Semi-quantitative analysis of Ruminococcus flavefaciens, Fibrobacter succinogenes and Streptococcus bovis in the equine large intestine using real-time polymerase chain reaction

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There is a need to further our understanding of the role that the equine hindgut ecosystem plays in digestive processes and diseases. The aim of the present study was to utilise the real-time PCR technique to determine the abundance of candidate cellulolytic (Ruminococcus flavefaciens; Fibrobacter succinogenes) and non-cellulolytic (Streptococcus bovis) bacteria in lumen contents from the caecum, ventral and dorsal colon, and rectum of healthy horses (n 14). Total DNA was extracted from frozen and lyophilised lumen contents, and PCR primers and Taqman® probes were designed based on 16S rDNA sequences for specific detection of candidate bacterial species. Overall, in frozen and lyophilised digesta, there were significantly (P<0·01) fewer candidate bacteria in the caecum than the dorsal colon and rectum. In frozen digesta, candidate bacteria levels were similar between the ventral colon, dorsal colon and rectum, but in lyophilised digesta there were significantly (P<0·05) higher levels of bacteria in the dorsal colon and rectum. Frozen digesta contained disparate levels of candidate bacteria such that R. flavefaciens > F. succinogenes > S. bovis (P<0·05), while in lyophilised digesta R. flavefaciens was present in significantly (P<0·05) greater amounts than F. succinogenes and S. bovis. R. flavefaciens and F. succinogenes were abundant at significantly (P<0·05) greater levels in lyophilised digesta v. frozen digesta, with no difference in S. bovis levels. These data indicate that for these bacteria at least, faeces are a suitable model for studying the bacterial ecosystem within the equine colon. The present study also indicates that the preservation method of digesta affects levels of bacteria detected.

Horses: Intestine: Bacteria: Real-time polymerase chain reaction

Horses have evolved from ancestors with an intestinal system designed to process large quantities of low-quality forage, containing high levels of structural plant polysaccharides, ingested on an almost continuous basis to meet their nutrient demands. The large intestine (hindgut) of the horse is anatomically specialised to accommodate microorganisms capable of degrading and fermenting structural polysaccharides of the plant cell wall, which are generally resistant to pre-caecal digestion1). The fermentation of feed-stuffs in the hindgut results in the production of volatile fatty acids that, when absorbed, constitute a significant proportion (30%) of the digestible energy intake of the animal; particularly in horses fed high-fibre diets2). Fibre-based diets are known to maintain normal fermentation conditions within the large intestine whilst, in contrast, diets containing high levels of concentrates (starch) can be detrimental to the maintenance of a homeostatic hindgut environment3), and can lead to a number of metabolic disorders such as acidosis and laminitis4–7).

Despite the importance of the intestinal microbial ecosystem in many aspects of host animal health and performance in other species, particularly ruminants, there is a dearth of information regarding the microbial ecology of the equine hindgut. A greater understanding of the microbial diversity of the hindgut is essential for improving our knowledge of digestive processes, and for the future prevention and treatment of diseases involving the gastrointestinal tract, for example, laminitis and grass sickness. However, knowledge of the bacterial populations present in the large intestine of the horse is very limited, compared, for example, with the rumen8) and the caecum and colon of humans and pigs9,10). Current knowledge of gut microbial ecology and diversity is almost exclusively based on the use of classic culture-based methods that are often laborious, time consuming and may only recover a fraction of the microbial diversity present within the gut11). However, advanced modern molecular methods, such as real-time semi-quantitative PCR (Q-PCR), are culture-independent tools for accurate and sensitive

Abbreviations: Cx, cycle threshold; Q-PCR, semi-quantitative PCR.
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quantification of individual bacterial species as well as total bacterial numbers\(^{12,13}\). Limited studies have reported the bacterial diversity within the large intestine of the horse using more conventional molecular methods such as end-point PCR or use of oligonucleotides\(^{11,14,15}\), with these studies identifying the fibrolytic bacteria *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* predominating. In these early hybridisation studies investigating the equine hindgut, some authors have lyophilised the material in order to account for the DM increase along the intestinal tract\(^{16}\), whilst others have extracted DNA from frozen material\(^{11,15}\). However, it is unclear if the preservation method affects the data obtained from studies using PCR methodologies and whether this should be considered when interpreting results from frozen and lyophilised material.

