From diversity to disease; meta-transcriptomic insights into the calf enteric microbiome

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Microbial communities in dairy calves significantly impact their health, influencing the occurrence of enteric and respiratory diseases. A deeper understanding of microbial diversity in health and disease is critical for improving diagnostic and treatment approaches. This study aimed to explore the microbial diversity and gene expression patterns of various virulence and antimicrobial factors in rectal samples from dairy calves with varying health conditions and four age groups (from 0-7, 8-14,15-42 and >42 days old) sampled across 72 farms in New South Wales, Australia. A total of 503 rectal samples including 144 from animals with diarrhoea were analysed using high-throughput RNA sequencing. Bacterial taxonomy was assessed using MetaPhlAn-4, calculating alpha and beta diversity. Virulence factors and antimicrobial resistance gene expression were also assessed within each age group and disease status. The analysis revealed a complex microbial landscape characterised by approximately 1,000 distinct bacterial genera with top genera including *Anaerovibrio*, *Prevotella*, and *Segatella*. Healthy calves had significantly higher diversity compared to symptomatic animals. Differential expression analyses showed that although antimicrobial resistance genes (e.g., *cfxA*, *Sul2*, *tet*(Q), *dfrA12*, and *APH(6)-Ia*) were present across different age groups, they were significantly more abundant in diarrhoeic calves. Similarly, virulence factors such as afaE VIII, espB, espD, ompA 28, and PorA were distinctly elevated in symptomatic animals compared to asymptomatic ones. Correlation network analysis further demonstrated age-dependent microbial interactions. *Escherichia* and *Campylobacter* showed consistent positive correlations with several virulence factors and host expression genes across groups. Each age group exhibited distinct microbial, viral, and parasitic compositions, with the 15–42-day group showing particularly strong correlations between Bovine enterovirus and uncultured bacteria. Together, by identifying age-specific microbes and resistance patterns, this study provides potential targets for improving diagnostics, guiding antimicrobial use and developing management strategies to support calf health and reduce disease burden in dairy herds.

Keywords: metatranscriptomics, virulence factors, enteric disease