

## Gut microbiome and intestinal dysbiosis analysis in horses



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Clinical signs of gastrointestinal complaints in horses are manifold. They include colic, diarrhoea, constipation or flatulence. Here, infections with bacteria, viruses and parasites are of particular importance. However, non-infectious factors such as feeding management and husbandry often play a role as well. In horses, too, more and more focus is being placed on microbiome analysis in order to identify intestinal dysbiosis. Dysbiosis describes a condition of microbial overgrowth in the gut. In addition to gastrointestinal complaints, other clinical diseases can now also be associated with intestinal dysbiosis in horses.

### **Gut microbiome**

The intestine is the largest surface area of the body and provides the largest contact surface with the environment. The gut microbiota is composed of bacteria, fungi and protozoa. Viruses and archaea are also found. The gut microbiome is defined as the total gut microbiota. There are up to  $10^{12}$  bacteria per gram of faeces in the large intestine, making it the most densely populated ecosystem in the world. The gut microbiota fulfils various tasks. First and foremost, it has a protective function, because on the one hand, nutrients from food should be absorbed through the intestine, but

on the other hand, harmful substances should not be able to penetrate through the intestinal wall into the organism. A mucosal barrier is formed, which is, amongst others, sustained by bacteria. The microbiota also influences the intestinal immune system. It ensures a constant balance between tolerating commensals and the defence against pathogens.

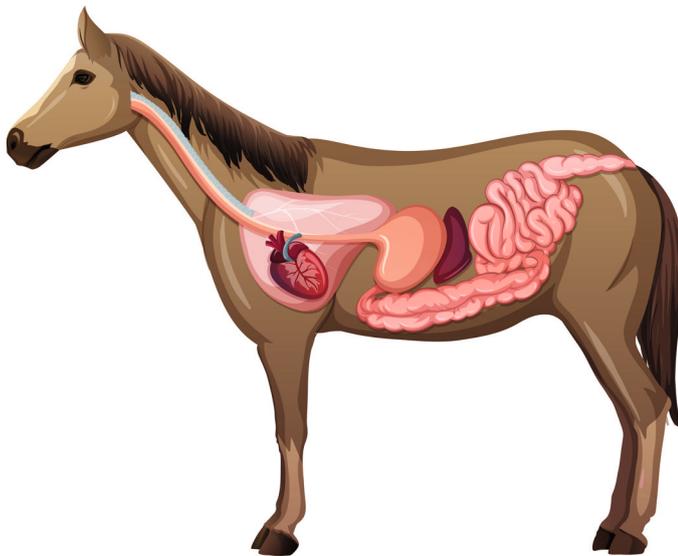


Fig. 1: Equine gastrointestinal tract

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Furthermore, intestinal bacteria play a decisive role in metabolic processes. In the foregut, the enzymatic digestion of fats, proteins and sugars takes place and the mash passes through within a few hours. Carbohydrates and raw fibre components, such as cellulose and hemicellulose, which cannot be digested in the small intestine, enter the large intestine undigested. In the large intestine, microbial fermentation of the fibre components occurs, which leads to the production of the short-chain fatty acids butyrate, propionate and acetate. Short-chain fatty acids primarily provide energy. In horses, this can account for up to 65% of the total energy requirement. An intact gut microflora is therefore very important for horses.

Depending on the type of feed, the digestion process can take up to 50 hours. During this process, the feed or the mash passes through the foregut, which includes the mouth, oesophagus, stomach and small intestine,

within a few hours (approx. 2 – 4 hours). When entering the large intestine, which is composed of the caecum and the colon, microbial fermentation begins. In this part of the intestine, the mash can remain for up to 48 hours.



Fig. 2: Equine faeces

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## Microbiological analysis of faecal samples

Faecal samples are an interesting material for analysis. They are easy to obtain and can provide a lot of information.

Bacterial culture tests are a long-established and proven method. They are used to detect living microorganisms capable of reproduction and are very suitable for the detection of obligate pathogenic bacteria such as Salmonella. Serotyping and susceptibility testing are only possible when a culture is grown as well. In addition, bacterial isolates from culture are used as a basis for producing an autovaccine. However, it is usually only possible to cultivate bacteria that grow under aerobic conditions on the culture media used. In individual cases, it is possible to detect some anaerobes, but doing so in culture is complex, time-consuming and only possible for a few bacterial species. The gut microbiome consists of almost 100% anaerobically growing bacteria. Thus, the majority cannot be detected by culture.

Dysbiosis analysis is carried out using real-time PCR, a quantitative PCR (qPCR) in which the 16s rDNA copy numbers of certain key bacteria groups are determined (Fig. 3). PCR is a culture-independent method. It has high specificity, is quantifiable and can be used to detect anaerobic bacteria with unknown growth conditions.

The aim of dysbiosis analysis is to identify whether there is a normobiotic or a dysbiotic state. For this, the bacterial strains that were most prevalent in previous scientific studies are grown. In horses, this is currently: Firmicutes, Bacteroidetes, Verrucomicrobia and Proteobacteria. The qPCR is performed according to a standardised protocol. A standard curve with a known concentration is used to quantify the individual key bacteria groups.