Furthermore, to our knowledge, there are no published data on the identification or quantification of equine intestinal bacteria using real-time PCR technology, which is a more accurate and sensitive alternative to conventional end-point PCR-based methodologies, and has recently been applied to study diet-dependent shifts in the bacterial populations of the rumen\(^{17}\) and infant gut\(^{17}\). Moreover, previous studies investigating microbial diversity and fermentation characteristics within the equine hindgut typically used animals specifically euthanased for the purpose, or surgically modified animals. Whilst these methods have provided important insights into the equine microbial ecosystem, they can be expensive and there is a consensus nowadays to adopt, where possible, cost-effective welfare-friendly alternatives.

Consequently, the objectives of the work reported here were to: (1) optimise real-time Q-PCR methodologies for quantifying changes in relative amounts of *R. flavefaciens*, *F. succinogenes* (fibrolytic bacteria) and *Streptococcus bovis* (non-fibrolytic bacterium) in the luminal contents of the equine caecum, ventral colon, dorsal colon and rectum; (2) compare the relative amounts of these candidate bacteria in frozen and lyophilised samples; (3) establish whether faeces are a suitable model of hindgut function in the horse. The candidate bacteria are likely to play key roles in equine digestion and health, given that *F. succinogenes* and *R. flavefaciens* are key fibrolytic bacteria, whilst the saccharolytic bacterium *S. bovis* has been proposed as having a role in hindgut acidosis and laminitis\(^{7,14,18}\).

**Materials and methods**

**Collection and processing of samples**

Samples of luminal contents (caecum; ventral colon; dorsal colon; rectum) were taken from fourteen freshly slaughtered horses (unknown age and breed), not suffering from any known intestinal diseases, obtained from the local abattoir. Upon recovery, lumens contents were placed in individually labelled grip-top bags and immediately placed on dry ice. At the laboratory, each sample was sub-divided into two groups; one of which remained frozen and was stored at \(-80^{\circ}C\) until required, whilst the other was lyopholised to constant weight before storage at \(-80^{\circ}C\). Quantification was carried out in luminal contents only, as previous work has established that microbial community structure between the hindgut wall and lumen contents is not different in equines\(^{13}\).

**DNA extraction**

Total DNA extraction from frozen and lyophilised luminal samples was carried out using the QIAamp\(^{\circledR}\) DNA stool kit (Qiagen Ltd, Crawley, West Sussex, UK). This purifies genomic, bacterial, viral and parasite DNA from stool samples and was used in accordance with the manufacturer’s recommendations, with some modifications. The following procedure was carried out for each sample of frozen and lyophilised lumen contents.

Lumen contents (180–220 mg) were homogenised in 1·4 ml of buffer ASL using a RiboLyser (Hybaid Ltd, Ashford, Middlesex, UK). Following homogenisation each sample was heated at 95\(^{\circ}C\) for 5 min to lyse the bacteria, and then centrifuged for 3 min at 14 000 g. The supernatant fraction was removed and placed into a microcentrifuge tube where it was vortexed with an InhibitEX tablet for 1 min, or until the tablet was completely suspended. The suspension was then incubated for 1 min at room temperature to allow potential PCR inhibitors or DNA-degrading substances to absorb to the InhibitEX matrix. The InhibitEX reagent was then pelleted by centrifugation for 3 min at 14 000 g, after which 200 \(\mu\)l of the supernatant fraction was then removed and placed in a new microcentrifuge tube containing 15 \(\mu\)l proteinase K. Then 200 \(\mu\)l buffer AL was added and the mixture was thoroughly vortexed for 15 s. After further heating at 70\(^{\circ}C\) for 10 min to allow protein digestion and degradation under denaturing conditions, 200 \(\mu\)l absolute ethanol was added. The resultant mixture was then loaded onto a QIAamp\(^{\circledR}\) spin column (Qiagen Ltd) and centrifuged for 3 min at 14 000 g. The DNA bound to the spin column was then washed in two centrifugation steps, first with 500 \(\mu\)l of buffer AW1 followed by 500 \(\mu\)l buffer AW2, at 14 000 g for 1 min and 3 min, respectively. Finally, purified DNA was eluted from the spin column in 200 \(\mu\)l of buffer AE by allowing it to incubate for 1 min at room temperature, followed by centrifugation at 14 000 g for 1 min. DNA was stored at \(-20^{\circ}C\) until required for real-time PCR.

