie (Sc) and control (C) group.

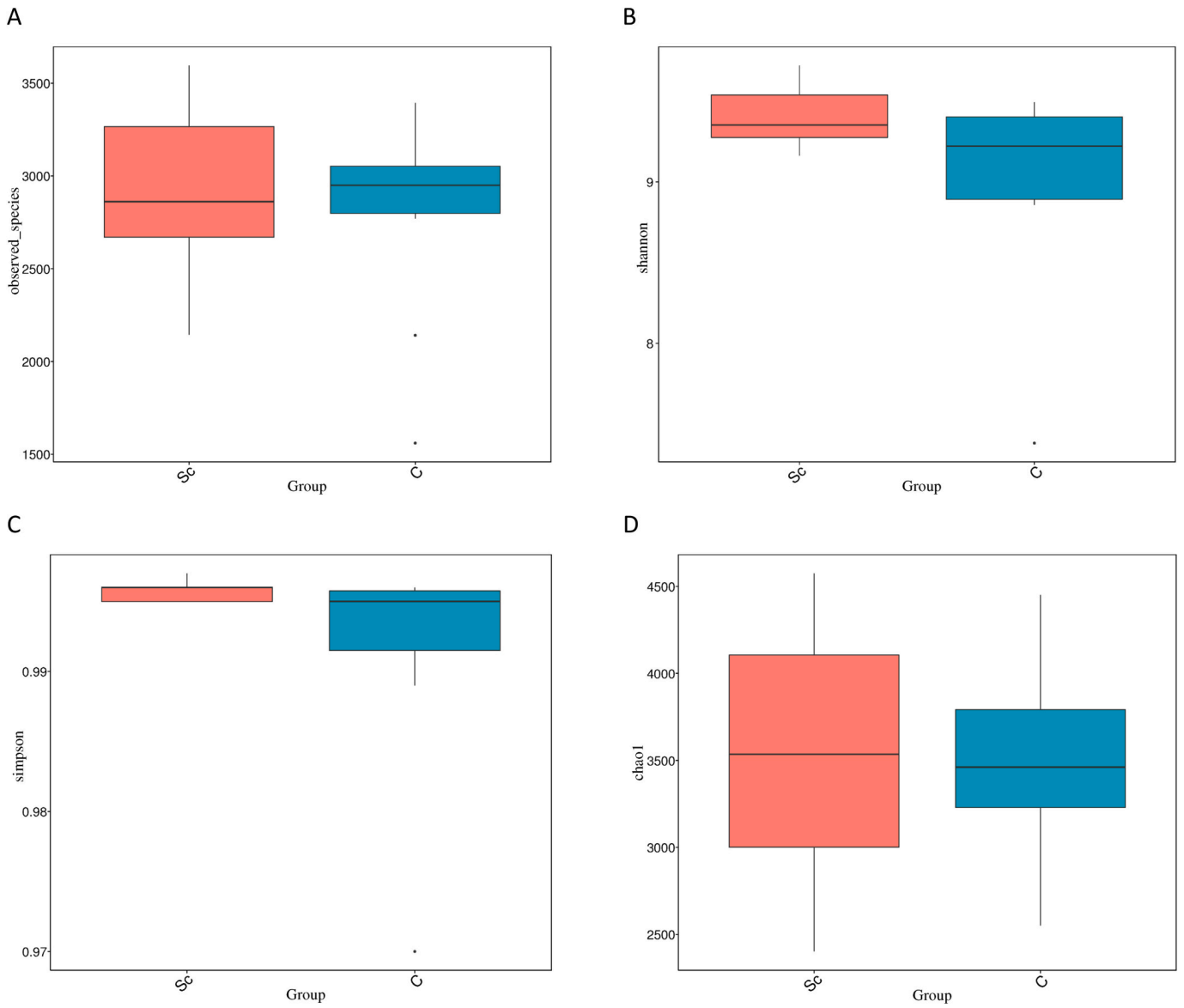

Fig. 3. Boxplots showing alpha diversity indices (A) observed species, (B) Shannon, (C) Simpson and (D) Chao1 in scrapie (Sc) and control (C) groups.

