THESIS

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Department of Physiology and Biochemestry

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Fecal microbiota transplantation as a treatment of hepatic encephalopathy in dogs

by

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Thesis Topic Declaration Form

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THESIS TOPIC DECLARATION FORM

I hereby request approval from the Head of Department of the Department of/and Physiology and Biochemistry

to prepare a thesis based on a topic announced and supervised by said Department as follows.

Date: Budapest, 20. March 2021

Student signature

Thesis topic:

Hepatoencephalopathy in dogs - faecal transplantation

Title of Thesis (English title as well):

Faecal microbiota transplantation as a treatment of hepatoencephalopathy in dogs

Supervisor signature:

Approved by:

Head of Department signature

1. List of abbreviations

Abbreviation	Meaning
AAA	aromatic amino acid
ALKP	alkaline phosphatase
ALT	alanine aminotransferase
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AST	aspartate aminotransferase
ATP	adenosine triphosphate
BBB	blood-brain barrier
BCAA	branched-chain amino acids
BUN	blood urea nitrogen
CLF	chronic liver failure
CNS	central nervous system
CT	computertomography
CTP	Child-Turcotte-Pugh
DNA	desoxyribonucleic acid
EAAT	exctiatory amino acid transporter
FMT	fecal microbiota transplant
FMTcr	fecal microbiota transplant colonic release
FMTgr	fecal microbiota transplant gastric release
GABA	gamma-Aminobutyric acid
GIT	gastrointistinal tract
GLDH	glutamate dehydrogenase
HE	hepatic encephalopathy
ICP	intracranial pressure
IL-6	interleukin-6
Mn	manganese
MELD	model of end-stage liver disease
MHE	minimal hepatic encephalopathy
MRI	magnetic resonance imaging
NMDAR	N-methyl-D-aspartate receptor

OHE	overt hepatic encephalopathy
PCR	polymerase chain reaction
PHE	phenylalanine
PSS	portosystemic shunt
RNA	ribonucleic acid
ROS	reactive oxygen species
SIRS	systemic inflammatory response syndrome
T4	thyroxine
TNF-α	tumor necrosis factor-alpha
TRP	tryptophan
TYR	tyrosine

2. Abstract

Hepatic encephalopathy (HE) is not a disease on its own, it is a dysfunction of the brain accompanying advanced liver diseases. Due to various causes, the liver is not able to detoxify ammonia, which leads to the accumulation of ammonia in the blood circulation and consequently to the accumulation in the brain, where it leads to cognitive dysfunction of the patient. These deficits have not only been observed in humans but also in dogs, for example the Author of this thesis has included a case study of a french bulldog named Bella, which was suffering from HE.

As a newly invented treatment, fecal microbiota transplantation (FMT) is already used in human medicine as a therapy to alleviate the symptoms. FMT is a method to directly manipulate the gut microbiome of a patient with concurrent dysbiosis, which causes a disease, to a more beneficial gut microflora. The corrected dysbiosis can reduce symptoms of a current disease, which can improve the quality of life of the patient.

This thesis was concerned with HE in general and the newly assayed FMT treatment as a solution of the concurrent clinical symptoms of HE. In the first part of this thesis the physiological and pathophysiological aspects of the disease are explained for further understanding of the second part, which focuses on the aspects of FMT. The second part of this thesis includes the principle on how FMT modulates the gut microbiome, studies in humans and in dogs, a case report, and how the studies in dogs can establish as an encouragement for further studies.

3. Physiology of the ammonia metabolism

Ammonia is the most important metabolic product in the course of HE. Several biochemical systems naturally produce Ammonia within the body, which can be in a healthy organism detoxified by several organs, like the liver and the kidney. In case of HE, surplus ammonia circulates in the systemic circulation and also crosses the blood brain barrier (BBB), which produce the HE characteristic neurological symptoms. In the following Section the physiological processes are discussed to simplify the understanding of the pathophysiology of HE. Furthermore, it is helpful to understand the new therapeutic approach, which will be discussed in Section 5.

3.1 Ammonia source

Protein digestion within the gastrointestinal tract (GIT) has the main effect on the ammonia household. Large amounts of free ammonia are produced by urease-producing bacteria within in the GIT, due to absorption of the enterocytes, the ammonia enters the portal vein and afterwards the liver, where the urea cycle will detoxify the free ammonia (5). The detoxification of ammonia is discussed in Section 3.2.

3.1.1 Urease-producing bacteria

Eubacteriaceae, Peptostreptococcaceae, Bifidobacteriaceae, Bacteroides, Clostridia, Fusobacteriaceae, Lactobacillaceae, and *Peptococcus* are anaerobic intestinal bacteria, which produce urease (1). Gram-negative anaerobes, like *Clostridia, Enterobacteriaceae,* and *Bacillus spp.* produce the largest amount of ammonia. Gram-positive non-sporing anaerobes, like *Streptococcus* and *Micrococcus* produce intermediate amount of ammonia, whereas *Lactobacilli* and yeasts produce the least amount of ammonia. Furthermore, ammonia is produced during the endogenous metabolism of bacterial cells (5). Figure 1 describes the ammonia production of an urease producing bacteria, where the enzyme urease converts urea into two ammonia molecules and one carbonic acid by adding on nitrogen molecule (2).

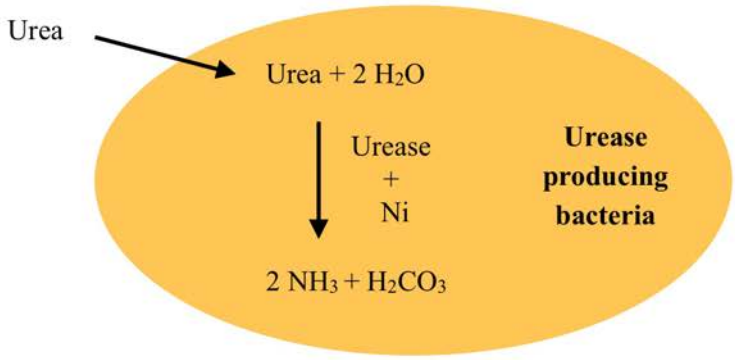


Figure 1: Urease producing bacteria within the GIT — ammonia production of an urease-producing bacteria. Ingested urea is absorbed by the intestinal bacteria, the enzyme urease hydrolyzes the urea by adding two water molecules and one nitrogen molecule, which will result in two ammonia molecules and one carbonic acid molecule (2).

3.2 Urea cycle

The liver is able to convert ammonia to a less toxic substance, called urea, by passing ammonia through the urea cycle, which is located within the liver within the periportal hepatocytes. Ammonia and carbon dioxide are formed into carbamoyl phosphate that is synthesized by the carbamoyl phosphate synthetase utilizing 2 adenosine triphosphate (ATP). The next step, carbamoyl phosphate, is combined with ornithine to citrulline catalyzed by ornithine transcarbamoylase. Finally, aspartate is added to citrulline by using ATP to argininosuccinate. The enzyme of this third step includes arginine succinate synthetase. Argininosuccinate lyase split off fumarate, and the product is arginine. While in the last step, the enzyme arginase hydrolysis arginine into ornithine by splitting urea. Urea enters the bloodstream, and its excretion is via the kidney. This way of ammonia detoxification has a high capacity but a low affinity (4).

Another way of detoxifying ammonia within the liver is the glutamine synthesis within the perivenous hepatocytes. The enzyme glutamine synthetase combines ammonia and glutamate, which forms glutamine, this process can be seen in figure 2 (6).

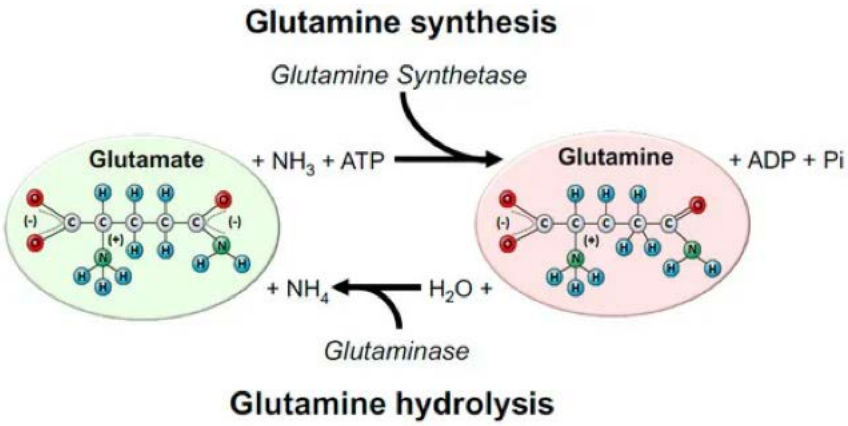


Figure 2: glutamine synthesis — ammonia is added to glutamate by the glutamine synthetase using one ATP, which results in one glutamine molecule, one adenosine diphosphate, and one inorganic phosphate (21).