**Real-time polymerase chain reaction**

Semi-quantitative real-time PCR was performed on extracted DNA from the frozen and lyophilised luminal contents for *R. flavefaciens*, *F. succinogenes*, *S. bovis* and total bacterial load, using the MX3000P Q-PCR system (Stratagene Ltd, Cambridge, Cambs, UK). The PCR reaction contained 10 \(\times\) Thermo-Start\(^{\circledR}\) standard buffer, 25 mM-MgCl\(_2\), 5 mM each dNTP, Thermo-Start\(^{\circledR}\) DNA polymerase (Abgene Ltd, Epsom, Surrey, UK), 300 mM each forward and reverse primer, 200 mM probe, DNA template (from frozen or lyophilised contents) and molecular biological-grade water (BDH, Poole, Dorset, UK). Thermal cycling conditions were 2 min at 50\(^{\circ}C\) followed by 10 min at 95\(^{\circ}C\) and forty cycles of 15 s at 95\(^{\circ}C\) and 2 min at 60\(^{\circ}C\). Samples were run in duplicate for each quantification assay.

Tagman\(^{\circledR}\) probes and oligonucleotide primers for *R. flavefaciens*, *F. succinogenes* and *S. bovis* were designed using Primer Express\(^{\circledR}\) software (PE Applied Biosystems, Warrington, Ches, UK). Probe and primer sets were designed based on *R. flavefaciens*, *F. succinogenes* and *S. bovis* 16S rDNA sequences published in GenBank\(^{\circledR}\). Probes and primers were
tested for specificity using the Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information (NCBI), Bethesda, MD, USA). A previously published universal primer and probe set was used for the determination of the total bacterial load\(^{13}\). The probes and primers were synthesised by MWG-Biotech AG (Ebersberg, Germany). All probes contained 6-carboxy-fluorescein as the 5’ reporter and 6-carboxy-tetramethyl-rhodamine (TAMRA) as the 3’ quencher. Details of primers and probes are given in Table 1.

For relative quantification of *R. flavefaciens*, *F. succinogenes* and *S. bovis* the comparative cycle threshold (CT; Fig. 1) method was used\(^{19}\), which involved normalisation of the number of target copies to total bacterial load (universal). The \(\Delta\text{CT}\) was first calculated (universal mean CT – specific bacteria mean CT). The normalised level of abundance was calculated using the formula 
\[
\frac{1}{1.78^{\Delta\text{CT}}},
\]
where 1.78 was derived from Nadkarni *et al.*\(^{13}\) as the response to standard amounts of DNA obtained with the universal primer and probe set. As the target bacteria were generally represented in small proportions relative to total bacterial load, data were transformed by multiplying by 1000 to allow for ease of data handling. Validation studies were carried out to demonstrate that the amplification efficiencies of the universal primers and probe set and specific bacteria were equivalent. This involved generating relative standard curves for each primer and probe set using serial dilutions of purified DNA. The \(\Delta\text{CT}\) (\(y\)) between the universal set and each specific bacterium was plotted v. log (dilution; \(x\)) to calculate the slope of the line (by linear regression analyses). Slopes for *R. flavefaciens*, *F. succinogenes* and *S. bovis* were all \(\pm 0.1\) as required (Fig. 2).