2016), was amplified using a specific primer (515F-806R) (Caporaso et al., 2011) with a barcode. PCR reactions were performed using the Phusion® High-Fidelity PCR Master Mix (New England Biolabs, UK). PCR products were then mixed with the same volume of 1 × loading buffer containing SYBR green, and amplicons were detected by electrophoresis on 2 % agarose gel. Samples with a bright main strip between 400 and 450 bp were selected for further experiments. PCR products were mixed in equal density ratios and the resulting mixture was purified with the Qiagen Gel Extraction Kit (Qiagen, Germany).

Sequencing libraries were generated with the NEBNext® UltraTM DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA), following the manufacturer's recommendations. The library quality was assessed with the Qubit 2.0 Fluorometer and Agilent Bioanalyzer 2100 system (Agilent, Santa Clara, CA, USA). Afterwards, the library was sequenced on an Illumina MiSeq platform and 250 bp paired-end reads were generated. The sequence data are available at NCBI Sequence Read Archive (SRA), BioProject ID PRJNA1161989.

2.4. Bioinformatic analysis

Paired-end reads were merged using FLASH analysis tool (v1.2.7) (Magoč and Salzberg, 2011), and the splicing sequences were called raw tags. Quality filtering of the raw tags was performed with QIIME (v1.7.0) (Caporaso et al., 2010) to obtain the high-quality clean tags (Bokulich et al., 2013). The clean tags were then compared with the reference database (Gold database) using UCHIME algorithm (Edgar et al., 2011) to detect chimera sequences. Finally, the chimera sequences were removed (Haas et al., 2011) to obtain the effective tags.

All effective tags were used to perform sequence analysis with Uparse software (v7.0.1001) (Edgar, 2013). Sequences with ≥97 % similarity were assigned to the same Operational Taxonomic Units (OTUs). The representative sequence for each OTU was screened for further annotation. For each representative sequence, Mothur software was performed against the SSUrRNA database of SILVA Database (Quast et al., 2013) for species annotation at each taxonomic rank (Threshold: 0.8–1) (Wang et al., 2007). To obtain the phylogenetic relationship of all OTUs representative sequences, the MUSCLE program (v3.8.31) (Edgar, 2004) was used. OTUs abundance information was normalised using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analyses of alpha and beta diversities were performed based on this output of normalised data.

Alpha diversity was used to analyse within-groups microbial

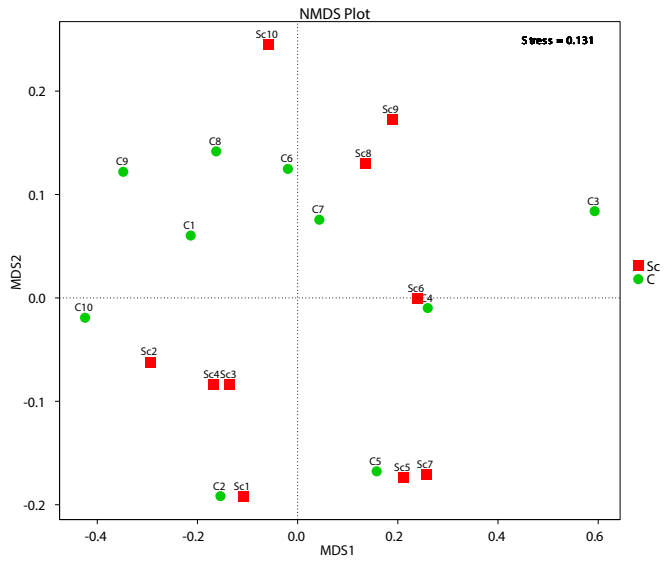

Fig. 4. Non-metric Multidimensional Scaling (NMDS) plot of scrapie (Sc) and control (C) samples.