The physiology of ammonia production and detoxification is in physiological balance to protect the organism from the neurotoxic effects of ammonia. The mammalian gut microbiome produces large amounts of ammonia, whereas the the urea cycle and the glutamine synthesis detoxifies the neurotoxic ammonia, to keep the blood ammonia concentration on a level where it has no harmful effects on the organism. The following Section 4, focuses on the pathophysiology of HE, on the causes, which lead to an increased blood ammonia concentration and its consequently effect on astrocytes, neurotransmission, and the eubiosis of the microbiome of the mammalian gut.

4. Pathophysiology of hepatic encephalopathy

HE is a common complication in patients with liver diseases and is a standard description of neuropsychiatric abnormalities, accompanied by the pathophysiology of the liver disease (7).

4.1 Etiology

In human medicine, HE is divided into three types, based on the primary liver disease.

Type A: acute liver failure

Type B: intra- or extrahepatic portosystemic shunt (PSS)

Type C: severe parenchymal liver disease, combined with portal hypertension and an acquired PSS

In type A liver failure, the disease is quick and progressive. The symptoms occur suddenly. The necrotizing liver releases neurotoxic substances into the circulation. Additionally, complications arise due to systemic inflammatory response syndrome (SIRS), which additionally causes multi-organ failure. The systemic infection enhances the ammonia-induced cerebral blood flow redistribution. The cerebral blood flow is decreased, leading to a decreased glucose and oxygen supply of the brain. Cytopathic hypoxia will lead to anaerobic metabolism of the neurons, causing lactate accumulation within the cerebral cortex. This severe condition results in the progressively decreasing activity of the neurons and the progressively worsened cerebral microcirculation dysperfusion. Accompanied fever will result in increased protein metabolism, producing ammonia precursors and aromatic amino acids. Hypoglycemia worsens the progression of the disease. Usually, the prognosis for these patients is lacking. The reason for acute liver failure are toxins, for example, acetaminophen, aflatoxins, and xylitol, but it could also be caused by diseases, like copper storage disease in Bedlington terriers, canine hepatitis, toxoplasmosis, leptospirosis, lymphoma, hemolytic anemia, neoplasia, severe hypertension, and arterial and venous occlusions (8).

The most common type in small animals is Type B. This type is also called bypass type caused by an abnormal connection between the vena portae and the systemic circulation, causing an atrophied liver. There are two types of bypasses. On the one hand, it can be an intrahepatic shunt, and on the other hand, it can be an extrahepatic shunt. The intrahepatic shunts are congenital, so some dog breeds are predisposed to this disease, for example, German Sheppard, Dobermann Pinscher, Golden Retriever Labrador, Australian Sheppard,

Wolfhound, and toy breeds like Yorkshire Terriers. They develop during fetal development from hepatic sinusoids or the portal veins or persistent ductus venosus. The extrahepatic shunt connects the vena portae to the vena cava caudalis or the vena azygos sinistra. Cairn terriers often have portal hypoplasia, causing a secondary microvascular dysplasia, also called a microscopic intrahepatic shunt. This malformation is in this breed inheritable, where the portal vein is deformed or portal vein aplasia. Multiple extrahepatic shunts are the acquired form of PSS. They result from end-stage liver disease, for example liver cirrhosis or portal hypertension. In this case, the shunt is microscopically connecting the vena portae with the vena cava caudalis. An increased blood pressure of the portal vein leads to the restarting of the blood flow through these veins. Consequently, detoxification does not take place (8).

The most common type of PSS in humans is type C. In this case, the liver cells fail in their function. Another cause is the pre-existing shunt between the portal vein and the systemic circulation (9).

The lack of detoxification of ammonia due to the PSS, will result in histopathological changes of the astrocytes, which will be discussed in further detail in the next Section.

4.2 Histopathology

HE is the result of the above mentioned liver diseases, and it is usually accompanied with impaired brain function. This Section focuses on the histopathological changes within the neurons, which are related to HE and which will cause the typical neurological clinical signs of the disease (24).

The reason for this abnormal brain activity can be traced back to the increased blood concentration of ammonia. Ammonia has an osmotic character and is able to cross the BBB in high concentrations, where ammonia is absorbed by the astrocytes and causing brain edema and histopathological changes of the astrocytes itself (3), where they show a paler appearance. The nuclei of the astrocytes become more prominent, and the chromatin migrates to the cell's perimeter, which forms a ring of chromatin at the margin of the astrocytes. It can also be seen, that astrocytes form pairs or triplets with other astrocytes. These changes of the cell nuclei and the chromatin is comparable to Alzheimer's type 2 disease, which can be seen in figure 3 (10). In severe HE, brain edema can be seen due to the perivascular astrocyte swelling, with an increased number of vesicles within the cytoplasm (11). Also, the nucleus appears lobulated, containing glycogen granules. Those changes are most commonly seen in the gray

matter of the brain tissue, especially in the pons, globus pallidus, putamen, thalamus, dentate nucleus, and in the deep layers of the cortical gray matter (12).

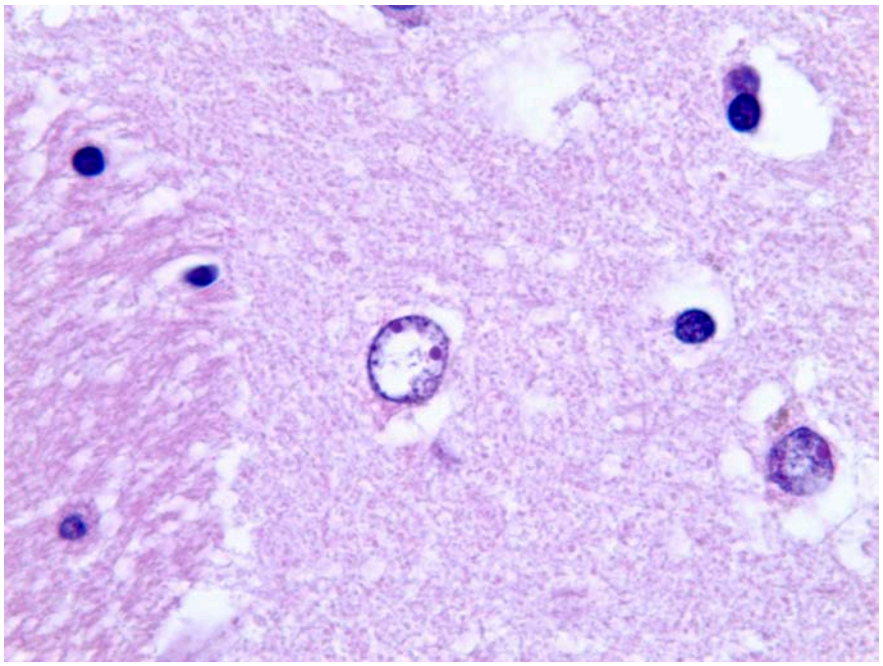


Figure 3: Alzheimer's type 2 degenerated astrocytes, 1,000x magnification, hematoxylin eosin staining — enlarged astrocytes nucleus with a rim of chromatin at the cell's perimeter and scant cytoplasm (12).

The next Section discusses the factors, which will lead to histopathological changes of the astrocytes, and the accompanied clinical signs of the patients.

4.3 Factors

Ammonia is not the only neurotoxic substance, which is a causative agent in HE. There are several other neurotoxic substances, which a healthy liver detoxifies to prevent the neurotoxic effects of these substances. Those substances are: manganese (Mn), mercaptans, short-chain fatty acids, tryptophan, glutamine, bile salts, gamma-Aminobutyric acid (GABA), and endogenous benzodiazepines (13).

4.3.1 Ammonia

The central molecule, which causes HE with accompanied neurological alterations is ammonia. The increasing ammonia concentration correlates with the course of the liver failure, which leads to hyperammonemia. Ammonia has a lipophilic character, so it can diffuse freely through the BBB. The astrocytes are the first barrier of the BBB. Absorbed ammonia into the cytosol of the astrocytes is transformed into glutamine by the enzyme glutamine synthetase using glutamate. This glutamine formation is the cerebral pathway to detoxify and reuse ammonia within the central nervous system (CNS) (13, 14). Hyperammonemia leads to an increased ammonia uptake within the astrocytes, causing the swelling of neurons, due to the osmotically effect of ammonia. Additionally, the increased ammonia concentration within the astrocytes will alter the neurotransmission, due to the detoxification way of transforming of ammonia into glutamine (15). The alteration of the neurotransmission is discussed in the Section 4.4.4. The course of the expanding astrocyte swelling will result in a cytotoxic edema of the brain (16). Studies have shown, that patients with acute liver failure have an increasing intracranial pressure (ICP) correlating with the increasing glutamine concentration within the brain (17).