### Statistical analyses

Data generated from real-time PCR were not normally distributed; a logarithmic (base 10) transformation was therefore performed on all of the data before statistical analysis. Values for the relative amounts of bacteria in the various regions of the hindgut were analysed for significant differences using two-way ANOVA in GenStat\textsuperscript{w} release 9.1 (Lawes Agricultural Trust, Harpenden, Herts, UK). This was done separately for both the frozen and lyophilised material. Values for bacterial species and preservation treatment (frozen or lyophilised) were also analysed for significant differences using two-way ANOVA. Comparisons between treatment groups were made by least significant difference equations. \(P\) values of \(<0.05\) were considered statistically significant.

### Results

The quantification of *R. flavefaciens*, *F. succinogenes* and *S. bovis*, involving the normalisation of the number of target copies to total bacterial load, confirmed the application of real-time PCR to successfully detect *R. flavefaciens*, *F. succinogenes* and *S. bovis* from the equine hindgut (Fig. 1). The relative quantification of *R. flavefaciens*, *F. succinogenes* and *S. bovis* 16S rDNA extracted from the large intestine of fourteen healthy horses confirmed that these bacterial species are all abundant at detectable levels throughout the equine hindgut, differing in relative quantification from region to region.
Analysis of real-time data showed no significant interaction between bacterial species and hindgut region; therefore, main effects were examined in isolation. Data derived from real-time PCR revealed that region of the equine hindgut significantly affected the overall bacterial load of \( \textit{R. flavefaciens} \), \( \textit{F. succinogenes} \) and \( \textit{S. bovis} \) in both frozen \( (P=0.011; \text{Table 2}) \) and lyophilised \( (P<0.001; \text{Table 3}) \) luminal contents. Overall, caecal samples had significantly \( (P<0.01) \) fewer \( \textit{R. flavefaciens} \), \( \textit{F. succinogenes} \) and \( \textit{S. bovis} \) than were present in the luminal contents of the ventral colon, dorsal colon and rectum in both the frozen and lyophilised samples. However, similar candidate bacterial loads were observed between the luminal contents of ventral colon, dorsal colon and rectum in the frozen samples \( (\text{Table 2}) \). In contrast, in the lyophilised samples significantly \( (P<0.01) \) fewer \( \textit{R. flavefaciens} \), \( \textit{F. succinogenes} \) and \( \textit{S. bovis} \) were present in the luminal contents of the ventral colon \( (P<0.05; \text{Table 3}) \), compared with the dorsal colon and rectum, which were similar.

With respect to the three individual bacteria, \( \textit{R. flavefaciens} \) was the predominant bacterial species within each region of the equine hindgut sampled, for both the frozen and lyophilised material, and overall was present in significantly greater amounts than both \( \textit{F. succinogenes} \) \( (P<0.05) \) and \( \textit{S. bovis} \) \( (P<0.01) \) \( (\text{Tables 2 and 3}) \). However, in the frozen samples values for \( \textit{F. succinogenes} \) and \( \textit{S. bovis} \) were similar \( (\text{Table 2}) \), whilst in the lyophilised samples \( \textit{F. succinogenes} \) was present at significantly \( (P<0.05) \) higher levels than \( \textit{S. bovis} \) throughout each region of the hindgut \( (\text{Table 3}) \).

Further analysis of real-time data revealed a significant \( (P<0.001) \) interaction between bacterial species and preservation treatment \( (\text{Table 4}) \). Values for \( \textit{R. flavefaciens} \) and \( \textit{F. succinogenes} \) were significantly \( (P<0.05) \) higher in the lyophilised material compared with the frozen samples, whereas similar values were obtained for \( \textit{S. bovis} \) in both the frozen and lyophilised material.

\[ \text{Fig. 1. A typical example of amplification plots obtained during the real-time PCR reaction for the universal (––), \textit{Ruminococcus flavefaciens} (–), \textit{Fibrobacter succinogenes} (–), and \textit{Streptococcus bovis} (–). The horizontal line represents the threshold fluorescence and corresponds to the cycle threshold value for a given sample. DΔR, baseline-subtracted fluorescence.} \]
equine-specific universal probe and primer set could be designed. Nevertheless, the data reported here are semi-quantitative and for the first time compare *R. flavefaciens*, *F. succinogenes* and *S. bovis* relative to the total bacterial load throughout the various regions of the equine hindgut.