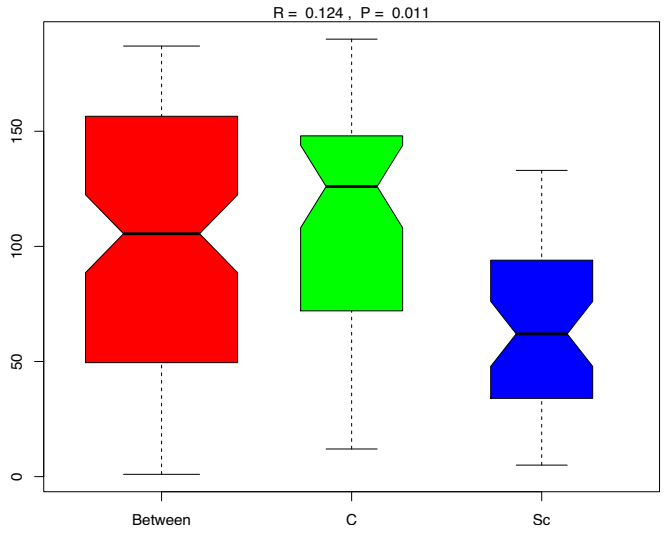

Fig. 5. Analysis of similarity (Anosim) displaying significant differences in the faecal bacterial communities between the control (C) and scrapie (Sc) group.

diversity. Observed species, Shannon, Simpson and Chao1 indices were calculated with QIIME (v1.7.0) and displayed with R software (v2.15.3). The differences in the alpha diversity indices between groups were analysed by *t*-test and Wilcoxon test ($p < 0.05$).

To study the beta diversity and estimate the dissimilarities between groups, a Non-metric Multidimensional Scaling (NMDS) analysis based on OTUs was represented and an Analysis of similarity (Anosim) was conducted using the vegan R package. To determine those taxa whose abundance varied significantly between groups, a t-test was carried out at various taxon ranks. The *p*-values obtained in the t-test were corrected for multiple comparisons using the Benjamini-Hochberg FDR correction, and adjusted p-values (q-values) ≤ 0.1 were considered statistically significant.

3. Results

3.1. Sequencing data metrics

The average of raw sequences found in all samples was 120,579 ± 22,540.35. After quality control and chimera removal, the average of high-quality sequences in the samples was 93,246 ± 17,398.73, which was indicative of a good sequencing performance.

The total number of identified OTUs was 8024, being 5592 shared between the two groups, 1191 only present in the control group and 1241 uniquely present in the scrapie group (Fig. 1).

3.2. Composition of gut microbiota in scrapie and control sheep

In terms of relative abundance, the phyla *Firmicutes* and *Bacteroidetes* were the most abundant phyla in the scrapie and control groups (Fig. 2a). The classes *Clostridia* and *Bacteroidia* (Fig. 2b), and the orders *Clostridiales* and *Bacteroidales* (Fig. 2c) were also the most abundant in both groups.

Within the phylum *Firmicutes*, *Ruminococcaceae* was the most abundant family, followed by the families *Lachnospiraceae* and *Christensenellaceae*. Other families, *Fibrobacteraceae*, *Rikenellaceae*, *Bacteroidaceae*, *Muribaculaceae* and *Prevotellaceae*, belonging to the phylum *Bacteroidetes, Akkermansiaceae* within the phylum *Verrucomicrobia* and *Methanobacteriaceae* within the phylum *Euryarcheota* were also present (Fig. 2d). Regarding the genera, *Rumincococcaceae_UCG-010*, *Ruminococcaceae_UCG-005* and *Christensenellaceae_R-7_group* were the most abundant genera in control and scrapie sheep (Fig. 2e).

3.3. Alpha diversity

As shown in Table 2 and Fig. 3, alpha diversity indices indicated that there was microbial diversity in the control and scrapie group, but no significant differences were observed between the two groups. Only the Shannon index displayed a trend to signification ($p = 0.089$).