4.3.2 Manganese

Mn is an important cofactor of several enzymes, like arginase and glutamine synthase. Additionally Mn has an important role to maintain the optimal physiology of several organs, like the brain. However, it is only from physiological importance, when absorption and excretion is balanced, otherwise it will cause Mn neurotoxicity. Until now, the development of Mn neurotoxicity in correlation with HE can not be fully clarified. One reasonable explanation is that the due to chronic liver diseases the excretion via the bile is disturbed and the Mn is reabsorbed into the blood stream (70). Mn will cross the BBB and accumulate

inside the basal ganglion and in the astrocytes. Increased Mn concentration within the basal ganglia will cause extrapyramidal symptoms, disturbing the dopaminergic neurotransmission (18). Extrapyramidal symptoms include rigidity, tremors, and akinesia (19). In the astrocytes, it causes Alzheimer's type 2 degeneration, leading to disturbed glutamate reuptake. Additionally, accumulated Mn disturbs the cerebral energy metabolism (20). Glucose influx is impaired due to the increased Mn concentration within the neurons. Oxidative stress is the consequence of the impairment of the mitochondria oxidative glucose metabolism, which the *de novo* synthesis of lactate compensates. This anaerobic lactate metabolism is the alternative pathway of energy production, but the increasing concentration of lactate in the neurons will lead to complications, like worsen the neuronal antioxidant capacity and energy metabolism (18).

4.3.3 Systemic inflammation

Systemic inflammation, in combination with increasing ammonia concentration, is related to the progressive worsening of the cognitive function, due to the degranulation of neutrophils. The neutrophils release reactive oxygen species (ROS), which are able to cross the BBB. Bacterial translocation, due to the developing dysbiosis, which will be further explained in Section 4.5, leads to endotoxin production and is followed by endotoxemia (22). Tumor Necrosis Factor-alpha (TNF-α) and interleukin-6 (IL-6) are the most important cytokines in combination with endotoxemia, which will occur in SIRS (23). The released inflammatory mediators in case of SIRS will lead to a redistribution of the cerebral blood flow (24) from the cerebral critical region to the basal ganglia, cerebellar and thalamic structures. The cerebral blood flow redistribution is associated with the brain glucose utilization (25). The energy deficiency is compensated by anaerobic lactate production, which is explained in Section 4.3.2 (18).

4.3.4 Neuroinflammation

Microglia are the macrophages of the CNS, and have an important role in the pathogenesis HE and in the worsening course of neuroinflammation. In the specific case of HE several triggers, like cerebral edema, systemic inflammation, and oxidative stress (27), activate the microglia by increasing them in their number (26). Activated microglia will

produce neurotoxic pro-inflammatory cytokines, like TNF-α and IL-6, glutamate, and ROS, which will lead to neuroinflammation. TNF-α supports the microglial glutaminase expression, which will accelerate the glutamate concentration, and it will increase the expression of gap junctions on the neuronal cell surface. Those two processes will lead to excitotoxic neuronal death, which describes the death of neurons due to intense exposure of the excitatory glutamate neurotransmitter. TNF-α will also lead to the impairment of the neuronal mitochondria, enhancing the neuronal cell death. Impaired axonal and dendritic transport will enhance the neuronal death. Increased concentrations of IL-6, produced by the activated microglia, are associated with the cognitive alteration of patients, due to its neurotoxicity. Mitochondrial oxidative metabolism as well as the microglia produce ROS, like superoxide anion, hydroxyl radical, and hydrogen peroxide, which will cause oxidative damage to lipids, proteins, DNA, and polysaccharides (28). Furthermore, the impairment of the oxidative metabolism of the mitochondria will lead to reduced cerebral glucose formation, which will cause an energy deficit within the CNS. The increased concentration of ammonia will lead to an increased expression of the toll-like receptor-4 and the production of cytokines, which will enhance the neuroinflammation. All together, these processes lead to an increased permeability of the BBB (29), which will increase the distance of the gaps of the endothelia cells of the BBB. The widen gaps of the BBB allow larger molecules to pass the BBB, enhancing the brain edema and may lead to cerebral herniation, which leads to fixed and dilated pupils (30).

4.4 Neurotransmitter

The typical neurological clinical signs are not only caused by the above mentioned substances. Another important factor, which will worse the course of the disease, is the imbalanced neurotransmission of excitatory and inhibitory neurotransmitter. The following Sections explain the pathways of several neurotransmitter which play an important role in the deteriorating course of the cognitive function of the patients.

4.4.1 Glutamine

Glutamine is the precursor of the neurotransmitters glutamate and GABA. The *de novo* synthesis of glutamine is the pathway of the cerebral detoxification of ammonia within

the CNS. As already mentioned in Section 3.2, the glutamine synthetase produces glutamine by adding ammonia to glutamate. Excessive blood ammonia concentration is compensated by excessive *de novo* glutamine synthesis. Glutamine is an osmotically active agent, which promotes the further swelling of the astrocytes. Due to the increased permeability of the BBB ammonia is able to diffuse in large amounts into the astrocytes, which leads to an overwhelmed ammonia metabolism within the astrocytes. The excessive ammonia, which can not be transformed into glutamine, diffuses freely into neighboring neurons. It will result in further cerebral swelling, neuritis, and an imbalance of excitatory and inhibitory neurotransmitter (20, 31).

4.4.2 Glutamate

Glutamate is a physiologically excitatory neurotransmitter and is produced by the phosphate activated glutaminase from glutamine. Glutamate is stored in vesicles within the presynaptic terminal and is released in the synaptic cleft due to the fusion of the vesicle with calcium to the presynaptic membrane. Into the synaptic cleft released glutamate binds to N-methyl-D-aspartate receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), and kainate receptors. The physiologically synaptic responses of these three receptors differ. NMDAR has a slow synaptic response, and AMPAR has a fast synaptic response. The physiological function of the kainate receptor is not known, but it is known that is implicated in epiletogensis and cell death (32). The excessive glutamate in the synaptic cleft is reabsorbed from the presynaptic cleft by the excitatory amino acid transporter (EAAT) to keep the extracellular glutamate levels low (33). In case of HE, the EAAT are blocked due to unknown reasons, which leads to the excessive accumulation of glutamate in the synaptic cleft. The consequences of the accumulation of glutamate in the extracellular space leads to astrocyte-mediated inflammation, formation of ROS, and the further realease of glutamate into the synaptic cleft, which leads to excitotoxicity. The excitotoxicity is in physiologically circumstances prevented by the glutamate reuptake of the EAAT in the presynaptic terminal. These alteration is usually associated with clinical signs like cognitive and motor alterations (34).

4.4.3 GABA

GABA is an inhibitory neurotransmitter, which can induce coma or decreased consciousness in case of HE. Physiologically, GABA is produced by the gut bacteria and metabolized within the liver. In case of HE, the liver is not able to metabolize the large amounts of gut bacteria derived GABA. The isomer of GABA, alpha-amino-isobutyric acid, is able to cross the BBB in an abnormally large quantity, due to the increased permeability of the BBB. An increase of the density of the GABA receptors is the consequence, due to the increased amount of accumulating GABA within the CNS (35). The synthesis of neurosteroids are effectively ligands of the neuronal GABA receptors, which are synthesized by the increased density of in the astrocytic mitochondria localized peripheral-type benzodiazepine receptors. The ligands enable an intensified receptor neurotransmitter binding of GABA and its corresponding receptor, which results in an increased influx of chlorine in the postsynaptic membrane (20). As already mentioned, the inhibitory action of GABA leads to changes of the consciousness, or can even lead to HE coma (35).

4.4.4 False neurotransmitter

The theory of false neurotransmitter is based on the malnutrition of patients with chronic liver failure (CLF), which is associated with the concurrent inflammation, hypercatabolism, malabsorption and maldigestion, and impaired glycogen storage (36). Muscle degradation into aromatic amino acids (AAA) is the consequence of the lacking nutrients, which will increase the plasma concentration of AAAs, like phenylalanine (PHE), tyrosine (TYR), and tryptophan (TRP). The equilibrium of AAAs and branched-chain amino acids (BCAA) becomes unbalanced, the plasma concentration of AAAs are increasing and the plasma concentration of BCAA is decreasing. The physiologically metabolism of BCAA are oxidized to produce energy within the peripheral tissue and they are anticatabolic factors, which reduce the degradation of muscle fibers. The pathologically increase of the plasma concentration of AAAs cause the accumulation of AAAs within the brain, which leads to an impairment of the neurotransmitter synthesis. This impairment of neurotransmitter synthesis plays a key role in the course of HE. False neurotransmitters, like octopamine and phenylethanolamine, are the end products of the impaired neurotransmitter synthesis (37). They bind to the catecholamine receptors and block the catecholamine action of the proper neurotransmitters, which decrease the intrinsic activity of these receptors (38).