From the early hybridisation studies investigating the equine hindgut, some authors (14) have lyophilised the material in order to account for the DM increase along the intestinal tract. However, there is no information available in the literature on the effect of preservation method on data obtained from studies using PCR methodologies. One of the aims within the present study was to investigate the possible implications of lyophilising material before extracting the DNA. A very important finding in the present study was that significantly higher values were obtained for *R. flavefaciens* and *F. succinogenes* in the lyophilised material compared with the non-lyophilised samples, with no differences detected between preservation methods for *S. bovis*. It is unclear why these differences occurred; however, they may be attributed to differences in the liquid- and solid-associated bacteria. For instance, higher fibrolytic activities have been reported in the solid-associated bacteria in the equine hindgut (21). In addition, freezing per se is unlikely to explain the differences observed, as the freezing occurs in both methods of preservation. Rather, it is possible that differences in preservation method may also be attributable to enzymic degradation of DNA, which may be released from lysed cells during subsequent thawing of frozen material before sub-sampling. Consequently, the preservation method of samples appears to be an important consideration in the enumeration of bacteria using this methodology as this could potentially have implications for quantifying and comparing results obtained within and across studies. It is also important to note that data from frozen samples in the present study were comparable with previous reports in the literature (14), whilst the abundance of the three candidate bacteria determined from lyophilised material were markedly higher than previous reports.

![Fig. 2. Validation studies demonstrating the amplification efficiencies of the universal primer and probe set to those of *Ruminococcus flavefaciens* (A; *y* = −0.0401x), *Fibrobacter succinogenes* (B; *y* = 0.0093x) and *Streptococcus bovis* (C; *y* = 0.0486x). The slope of each line was calculated by linear regression analysis. The absolute value of the slope was close to zero (*y* = <0.1), therefore the efficiencies of the target and reference genes were similar. CT, cycle threshold.](image)

### Table 2. Semi-quantitative levels of *Ruminococcus flavefaciens*, *Fibrobacter succinogenes* and *Streptococcus bovis* in frozen luminal contents of the equine caecum, ventral colon, dorsal colon and rectum (*n* 14)*

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Hindgut region</th>
<th>Mean relative level</th>
<th>%</th>
<th>Mean relative level</th>
<th>%</th>
<th>Mean relative level</th>
<th>%</th>
<th>Region mean</th>
<th>Mean relative level</th>
<th>%</th>
<th>P</th>
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</tr>
<tr>
<td><em>R. flavefaciens</em></td>
<td>Caecum</td>
<td>1.767</td>
<td>5.85</td>
<td>1.529</td>
<td>3.38</td>
<td>1.426</td>
<td>2.67</td>
<td>1.574*</td>
<td>3.75</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Ventr al colon</td>
<td>1.987</td>
<td>9.71</td>
<td>1.826</td>
<td>6.70</td>
<td>1.748</td>
<td>5.60</td>
<td>1.854*</td>
<td>7.14</td>
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</tr>
<tr>
<td></td>
<td>Dorsal colon</td>
<td>1.891</td>
<td>7.78</td>
<td>1.676</td>
<td>4.74</td>
<td>1.745</td>
<td>5.60</td>
<td>1.771†</td>
<td>5.90</td>
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<tr>
<td></td>
<td>Rectum</td>
<td>1.969</td>
<td>9.31</td>
<td>1.743</td>
<td>5.53</td>
<td>1.837</td>
<td>6.87</td>
<td>1.859‡</td>
<td>7.08</td>
<td></td>
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<tr>
<td><em>F. succinogenes</em></td>
<td>Caecum</td>
<td>1.903*</td>
<td>8.00</td>
<td>1.694*</td>
<td>4.94</td>
<td>1.689*</td>
<td>4.89</td>
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</tr>
<tr>
<td><em>S. bovis</em></td>
<td>Caecum</td>
<td>1.903*</td>
<td>8.00</td>
<td>1.694*</td>
<td>4.94</td>
<td>1.689*</td>
<td>4.89</td>
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SED, Standard error of the difference. CT, cycle threshold.