3.4. Beta diversity

To assess the overall difference of bacterial community between the control and scrapie groups, NMDS and Anosim analyses were performed. The NMDS plot showed a reliable representation of inter-sample variation (stress = 0.131) with no clear separation between the two groups of samples (Fig. 4). Anosim analysis ($R = 0.124$, $p = 0.011$), however, showed significant bacterial community differences between the scrapie and control group (Fig. 5).

Specifically at phylum, class and order level, *t*-test analysis evidenced significant differences between the two groups. Regarding the phyla, an abundance increment of *Cyanobacteria* (q = 0.03) and *Lentisphaerae* (q = 0.1) was observed in scrapie-affected animals compared to the control group (Fig. 6a). The classes *Melainabacteria* (q = 0.06) and *Lentisphaeria* (q = 0.06) (Fig. 6b), and the orders *Gastranaerophilales* (q = 0.1) and *Victivallales* (q = 0.1) (Fig. 6c) were also more abundant in the scrapie group compared to the control one. Within the phylum *Lentisphaerae*, the family *Victivallaceae* was found to be more abundant in the scrapie group, being this increment statistically significant in terms of *p*-value ($p = 0.001$), but not after FDR correction.

4. Discussion

Dysbiosis in gut microbiota composition has been linked to different neurodegenerative diseases including prion-like diseases such as PD and AD (Heravi et al., 2023; Zhang et al., 2020; Zhuang et al., 2018), and prion diseases such as CJD (Guo et al., 2022; Kong et al., 2023) and CWD (Didier et al., 2024; Minich et al., 2021). Although two recent studies have reported changes in the gut microbiota of mice intracerebrally inoculated with scrapie prions (Losa et al., 2024; Yang et al., 2020b), to our knowledge no studies in a natural model of scrapie disease have been performed to date.

In the present work, a 16S rRNA sequencing of faecal samples from sheep naturally infected with classical scrapie and control sheep was performed to compare the gut microbiota composition between the two