4.5 Dysbiosis

HE has several stages in human medicine, minimal (MHE) and overt (OHE) hepatic encephalopathy. Those stages describe an impaired gut-liver-brain axis in the case of liver diseases. MHE and OHE are associated with microfloral changes, leading to poor cognition, endotoxemia, and inflammation. Recent human studies focused on the fecal microbiota analysis of patients with liver diseases. Patients in the group of OHE have significantly higher *Enterobacteriaceae*, *Alcaligeneceae*, and *Fusobacteriaceae* (39).

Lower autochthonous taxa are also very notable. The autochthonous taxa include *Lachnospiraceae*, *Ruminococceae*, *Porphyromonodaceae,* and *Clostridiales XIV*. In comparison to cirrhotic patients without HE, the group OHE has a higher *Veillonellaceae*. The increased population of *Alcaligenaceae* and *Porphyromonodaceae* correlate with impaired cognition. *Streptococcus salivarius* produces, due to its high urease activity, a considerable amount of ammonia within the gut, which is linked to impaired cognition. This increase of this species can be seen in MHE patients (39).

Child-Turcotte-pugh (CTP) and Model for end-stage liver disease (MELD) are scoring systems that measure the severity of cirrhosis. CTP measures the prothrombin-time, serum albumin, bilirubin, HE, and ascites severity. MELD calculates the logarithmic score of bilirubin, creatinine, and the INR of prothrombin-time. An increased population of *Streptococcaceae* is positively related to CTP, meaning worsening of HE. The expanding population of *Enterobacteriaceae* can cause an increased MELD value (39).

The cirrhosis dysbiosis ratio is the ratio of the autochthonous taxa and the non-autochthonous taxa. The non-autochthonous taxa include pathogenic bacteria like *Enterobacteriaceae* and *Bacteroidaceae*. In the case of dysbiosis, the ratio shifts towards the non-autochthonous taxa. This shift leads to hyperammonemia, endotoxemia leading to systemic inflammation, inflammatory cytokine release, and bacteremia, progressing the already impaired cognition (39).

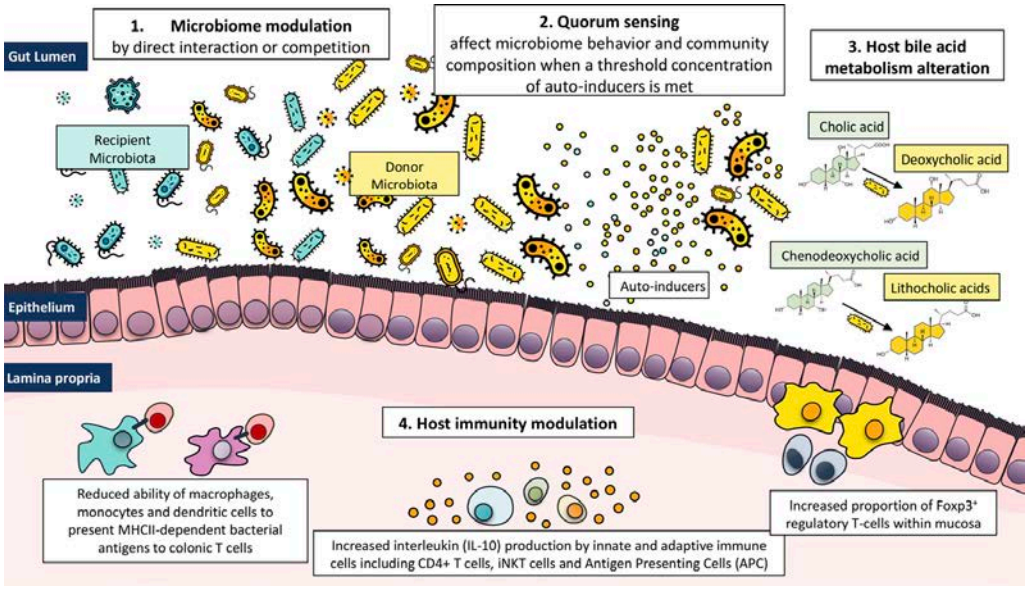
FMT is a newly assayed method in the treatment of HE. The aim is to restore gut microfloral dysbiosis, shifting the ratio to the site of the autochthonous taxa. These taxa have a low urease activity and reestablish the integrity of the intestinal barrier, which is beneficial for the liver (45). The following Section explains the mechanism of FMT, and how it is in correlation with the improvement of the course of HE.

5. Mechanism of the transplanted fecal microbiota

The aim of treating HE is to decrease the ammonia load from the GIT to the liver. To achieve this goal, the above mentioned dysbiosis has to be corrected, which can be achieved by transplanting the microbiome of healthy donor gut into the recipient gut. This Section focuses on the specific mechanism of FMT, how it will affect the gut microbiome and the improvements, which will result from the restored equilibrium of the intestinal microbiome in patients with HE. It also includes a study of two groups of rats with induced hapatosis and accomapnied HE. To clarify the effects of FMT on cognition and on the microflora of the gut, the rats were devided into two groups, where one group was treated with FMT and the other group did not receive any treatment.

To explain the mechanism of FMT in the recipients gut, we have to focus on the basic principles of competitive exclusion principle, which will achieve a homeostasis of the two rivals (49). To relate it to FMT, the transplanted micro- organisms either compete or directly interact with the recipient's gut microbiota, where one species will increase, and the other species will reduce in its population (55). Figure 4 shows the competition mechanism between the donor and the recipient microbiome. Bacteriocins are one possibility of the gut microbes to compete against each other, which is based on the theory „survival of the fittest“. They are antimicrobial peptides, synthesized by Ribosomes of gram-positive and gram-negative bacteria (49), which will cause pore formation of the bacterial cell wall, and inhibit the synthesis of cell wall, nucleic acid, and proteins (64). These peptides only have a narrow antibacterial effect, which is only crucial for closely related strains, and will improve their own performance (65). Consequently, toxin producing bacteria are killed, which will limit the toxin production of the microbial flora. The increasing variety of bacteriocins, leads to a more extensive spectrum of the antibacterial effect (49). The host will benefit from this competitive mechanism, because the ongoing imbalance of autochthonous taxa and non-autochthonous taxa is shifted towards the beneficial autochthonous taxa. The bacteria of the autochthonous taxa are low in urease activity, hence the microbial ammonia production within the GIT is reduced, which will consequently reduce the ammonia blood concentration (45). The concurrent dysbiosis disrupts the tight junctions between enterocytes, which will consequently decrease the integrity of the intestinal barrier. It is proven by a study that the integrity of the intestinal mucosa can be restored due to the induced equilibrium of the

intestinal microflora by FMT. This study included laboratory rats, where acute hepatosis was induced with carbon tetrachloride injections. The rats had accompanied HE due to the induced liver failure. Histological examination of the intestinal mucosa, shows that the rats, treated with FMT, have a decreased mucosal damage, edema, and inflammatory cell infiltration compared to the rats, which were not treated with FMT. The study also compared the systemic inflammation mediators, like TNF-α, IL-1, and IL-6, of the two trial groups. There was a significant difference between the rats, which received FMT treatment and the rats, which were not treated. The concentration of the pro- inflammatory mediators were remarkable low in the group treated with FMT. Rats treated with FMT show an increased learning ability, they were able to understand and learn things significantly faster than the rats without FMT treatment. FMT has also a beneficial effect on the liver, the study shows that the FMT treated rats did have less hepatic necrosis than the rats, which did not received FMT treatment (56). Similar to cognitive testing in rats, there are some studies in human medicine, which test the attention and the response time of patients with HE (57). Also in human medicine the results show clear outcomes, FMT causes improved cognitive function of patients with HE. This human study did not only detect the beneficial effect of FMT on the expression of the tight junction proteins within the GIT, but also on the endothelial cells of the BBB. The increased formation of tight junctions in the BBB will decrease the permeability of the BBB, which restores the health of the brain (66).



<u>Figure 4:</u> mechanism of FMT — competition between donor and recipient gut microbiome to achieve balance between autochthonous and non-autochthonous taxa and the effect on the hosts immunity (49).

In order for the FMT mechanism to function, the transplant has to be administered to the recipient, which will be explained in more detail in the next Section.