*Mean values within a column with unlike superscript letters were significantly different (P < 0.05).

Mean values within a row with unlike superscript letters were significantly different (P < 0.00).

Data (log-transformed) are expressed relative to mean total bacterial load. Actual percentages of total bacterial load are also shown. Log-transformed data were derived according to the formula $\text{log}_{10}(1000(1.78^{-\Delta C_T}))$.
Table 3. Semi-quantitative levels of *Ruminococcus flavefaciens*, *Fibrobacter succinogenes* and *Streptococcus bovis* in lyophilised luminal contents of the equine caecum, ventral colon, dorsal colon and rectum (*n* 14)*

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Hindgut region</th>
<th>Ventral colon</th>
<th>Dorsal colon</th>
<th>Rectum</th>
<th>Bacteria mean</th>
<th>Region mean</th>
<th>Bacteria × region mean</th>
<th>Mean relative level</th>
<th>%</th>
<th>Mean relative level</th>
<th>%</th>
<th>Mean relative level</th>
<th>%</th>
<th>Mean relative level</th>
<th>%</th>
<th>P</th>
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<tbody>
<tr>
<td><em>R. flavefaciens</em></td>
<td>2.219</td>
<td>19.82</td>
<td>2.297</td>
<td>2.453</td>
<td>2.353d</td>
<td>2.353d</td>
<td>NS</td>
<td>0.0884</td>
<td>&lt;0·001</td>
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<td></td>
<td>2.219</td>
<td>19.82</td>
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<td>2.353d</td>
<td>2.353d</td>
<td>NS</td>
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<tr>
<td><em>F. succinogenes</em></td>
<td>1.808</td>
<td>14.96</td>
<td>2.175</td>
<td>2.355</td>
<td>1.2157</td>
<td>2.157a</td>
<td>4·11</td>
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<td>1.808</td>
<td>14.96</td>
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<td>2.157a</td>
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<td></td>
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<tr>
<td><em>S. bovis</em></td>
<td>1.396</td>
<td>6.43</td>
<td>1.614</td>
<td>2.007</td>
<td>1.7478</td>
<td>5·58</td>
<td>10·16</td>
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SED. Standard error of the difference, CT, cycle threshold.

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<th>Treatment</th>
<th>Mean relative level</th>
<th>%</th>
<th>Mean relative level</th>
<th>%</th>
<th>Mean relative level</th>
<th>%</th>
<th>Mean relative level</th>
<th>%</th>
<th>P</th>
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<tbody>
<tr>
<td>Frozen</td>
<td>1·903</td>
<td>&lt;0·001</td>
<td>1·694</td>
<td>&lt;0·001</td>
<td>1·689</td>
<td>&lt;0·001</td>
<td>1·786</td>
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<td>Lyophilised</td>
<td>2·353d</td>
<td>0·001</td>
<td>2·157d</td>
<td>0·001</td>
<td>1·747d</td>
<td>0·001</td>
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<td>1·926</td>
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<td>&lt;0·001</td>
<td>1·694</td>
<td>&lt;0·001</td>
<td>1·689</td>
<td>&lt;0·001</td>
<td>1·786</td>
<td>6·11</td>
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<tr>
<td>Lyophilised</td>
<td>2·353d</td>
<td>0·001</td>
<td>2·157d</td>
<td>0·001</td>
<td>1·747d</td>
<td>0·001</td>
<td>2·086</td>
<td>12·20</td>
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<tr>
<td>Bacteria mean</td>
<td>0·0658</td>
<td></td>
<td>1·926</td>
<td></td>
<td>1·718</td>
<td></td>
<td></td>
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<tr>
<td>Treatment mean</td>
<td>0·0537</td>
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<td></td>
<td></td>
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<td>Bacteria × treatment mean</td>
<td>0·0931</td>
<td></td>
<td></td>
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One of the novel findings presented in the present study was the determination of the main lactic acid bacteria, S. bovis. To the best of our knowledge the abundance of this bacterium has yet to be determined within the equine hindgut, although several cultivation studies have focused on the lactobacilli and streptococci bacterial groups as a whole. Real-time PCR data revealed S. bovis to be present in lower amounts in the caecum compared with the ventral colon, dorsal colon and rectum in both the frozen and lyophilised material. This concurs with the findings of culture-based experiments, whereby, on average, the concentration of lactobacilli and streptococci tends to be lower in the caecum than the colon, which has been attributed to the faster rate of passage of soluble carbohydrate and undigested starch through the caecum compared with the colon.