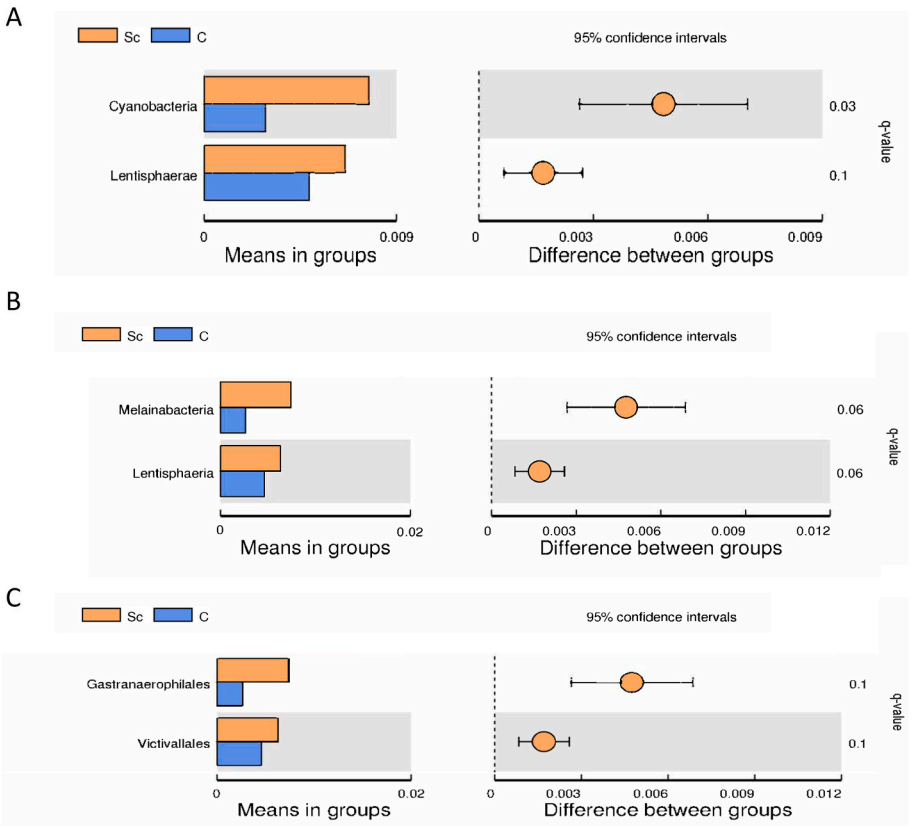

Fig. 6. *t*-test analysis results at phylum (A), class (B) and order (C) level between scrapie (Sc) and control (C) groups. The left panel shows the abundance of each specific taxa, representing each bar the abundance mean value in each group. The right panel displays the confidence interval of between-group variation: in each circle, the left-most part stands for the lower limit of the 95 % confidential interval, while the right-most part is the upper limit. The centre of the circle is the difference of the mean value. The colour of the circle agrees with the group whose mean value is higher. The right-most value represents the q-value.

groups and identify alterations that could be associated with scrapie disease.

In terms of relative abundance, as previously observed in other studies of gut microbiota from sheep (Mamun et al., 2020; Tanca et al., 2017; Wang et al., 2021; Yang et al., 2020a), *Firmicutes* and *Bacteroidetes* were the most prevalent phyla in both scrapie and control sheep. These findings are in accordance as well with other studies performed in AD, PD and CJD patients (Heravi et al., 2023; Kong et al., 2023; Li et al., 2017; Ubeda et al., 2022; Zhang et al., 2020; Zhuang et al., 2018) and in CWD-affected deer (Didier et al., 2024), in which these two phyla were also the most abundant in healthy and diseased individuals. In addition, the richness in scrapie and control sheep were similar, with no significant differences in the alpha diversity metrics. No differences in alpha diversity between case and control groups were also found in CWD studies (Didier et al., 2024; Minich et al., 2021). Conversely, in CJD patients (Guo et al., 2022; Kong et al., 2023), a significant increase in species richness was found and in scrapie-infected mice (Yang et al., 2020b), a decrease in richness and diversity was observed. Different results have also been found in AD and PD, with some studies reporting no changes in the alpha diversity between patients and controls (Baldini et al., 2020; Heravi et al., 2023; Xi et al., 2021), and others reporting higher or lower alpha diversity in PD (Barichella et al., 2019; Pietrucci et al., 2019) and lower alpha diversity in AD (Chen et al., 2022; Ubeda et al., 2022).

In relation with beta diversity, we observed that the gut bacterial profile of scrapie-infected sheep differed significantly from that of the control group. Similar changes have been reported in patients with AD (Chen et al., 2022; Ubeda et al., 2022), PD (Scheperjans et al., 2015; Zhang et al., 2020) and CJD (Guo et al., 2022), in deer with CWD (Didier et al., 2024; Minich et al., 2021) and in scrapie-inoculated mice (Losa et al., 2024; Yang et al., 2020b), suggesting a possible trend worth further investigation.

Furthermore, the phyla *Cyanobacteria* and *Lentisphaerae* were significantly increased in scrapie animals. *Cyanobacteria* are gram-negative photosynthetic bacteria that inhabit terrestrial environments and fresh, transitional, and marine ecosystems, and include species that produce cyanotoxins (Moreira et al., 2013). These cyanotoxins are grouped in neurotoxins, hepatotoxins, cytotoxins, irritants, and gastrointestinal toxins (Sini et al., 2021). The exposure to cyanotoxins through different routes (orally, dermally or by inhalation) causes acute or chronic poisonings of animals and humans (Drobac et al., 2013). Among the different types of neurotoxins, the neurotoxin β-*N*-methylamino-L-alanine (BMAA) could play an important role in prion-like neurodegenerative diseases (Nunes-Costa et al., 2020; Sini et al., 2021). In fact, BMAA has been detected in brain proteins from PD, AD, and amyotrophic lateral sclerosis (ALS) patients but not in healthy individuals (Dunlop et al., 2013) and in the cerebrospinal fluid of ALS patients (Berntzon et al., 2015). Moreover, rats treated with BMAA display ALS-like neurological impairment (De Munck et al., 2013). BMAA could also be associated with the proinflammatory profile, and the mitochondrial dysfunction observed in neurodegenerative diseases. BMAA caused a decrease in oxidative phosphorylation, altered calcium homeostasis and exacerbated reactive oxygen species (ROS) production in a cell line of motor neurons in vitro (Beri et al., 2017) and administration of this toxin to rats was able to reproduce the neuronal phenotype of ALS, along with the expression of proinflammatory cytokines (Michaelson et al., 2017). Although our taxonomic resolution did not allow determining specific toxin-producing strains, the observed increase in *Cyanobacteria* in scrapie-affected sheep prompts further investigation into potential neuroactive metabolites that may contribute to the disease