6. Fecal microbiota transplant

It is now clear how FMT works in the gut and how it is able to improve the course of the disease by modulating the gut microbiome, but this Section focuses on the transplant itself, which analyses the way of application, the preparation of the donors and the recipients, and the sampling and transplantation process (46). Most of the data, which are included in this Section, originate from studies in human medicine (57). Nevertheless, there is one published clinical study about FMT in parvovirus-infected puppies. I have included this study into my thesis, because several data can be very useful for further studies in this field of scientific research. Those crucial data from this study are the selection of a donor dog and how to prepare the feces material for transplantation (58). If there are no similarities between the human and veterinary medicine, the differences of FMT will be explained in this Section. The reason for choosing human studies as an example for this thesis is because of the large amount of similarities of the human GIT and the GIT of dogs. One study found significant correlations of these two carnivore guts. Dogs are adapted to live in the same environment as humans, and to eat similar diets. Therefore, humans and dogs live in a cohabit with almost the same environmental factors. So, the mammalian gut microbiome of carnivores adapted to similar conditions (59), resulting in a similar microbial flora consisting of bacteria, fungi, protozoa, and viruses. The most commonly represented bacterial phyla in the mammalian carnivore gut are *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, and *Actinobacteria* (58).

6.1 Delivery Route

Analyzing the route of delivery, I have come across several different techniques, which are divided into upper application routes and lower application routes. The upper route of application include gastroscopy, nasoenteric tube, and oral capsules. Enema and colonoscopy belong to the lower application route (46). Oral capsules contain either frozen stool or lyophilized stool, which both have similar efficacy. The stool is mixed with a cryoprotectant, which can be, for example, glycerol. A double or triple encapsulation of the FMT protects the FMT from the low pH within the stomach. The capsules can target the stomach (fecal microbiota transplant gastric release, FMTgr) or the intestines (fecal microbiota transplant colonic release, FMTcr), where the intestines are the better target for the FMT release. When the FMT is released within the stomach, it will be chemically and

enzymatically degraded. The extra layers of encapsulation are essential for the FMTcr. Short-term outcome of only eight weeks effectiveness is the result of oral capsule application, which can be considered as a disadvantage. The traditional ways are enema, colonoscopy, gastroscopy, and nasoenteric tube. In the case of gastroscopy, there is the risk of developing aspiration pneumonia (46), due to developing nausea, which is followed by vomiting (60). Trails have shown that enema is the least effective method in FMT because it has shown a low response rate. The colonoscopy has shown "excellent cure rates", in which the FMT is transplanted into the terminal ileum (47). In small animals, the most common route of FMT administration is with fresh feces. Fresh feces is collected from the donor dogs and has to be processed within 6 to 12 hours. The feces solution is administered via enema or colonoscopy at a dose of 5 mg per bodyweight. Sedating difficult dogs is debated by several veterinarians. Keeping the recipients quiet for at least 30 minutes is necessary to prevent the freshly administered solution from being removed (58).

6.2 FMT formulation

There are three forms of how the stool for FMT can be prepared. The first option is to use fresh stool. This method is used in direct transplantation. In human medicine, relatives are used for frozen FMT. After collection, the fresh stool is homogenized with phosphate-buffered saline in a ratio of 1:3 until 1:5. Once homogenized, the suspension is filtered through sterile gauze to remove particles. The administration should be within two till eight hours, but at lower temperatures, four degrees, it can be stored up to 24 hours. The frozen stool FMT is stored at -80 °C and can be kept for up to a year. It is also called 'ready to use' stool. During preparation, the fresh stool is mixed with 10% glycerol, which serves as a precipitant, preserving the long-term storage of microorganisms. The last method for the preservation of FMT is lyophilized stool. The lyophilization process removes the water and other solvents from the fresh stool under low pressure and temperature. The first step is to freeze the filtered stool, followed by freeze-drying. This means that ice enters the gaseous state without passing the liquid state, also called sublimation. A stable product easily stored at room temperature is produced (48). There is one study of applying FMT to dogs, but it is not for treating HE, instead the study used FMT application for parvovirus- infected puppies.This study used the method of diluting the feces of the donor dogs with saline, which can also be considered as a possible formulation of the donor feces. The volume for the saline is

calculated by the feces weight of the donor dog multiplied by four. The solution is mixed and filtered through a layer of gauze. The filtered feces solution has to be directly administered to the recipient dog (58). The clinical study of human patients with HE extracted the DNA of the microbiome by homogenizing the collected stool. 0.2 mg of stool was suspended in argininosuccinate lyase buffer with 0.75 g of 0.1 mm zirconia/silica beads. This suspension was put in the centrifuge for one minute at 4.800 revolutions per minute to disrupt the cells, followed by a 90 °C water bath for ten minutes. The last step for identification was to perform a length heterogeneity PCR to fingerprint the 16 subunit ribosomal ribonucleic acid of the fecal microbiota (57).

6.2.1 Factors influencing formulation of FMT

As previously mentioned in Section 6.2, the sample can be stored frozen for the next application in the future. On the contrary, it has to be considered that in frozen stool, microbial viability will decline with time, which has been shown by quantitative PCR. The PCR results of 9 months old frozen samples have shown a significant decrease in populations of *Bifidobacterium* and *Lactobacillus,* which are two important groups of bacteria in the course of HE (49).

6.3 Dose and frequency

Additionally, the optimal dosage of FMT application is an important role in order to get the best results of the treatment. The optimal dosage of FMT is 50 g. Lower doses have a lower efficacy, which has been reported. There are no information about the consequences of a FMT overdose (46). The frequency of FMT applications remains unclear until now, but studies have shown that a higher frequency improves the result of the FMT treatment (50).

6.4 Recipients

In recent human medicine studies, the recipients of the FMT received a pre five-day antibiotic treatment. This pretreatment increased the success of donor bacterial colonization compared to the patients without antibiotic pretreatment (51). The aim of the pretreatment with antibiotics decrease the bacterial load of the hosts GIT, which consequently decreases the diversity of the hosts gut microbiome, and enable the colonization of the donors microbiota (61). Studies used vancomycin, fidaxomicin, and metronidazole as a pretreatment of FMT in

patients with liver cirrhosis. The antibiotics were administered in this study via colonoscopy. One case reported a complication of *E. coli* bacteremia accompanied by fever (52). The pretreatment with vancomycin, fidaxomicin, and metronidazole, had the best outcome in reducing the diversity of the recipients gut microbiome (62).

6.5 Donors

To be considered as a potential stool donor, the donor will be screened on several diseases. This screening should minimize the transmission of infectious agents and reduce the risk of donor traits linked to dysbiosis. Following diseases are exclusion criteria: viral hepatitis, inflammatory bowel disease, irritable bowel disease, autoimmune or neurological disorders, immune comprehensive, cancer, obesity, HIV, *Chlamydia*, and enteric pathogens like *E. coli*, S*almonella*, *Campylobacter*, and *Clostridium difficile* (46). A study on humans and mice has shown that the circadian rhythm also influences the stool's microbial composition in a minor way. The diurnal oscillations depend on the feeding rhythms, leading to different stool profiles throughout the day. These profiles differ in composition and function (53). The bacterial load was the highest at eleven post meridiem, and at seven ante meridiem, it had the lowest bacterial load (54). Same as in human medicine, the donor dog is screened on several diseases. The body condition score has to be normal, in the range of four to five on a scale from one to nine. Donor dogs with gastrointestinal diseases, like vomiting and diarrhea, are excluded. Screening the feces for intestinal parasites is essential. PCR screening is performed to rule out enteropathogenic bacteria. Dogs treated with antibiotics in the last three to six months are also excluded from being fecal donors (58).

6.6 Possible adverse effects

FMT is usually considered as a safe therapy, but in some cases in human medicine it can lead to adverse effects, which may even kill the patient. The possible side effects of FMT therapy can either be short term or long-term events, which both are usually uncommon. Short-term adverse events can be transient fever, abdominal pain, bloating, flatulence, diarrhea, nausea combined with vomiting and the developing aspiration pneumonia, bowel perforation, bleching, constipation, hematochezia, endotoxaemia, and death. The long-term adverse events include obesity, immune mediated disorders, irritable bowel syndrome, and

inflammatory bowel disease (63). The veterinary study of parvovirus-infected puppies did not mention adverse reaction to the FMT treatment (58).