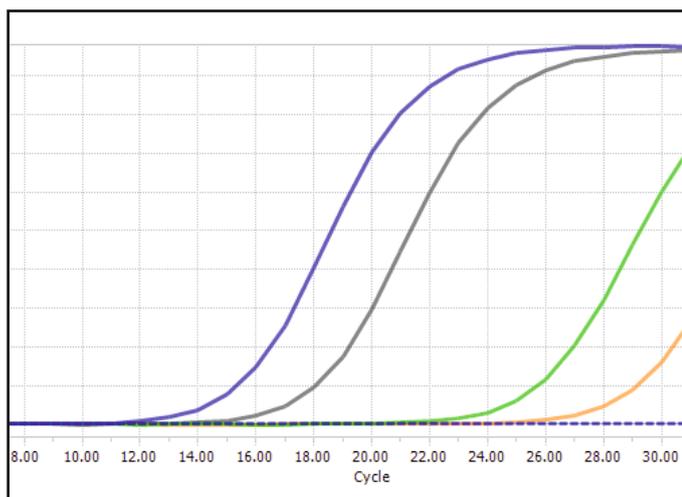


Fig. 3: qPCR amplification curve

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If gastrointestinal disorders are accompanied by poor general condition, infectious causes should also be clarified before or in addition to testing for dysbiosis.

Besides a faecal examination for parasites, which is always indicated in horses, viruses or bacteria can also lead to diarrhoea. For example, an infection with **coronaviruses** may also cause clinical signs in adult horses, although usually only individual animals are affected. Detection

is done from faecal samples by means of PCR. Moreover, various bacteria can cause diarrhoea in horses. The general condition is not always impaired. Bacterial diarrhoea, too, often only affects individual animals.

Horses, for example, are rather susceptible to **Salmonella** infections. Even a low bacterial count may be sufficient for transmission. Often, asymptomatic horses that carry and shed Salmonella are the source of infection. Particularly foals, immunocompromised and stressed animals can develop severe clinical signs.

**Clostridium perfringens**, a strictly anaerobic bacterium, is the most common bacterial species in the large intestine of our horses, as well as in other animals and humans. Colonisation takes place very early: In more than 90% of foals that are over 3 days old, *Clostridium perfringens* can already be detected in the faeces. Under certain circumstances, these bacteria can form enterotoxins which then cause diarrhoea.

### Dysbiosis analysis - interpretation of findings and treatment

To assess whether there could be a dysbiotic state, it is necessary to look at the test results of the individual groups of key bacteria. Are the values within the norm or are there any deviations from the reference range? Which key bacteria show changes and how great is the discrepancy?

This not only raises the question of how to interpret the results of a dysbiosis analysis but also of how to proceed with the treatment. Contrary to other laboratory analyses, it is not possible to give a general recommendation for treatment based on the findings. Each test result must be assessed individually, as the gut microbiome can be influenced by many factors (Fig. 4).

At the beginning, it should be clarified if there are any primary diseases.

Feeding has a considerable impact on the composition of the gut microbiota. An optimal supply of roughage, mineralisation tailored to the individual needs and drinking water of impeccable quality are prerequisites for a well-functioning and healthy digestive tract. If there is any uncertainty, feed and drinking water analyses can provide information. Feed changes should take place gradually. An example are the beginning and the end of the grazing season in spring and autumn. Food intake through the mouth with mechanical grinding is the first stage of digestion. Thus, dental health should be part of a thorough medical history. The medical history should also include an evaluation of how the animals are kept with a special focus on possible stress factors.

A critical and holistic look at factors that possibly have an impact provides the basis for an individual therapeutic approach.

**Factors that may influence the gut microbiome:**

- feeding
- drinking water
- husbandry
- age
- using the horse
- breed/type of horse
- pre-existing conditions
- antibiotics

Fig. 4: Factors influencing the gut microbiome

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## Pre- and probiotics

Pre- and probiotics are often used to treat digestive disorders. They are said to have a positive influence on digestion, the intestinal mucosa and the gut microbiota.

Prebiotics are dietary fibres that, similar to crude fibre in roughage, cannot be digested or are difficult to digest in the foregut and support bacteria in the large intestine. Prebiotics are also fermented by microbes. This is said to stimulate the growth of physiological microorganisms. Probiotics contain living organisms that are supposed to have beneficial effects on intestinal health. The most familiar example is the live yeast *Saccharomyces cerevisiae*.

In contrast to other species, there have only been a few studies in horses so far despite the frequent use of prebiotics and probiotics. It is a controversial issue whether their use has positive and/or negative effects.

## Summary

Intestinal dysbiosis can be associated with a variety of clinical disorders. The composition of the gut microbiota depends on many different factors and there is no general recommendation for treatment. Molecular biological methods provide a general overview of the gut microbiota. Evaluations are made based on current scientific knowledge. Nevertheless, further studies are needed to promote understanding and gain additional insights.

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## Further reading

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