pathogenesis. Further metagenomic studies with higher taxonomic resolution are needed to confirm any potential mechanistic roles.

On the other hand, an abundance increment of *Lentisphaerae,* a member of the *Planctomycetes–Verrucomicrobia–Chlamydiae* superphylum (Cho et al., 2004), has also been reported in the gut microbiota of PD patients, with a potential association of this phylum with a protective effect against PD (Ning et al., 2022). Further research is needed to unveil the possible role of these microorganisms in the pathogenesis of prion and pion-like neurodegenerative diseases.

It is important to mention some limitations in our study. Compared to other studies, the sample size was relatively small. Therefore, our findings need to be validated in a larger cohort of animals. Moreover, we were not able to establish a causal relationship between scrapie disease and the changes observed in the gut microbiota, as faecal samples were obtained from clinical animals with clear manifested symptoms. To verify the possible causal effect of these microbiota alterations, future longitudinal studies involving preclinical animals will be needed. Finally, using the 16S rRNA sequencing method limited the ability to analyse bacterial composition at the species or strain level, which is key for identifying the specific bacteria associated with scrapie. Addressing these limitations in future studies is crucial to understand the role of the gut microbiota and its underlying mechanisms in neurodegenerative diseases.

Nevertheless, we have reported for the first time a gut microbiota imbalance in sheep infected with classical scrapie, with an increased abundance of *Cyanobacteria* and *Lentisphaerae* phyla in scrapie-infected animals. Although no studies in other prion diseases have shown before changes in the abundance of these two phyla, studies in prion-like neurodegenerative diseases evidence a possible involvement of these microorganisms in the pathogenesis of these diseases. Our study contributes to the understanding of the role of the gut microbiota in prion diseases and provides new directions for further research on the pathogenesis of scrapie.

CRediT authorship contribution statement

Adelaida Hernaiz: Writing – original draft, Visualization, Investigation. **Laura Grasa:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition. **Paula De Diego Abejón:** Writing – review & editing, Investigation. **Celia León Huertas:** Writing – review & editing, Investigation. **Belén Marín:** Writing – review & editing, Investigation. **Juan José Badiola:** Writing – review & editing, Resources, Funding acquisition. **Rosa Bolea:** Writing – review & editing, Resources, Funding acquisition. **Pilar Zaragoza:** Writing – review & editing, Resources, Funding acquisition. **Inmaculada Martín-Burriel:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Funding

This work was supported by the Strategic Research Lines (LEI) “One Health” and “Gut Microbiota in Health and Nutrition” of the Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA), the Gobierno de Aragón consolidated research groups A19_20R and A20_23R, and by the Servicio Público de Empleo Estatal (PROGRAMA INVESTIGO-100-76) within the framework of the Recovery, Transformation and Resilience Plan (European Union-NextGenerationEU).

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This study has the support of the network REZGEN-IBA - Red iberoamericana sobre los recursos zoogenómicos y su resiliencia (123RT0139) financed by CYTED.

Data availability

The sequencing data are available at NCBI Sequence Read Archive (SRA), BioProject ID PRJNA1161989.

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