6.7 Human study results

Microbial analysis was used to identify the altered bacterial flora one month and one year after the FMT treatment of patients with HE, which show a clear success in the long-term modulation of the gut microbiome, further details can be seen in figure 5 (68). This long-term success of the modulated gut microbiota is in close association with cognition and quality of life of the patients. The MELD score was positively correlated with *Enterobacteriaceae*, *Porphyromonadaceae, Veillonellaceae,* and negatively correlated with *Ruminococcaceae.* The presence of *Enterobacteriaceae* was associated with TNF-α. An association was also recognized between worsened inflammation (IL-13, IL-6) and endotoxemia, as well as the population of *Veillonellaceae* and *Fusobacteriaceae*. In comparison, *Ruminococcaceae* were negatively correlated to endotoxemia. Poor cognition on individual tests was associated with the presence of the phyla of *Alcaligeneceae* and *Porphyromonadaceae*. The HE group showed a defined higher abundance of *Veillonellaceae* than the non-HE group. Within the HE-group, there is a significant correlation between IL-23 and the phyla of *Leuconostocaceae, Eubacteriaceae, Erysipelotrichaceae, Moraxellaceae, Streptophyta*, and *Streptococcaceae*. Further correlations between IL-2 and IL-13 and the phyla of *Fusobacteriaceae* and *Prevotellaceae* were found. Poor performance on the cognitive test was correlated to the presence of *Porphyromonadaceae* and *Alcaligenaceae* (57).

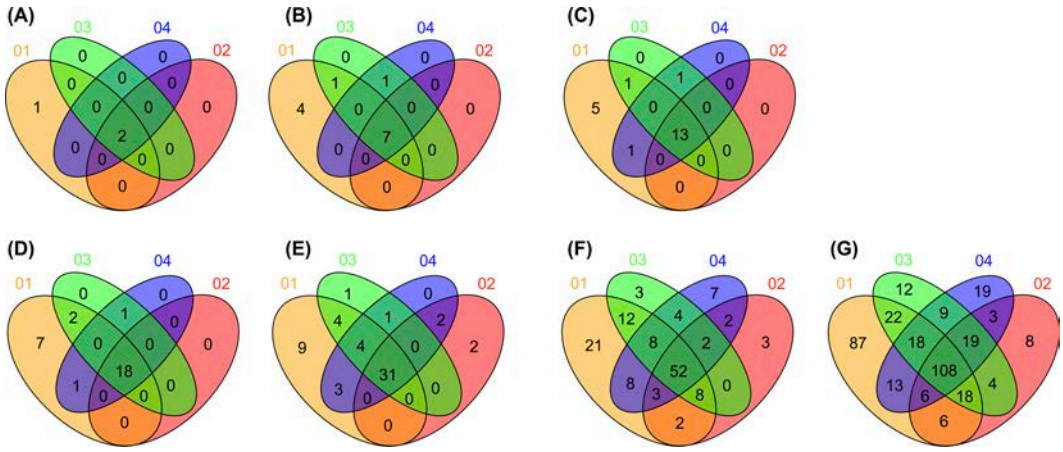


Figure 5: Venn diagram — human study, taxonomic classification of gut microbial composition is shown from kingdom to species pre- and post-FMT
01 corresponds to the donor, 02 to the recipient (pre-FMT), 03 to the recipient (at 1 month post-FMT), and 04 to the recipient (at 1 year post-FMT). Microbial-compositional structure is presented according to (A) kingdom (B) class, (D) order, (E) family, (F) genus, and (G) species. All of the diagrams of figure 5 show the accordance of (A) - (G) of the recipient in different time periods to the donor. To describe the diagram (G) more accurately, one month post-FMT 22 bacterial species of the donor gut microbiota were found in the recipients stool and also after one year post-FMT 18 equal bacterial species were still found in the recipients stool. The conclusion of this study is that FMT also have a long-term modulating effect on the recipients gut microbiome (68).

6.8 Future prospective of veterinary studies on dogs

The trend of using FMT as a treatment for several diseases is constantly increasing. There are several current studies in human medicine about the treatment of HE with FMT, and also in veterinary medicine concerning the treatment of several acute or chronic gastrointestinal disorders. The outcomes of these studies will be known in a few years, but the prospect of these studies is aspiring (58). However, this new treatment has already had remarkable good outcomes in treating HE human medicine, which are very promising. Starting new clinical trials on small animals could also be very promising due to the vast similarities of the microflora of the GIT between humans and dogs (59). The following case report should motivate more veterinarians to dedicate themselves to this new treatment method, in order to be able to treat just such cases in the near future. Especially because there are already promising results from human medicine, this topic should also be further researched in veterinary medicine, to enable dogs with HE a better quality of life.

7. Case report

This case report was presented to me by a German veterinarian, Reiner Hausmann. His veterinarian clinic is located in the northeast of Germany, in „Mühlheim an der Ruhr“. A french bulldog puppy was presented to him and he diagnosed a congenital hepatic shunt with accompanied HE. The previous owner could not afford the diagnostic testing and the treatment, hence Reiner Hausmann adopted her (69).

7.1 Anamnesis

Bella, a six-month-old french bulldog puppy, was presented to Reiner Hausmann on the 18th of October 2021 with neurological symptoms. She could not respond to environmental factors while she was awake and furthermore she was sleeping more than usual, and was less active than other puppies in her age. While walking her head was hanging down and she was not able to respond to her surrounding environment, which lead her to walk against objects. Compared to the other puppies in her age, her gait pattern was completely different. She also could not keep her legs in a straight position, which lead to a gait with spread legs. After she got adopted by the family from Reiner Hausmann, he noticed polydipsia and polyuria. This behavior was very prominent, because she had to urinate 30 times a day. Reiner Hausmann also changed her diet to a liver diet, which should improve the liver function, due to its decreased metabolic load of the liver. This prescribed diet has a decreased protein amount, but the proteins have a higher bioavailability. These proteins are egg or dairy-based proteins. A few days after changing her diet, her mental state improved, but it did not alleviate all of her symptoms (69).

7.2 Differential blood count

On the day of her presentation to the clinic on the 18th of October 2021, a blood sample was taken and sent to the laboratory. Reiner Hausmann took an EDTA blood sample, an EDTA frozen serum sample, and a standard serum samples. The laboratory performed a differential blood count. Until the 29th of October 2021, after her adoption, her symptoms worsened and a second blood sample was taken. The laboratory results from the 18th of October 2021 are listed in Appendix 1, and Appendix 2 shows the laboratory results from the 29th of October 2021.

7.2.1 Evaluation of the differential blood count, 18.10.2021

The decreased value of MCV indicates a microcytosis, which can be related to a PSS. Leucocytosis is an indicator that a current inflammation is present, which will lead to a mild non-regenerative anemia. In the haemogram, a mild non-regenerative anemia can be interpreted due to the combination of the decreased value of MCV, an average value of MCHC, and non elevated reticulocyte count. Failure of the liver function is indicated by a mildly increased level of the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), GLDH, and alkaline phosphatase (ALKP). In Bella´s case, all of the enzymes were mildly elevated, except ALT, which was 3.4 times elevated. Decreased cholesterol and triglyceride values indicate in general liver problems. Important parameters in the diagnosis of decreased liver function are blood urea nitrogen (BUN) and albumin values. Urea and albumin are synthesized in the liver, decreased values indicate decreased liver function, PSS, or acute inflammation. Additionally, decreased thyroxine (T4) values were noticed, this can be explained due the importance of the liver on the metabolism of thyroid hormones. T4 allows to conclude, that a decreased T4 correlates with the severity of a liver disease (67).

7.2.2 Evaluation of the differential blood count, 29.10.2021

The hematology shows that the microcytosis changed during this time period into a normocytosis, which also regenerated the anemia. The cholesterol and triglyceride values are still decreased, due to the persistent liver problems. BUN did also not improve, but the albumin was slightly elevated, which can be a consequence of the highly bioavailable proteins of the liver diet. This blood work also measured the blood ammonia concentration, which was increased by 3.6% than the normal value. This severe increased value does not stand in correlation with the severity of the clinical signs, it only indicates that there is a liver problem, and in case of Bella it is the result of the congenital PSS. The inflammation process lead the neutrophils to a right shift, which is indicated by the 1.4% times increased value of the segmented neutrophils and the 1.4% increased value of the monocytes. They are the indicators of a chronic inflammation. Band form neutrophils are elevated by 6%, which indicates the new neutrophil production of the bone marrow, due to the increased use of the neutrophils. The T4 levels did not improve, which also indicates the liver problems (67).

7.3 Diagnosis

Reiner Hausmann diagnosed Bella with a PSS with accompanied HE. Diagnostic imaging alone was not enough to diagnose the PSS, he needed to perform an explorative laparotomy to see the expansion of the PSS. It turned out that the PSS is build out of a network of arteries which connected the portal vein with the systemic circulation. In this case the congenital PSS is not easy operable and is highly complex. Based on the clinical signs and the increased blood ammonia concentration of the second laboratory results, accompanied HE was diagnosed. Concurrent chronic inflammation has been diagnosed by the elevated cell count of segmented neutrophils and monocytes (69).