Although S. bovis is a normal inhabitant of the gastrointestinal tract of the horse it has been implicated as a putative cause of laminitis, and other key bacteria, in different stages of gastrointestinal disease. Since 1952, S. bovis has been studied more extensively than any other lactic acid-producing bacteria of ruminal origin, with numerous strains having been isolated from cattle and sheep, characterised on both morphological and biochemical characteristics. However, several cases have been identified that do not cause disease. As such, cultivation methods cannot be used as a reliable way to detect rising levels of S. bovis as the cause of gastrointestinal disease. More recently, S. bovis has been characterised on a molecular level in other species, using similar techniques to those presented here. However, to date this is the only study that has determined the abundance of S. bovis in equine hindgut contents. Nevertheless, further studies are required in equines to investigate the role that S. bovis plays in fermentative acidosis, gastric ulceration and laminitis. Furthermore, studies also need to consider other lactate-producing bacteria, since S. bovis is unlikely to be the sole bacterial species involved in gastrointestinal disease.

By establishing a model of hindgut function using non-invasive techniques, further research can explore the role of S. bovis, and other key bacteria, in different stages of gastrointestinal disease, and not just at the terminal stages following euthanasia. Data from both the frozen and lyophilised samples showed similar levels of R. flavefaciens, F. succinogenes and S. bovis relative to total bacterial load in luminal contents obtained from the dorsal colon and rectum. These findings indicate that, similar to other single-stomached animals, equine faecal material could reflect the microbiological characteristics of the distal colon. This would subsequently allow faeces to act as a model for the distal colon, facilitating accurate determination of changes in gut microflora without the need for surgically modified animals or the use of slaughter material, which allows for no information on the animal’s health or dietary management. Furthermore, data from the frozen luminal contents indicated similarities between the three bacteria in the ventral colon, dorsal colon and rectum, potentially allowing faeces to be used as a model for the whole colon. However, more work is required to further develop real-time Q-PCR for quantification of a greater number of candidate bacterial species, in particular key fibrolytic species and lactate-producing bacteria (especially from the genus Streptococcus), and to assess the effect of environmental factors, such as diet, on the relationship between faecal and colonic bacterial populations. If a conclusive link can be established in healthy horses using faecal material to give an indication of bacterial community structure, then faecal material could potentially become a non-invasive tool to accurately monitor changes in the colonic bacterial populations in response to diet and other environmental factors, and allow for the accurate measurement of potential disease-causing bacteria, such as strains of S. bovis (and other bacteria) in the colon. In human subjects, reports have suggested a potential relationship between increased faecal carrier levels of S. bovis and human gastrointestinal disease. If a similar trend can be established in the equine, faecal material has the potential to be employed as a model for identifying and monitoring the level of S. bovis in the hindgut, thus detecting rising levels or imbalances at the early stages of disease when treatment can be more effective.

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References