7.4 Treatment

In Bellas case it is hard to decide if surgery would be helpful or if it will worsen her prognosis. The problem here is the intricate network of arteries, which forms the PSS. The shunt could be ligated with an ameroid ring to stop the abnormal circulation and restore the blood flow of the liver, which will improve the detoxification of ammonia and other toxic substances. It also may be, that several surgeries are needed and the fact that she is a brachycephalic breed makes the anesthesia difficult and more risky. Concerning the topic of this thesis, FMT could be used as a supportive treatment. Certainly, FMT is no treatment of the actual cause which induces HE, but it definitely will improve her mental health and improves her quality of life. FMT is an experimental treatment due to the fact that until now no studies have been published concerning FMT treatment of HE in dogs. However, the results of the human study show clear success in improving the mental state and cognitive behavior of the patients. As already in Section 6 mentioned, it is possible to correlate the human GIT microbiome with the microbiome of the carnivore GIT. Therefore, it would be possible to transfer the acquired knowledge from the human study onto veterinarian cases. FMT would decrease her blood ammonia concentration, because of the reduction of urease producing gut bacteria, and it also would decrease her systemic inflammation. IL appearance correlates with the abundance of some phyla within the GIT. IL production decreased by shifting the ratio back to the autochthonous taxa after FMT application, and fewer inflammation signs were present in the human patients (36). Furthermore, the liver-prescribed diet with proteins high in bioavailability should be continued to keep the decreased metabolic

load to the liver. Feeding the liver-prescribed diet is only a supportive treatment, but can not restore the liver's normal function.

8. Conclusion

This thesis aimed to research FMT as a new treatment for dogs with HE. As preciously mentioned in Section 6, there are 2 reasons, why I human study results in this thesis are included. Those 2 reasons are, that FMT treatment in veterinary patients with HE is not studied (36), and that the human and dog gut have similar microflora due to their adaption to the same environmental factors (38). The case report of the french bulldog puppy shows the appearance of liver diseases with accompanied HE in dogs and the possibilities of treatment, including FMT.

The main aspects of human research are shortly summarized as follows. Cognition is directly correlated with the abundance of *Porphyromonadaceae* and *Alcaligenaceae*. In the HE-group, the IL-23 is highly associated with several phyla of the GIT. Patients on lactulose and lactulose and rifaximin did not show any significant differences in their stool flora. The systematic withdrawal of lactulose had a minimal effect on the microbial flora. The problem concerning the study of associating the microbial gut flora to a disease is the individual differences between the patients (36).

Nevertheless, the study noticed a significant difference between the healthy control group and the cirrhotic group and between the non-HE and the HE groups. In the control group, *Ruminococcaceae* and *Clostridium XIV* were overrepresented and were proportional less present in the cirrhotic group. Increased *Alcaligenaceae* population was associated with poor cognition in the HE group. The abundance of *Enterobacteriaceae* correlates with the worsening of inflammation (36). The study shows outstanding outcomes of FMT by improving cognition, dysbiosis, and the accompanying inflammation.

The outcomes of this research have provided insight in positive results of human studies (68), and how these studies can be the basis and encouragement for studies in veterinary medicine on this field. However, the results are still uncertain about the long-term effects of FMT, but the current results are promising. In spite of the large similarities of the human and dog gut (59), those similarities do not cover every little detail about the individual digestion and the microflora of the GIT. It has to be conscious, that not everything can be transferred from humans to dogs. Nevertheless, including the human study results were a big help in several aspects in this thesis, and the missing data in the veterinary field should encourage further veterinarians or scientists to perform experiments on dogs with HE. In the

opinion of the Author of this thesis, this topic has a bright perspective, because FMT is a simple and non-invasive method with a big influence on the neurological status of the patients, which can improve the quality of life of dogs with HE. This is a big step not only for the dogs but also for the owners to not see their animals suffer and do not have to put them down due to the impaired cognitive function. Due to the promising outcomes in human medicine (57), the Author of this thesis is very optimistic about future results of experiments in veterinary medicine.

So all in all the Author of this thesis believes, that FMT could be a new treatment method of HE in the veterinary field, but the Author of this thesis also like to emphasizes that this requires a lot of experiments on dogs, and finding the suitable patients takes a lot of time, but this should not be a deterrent. Rather should the positive outcomes of previous studies in human medicine encourage veterinarians to bring this research into veterinary medicine.

9. List of Reference

1: Suzuki, K., Benno, Y., Mitsuoka, T., Takebe, S., Kobashi, K. and Hase, J. (1979). Urease-producing species of intestinal anaerobes and their activities. *Applied and Environmental Microbiology*, 37(3), pp.379–382. doi:10.1128/aem.37.3.379-382.1979.

2: Sigurdarson, J.J., Svane, S. and Karring, H. (2018). The molecular processes of urea hydrolysis in relation to ammonia emissions from agriculture. *Reviews in Environmental Science and Bio/Technology*, 17(2), pp.241–258. doi:10.1007/s11157-018-9466-1.

3: Norenberg, M.D., Rao, K.V.R. and Jayakumar, A.R. (2005). Mechanisms of Ammonia-Induced Astrocyte Swelling. *Metabolic Brain Disease*, 20(4), pp.303–318. doi:10.1007/s11011-005-7911-7.

4: Barmore, W. and Stone, W.L. (2019). *Physiology, Urea Cycle*. [online] Nih.gov. Available at: https://www.ncbi.nlm.nih.gov/books/NBK513323/.

5: Vince, A.J. and Burridge, S.M. (1980). Ammonia production by intestinal bacteria: the effects of lactose, lactulose and glucose. *Journal of medical microbiology*, [online] 13(2), pp.177–91. doi:10.1099/00222615-13-2-177.

6: Biga, L.M., Dawson, S., Harwell, A., Hopkins, R., Kaufmann, J., LeMaster, M., Matern, P., Morrison-Graham, K., Quick, D. and Runyeon, J. (2019). Anatomy & Physiology. Oregon: open.oregonstate.education, pp.1-1964. Available at: https://open.oregonstate.education/aandp/chapter/24-4-protein-metabolism/.

7: Elwir, S. and Rahimi, R.S. (2017). Hepatic Encephalopathy: An Update on the Pathophysiology and Therapeutic Options. *Journal of Clinical and Translational Hepatology*, [online] 5(2), pp.142–151. doi:10.14218/JCTH.2016.00069.

8: Bleibel, W. and Al-Osaimi, A.M.S. (2012). Hepatic Encephalopathy. Saudi Journal of Gastroenterology : Official Journal of the Saudi Gastroenterology Association, 18(5), pp.301–309. doi:10.4103/1319-3767.101123.

9: Ridola, L., Riggio, O., Gioia, S., Faccioli, J. and Nardelli, S. (2020). Clinical management of type C hepatic encephalopathy. *United European Gastroenterology Journal*, [online] 8(5), pp.536–543. doi:10.1177/2050640620909675.

10: Wouters, A.T.B., Wouters, F., Boabaid, F.M., Watanabe, T.T.N., Fredo, G., Varaschin, M.S. and Driemeier, D. (2017). Brain lesions associated with acute toxic hepatopathy in cattle. *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, [online] 29(3), pp.287–292. doi:10.1177/1040638717699242.

11: Kato, M., Hughes, R.D., Keays, R.T. and Williams, R. (1992). Electron microscopic study of brain capillaries in cerebral edema from fulminant hepatic failure. *Hepatology (Baltimore, Md.)*, [online] 15(6), pp.1060–1066. doi:10.1002/hep.1840150615.

12: Nisarga, P., Gyure, K. (2021). Hepatic encephalopathy. *PathologyOutlines.com website.* Available at: https://www.pathologyoutlines.com/topic/cnshepatic.html [Accessed 22 April 2022].

13: Lidbury, J.A., Cook, A.K. and Steiner, J.M. (2016). Hepatic encephalopathy in dogs and cats. *Journal of Veterinary Emergency and Critical Care*, 26(4), pp.471–487. doi:10.1111/vec.12473.

14: Suárez, I., Bodega, G. and Fernández, B. (2002). Glutamine synthetase in brain: effect of ammonia. *Neurochemistry International*, 41(2-3), pp.123–142. doi:10.1016/s0197-0186(02)00033-5.

15: Holecek, M. (2013). Evidence of a vicious cycle in glutamine synthesis and breakdown in pathogenesis of hepatic encephalopathy–therapeutic perspectives. *Metabolic Brain Disease*, 29(1), pp.9–17. doi:10.1007/s11011-013-9428-9.

16: Colombo, E. and Farina, C. (2016). Astrocytes: Key Regulators of Neuroinflammation. *Trends in Immunology*, [online] 37(9), pp.608–620. doi:10.1016/j.it.2016.06.006.

17: Tofteng, F., Hauerberg, J., Hansen, B.A., Pedersen, C.B., Jørgensen, L. and Larsen, F.S. (2006). Persistent arterial hyperammonemia increases the concentration of glutamine and alanine in the brain and correlates with intracranial pressure in patients with fulminant hepatic failure. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, [online] 26(1), pp.21–27. doi:10.1038/sj.jcbfm.9600168.

18: Zwingmann, C., Leibfritz, D. and Hazell, A.S. (2003). Energy Metabolism in Astrocytes and Neurons Treated with Manganese: Relation among Cell-Specific Energy Failure, Glucose Metabolism, and Intercellular Trafficking Using Multinuclear NMR-Spectroscopic Analysis. *Journal of Cerebral Blood Flow & Metabolism*, 23(6), pp.756–771. doi:10.1097/01.wcb.0000056062.25434.4d.

19: Ciećko-Michalska, I., Szczepanek, M., Słowik, A. and Mach, T. (2012). Pathogenesis of Hepatic Encephalopathy. *Gastroenterology Research and Practice*, [online] 2012, p.642108. doi:10.1155/2012/642108.

20: Cordoba, J. (2014). Hepatic Encephalopathy: From the Pathogenesis to the New Treatments. *ISRN Hepatology*, [online] 2014, pp.1–16. doi:10.1155/2014/236268.

21: Cruzat, V., Macedo Rogero, M., Noel Keane, K., Curi, R. and Newsholme, P. (2018). Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. *Nutrients*, [online] 10(11), p.1564. doi:10.3390/nu10111564.

22: Tranah, T.H., Vijay, G.K.M., Ryan, J.M. and Shawcross, D.L. (2012). Systemic inflammation and ammonia in hepatic encephalopathy. *Metabolic Brain Disease*, 28(1), pp.1–5. doi:10.1007/s11011-012-9370-2.

23: Azhari, H. and Swain, M.G. (2018). Role of Peripheral Inflammation in Hepatic Encephalopathy. *Journal of Clinical and Experimental Hepatology*, 8(3), pp.281–285. doi:10.1016/j.jceh.2018.06.008.

24: Ferenci, P. (2017). Hepatic encephalopathy. *Gastroenterology Report*, [online] 5(2), pp.138–147. doi:10.1093/gastro/gox013.

25: Butterworth, R.F. (2019). Hepatic Encephalopathy in Cirrhosis: Pathology and Pathophysiology. *Drugs*, 79(S1), pp.17–21. doi:10.1007/s40265-018-1017-0.

26: Nayak, D., Roth, T.L. and McGavern, D.B. (2014). Microglia Development and function. *Annual review of immunology*, [online] 32, pp.367–402. doi:10.1146/annurev-immunol-032713-120240.

27: Jaeger, V., DeMorrow, S. and McMillin, M. (2019). The Direct Contribution of Astrocytes and Microglia to the Pathogenesis of Hepatic Encephalopathy. *Journal of Clinical and Translational Hepatology*, 7(X), pp.1–10. doi:10.14218/jcth.2019.00025.

28: Mizuno, T. (2015). Neuron-microglia interactions in neuroinflammation. *Clinical and Experimental Neuroimmunology*, 6(3), pp.225–231. doi:10.1111/cen3.12228.

29: Jayakumar, A.R., Rama Rao, K.V. and Norenberg, M.D. (2015). Neuroinflammation in Hepatic Encephalopathy: Mechanistic Aspects. *Journal of Clinical and Experimental Hepatology*, 5, pp.S21–S28. doi:10.1016/j.jceh.2014.07.006.

30: Nguyen, J.H. (2012). Blood–brain barrier in acute liver failure. *Neurochemistry International*, [online] 60(7), pp.676–683. doi:10.1016/j.neuint.2011.10.012.

31: Raabe, W. (1987). Synaptic transmission in ammonia intoxication. *Neurochemical Pathology*, 6(1-2), pp.145–166. doi:10.1007/bf02833604.

32: Vignes, M. and Collingridge, G.L. (1997). The synaptic activation of kainate receptors. *Nature*, 388(6638), pp.179–182. doi:10.1038/40639.

33: Magi, S., Piccirillo, S., Amoroso, S. and Lariccia, V. (2019). Excitatory Amino Acid Transporters (EAATs): Glutamate Transport and Beyond. *International Journal of Molecular Sciences*, 20(22), p.5674. doi:10.3390/ijms20225674.

34: Limón, I.D., Angulo-Cruz, I., Sánchez-Abdon, L. and Patricio-Martínez, A. (2021). Disturbance of the Glutamate-Glutamine Cycle, Secondary to Hepatic Damage, Compromises Memory Function. *Frontiers in Neuroscience*, 15, p.578922. doi:10.3389/fnins.2021.578922.

35: Jones, E.A., Schafer, D.F., Ferenci, P. and Pappas, S.C. (1984). The GABA hypothesis of the pathogenesis of hepatic encephalopathy: current status. *The Yale Journal of Biology and Medicine*, [online] 57(3), pp.301–316. Available at: https://pubmed.ncbi.nlm.nih.gov/6093394/ [Accessed 30 April 2022].

36: Norman, K., Valentini, L., Lochs, H. and Pirlich, M. (2010). [Protein catabolism and malnutrition in liver cirrhosis - impact of oral nutritional therapy]. *Zeitschrift Fur Gastroenterologie*, [online] 48(7), pp.763–770. doi:10.1055/s-0029-1245388.

37: Dam, G., Aamann, L., Vistrup, H. and Gluud, L.L. (2018). The role of Branched Chain Amino Acids in the treatment of hepatic Encephalopathy. *Journal of Clinical and Experimental Hepatology*, 8(4), pp.448–451. doi:10.1016/j.jceh.2018.06.004.

38: Holecek, M. (2015). Ammonia and amino acid profiles in liver cirrhosis: Effects of variables leading to hepatic encephalopathy. *Nutrition*, 31(1), pp.14–20. doi:10.1016/j.nut.2014.03.016.

39: Bajaj, J.S. (2014). The role of microbiota in hepatic encephalopathy. *Gut Microbes*, 5(3), pp.397–403. doi:10.4161/gmic.28684.

40: Salgado, M. and Cortes, Y. (2013). Hepatic encephalopathy: diagnosis and treatment. *Compendium (Yardley, PA)*, [online] 35(6), pp.E1-9; E10. Available at: https://pubmed.ncbi.nlm.nih.gov/23677823/ [Accessed 1 May 2022].

41: Ojetti, V., Lauritano, E.C., Barbaro, F., Migneco, A., Ainora, M.E., Fontana, L., Gabrielli, M. and Gasbarrini, A. (2009). Rifaximin pharmacology and clinical implications. *Expert Opinion on Drug Metabolism & Toxicology*, 5(6), pp.675–682. doi:10.1517/17425250902973695.

42: Robins, G.W. and Wellington, K. (2005). Rifaximin. *Drugs*, 65(12), pp.1697–1713. doi:10.2165/00003495-200565120-00011.

43: Gillis, J.C. and Brogden, R.N. (1995). Rifaximin. *Drugs*, 49(3), pp.467–484. doi:10.2165/00003495-199549030-00009.

44: Weber, F.L. (1996). Lactulose and combination therapy of hepatic encephalopathy: the role of the intestinal microflora. *Digestive diseases (Basel, Switzerland)*, 14 Suppl 1, pp.53–63. doi:10.1159/000171583..

45: Madsen, M., Kimer, N., Bendtsen, F. and Petersen, A.M. (2021). Fecal microbiota transplantation in hepatic encephalopathy: a systematic review. *Scandinavian Journal of Gastroenterology*, 56(5), pp.560–569. doi:10.1080/00365521.2021.1899277.

46: Lazebnik, L., Sitkin, S., Kao, D. and Karakan, T. (2020). *WGO Handbook on gut microbiome. A Global Perspective*. Milwaukee: World Gastroenterology Organisation, pp.1–87. Available at: https://www.apage.org/files/WDHD-2020-hdbk.pdf.

47: Allegretti, J.R., Korzenik, J.R. and Hamilton, M.J. (2014). Fecal Microbiota Transplantation via Colonoscopy for Recurrent C. difficile Infection. *Journal of Visualized Experiments*, (94), e52154, pp.1-6. doi:10.3791/52154.

48: Gweon, T.-G. and Na, S.-Y. (2021). Next Generation Fecal Microbiota Transplantation. *Clinical Endoscopy*, [online] 54(2), pp.152–156. doi:10.5946/ce.2021.053.

49: Ng, S.C., Kamm, M.A., Yeoh, Y.K., Chan, P.K.S., Zuo, T., Tang, W., Sood, A., Andoh, A., Ohmiya, N., Zhou, Y., Ooi, C.J., Mahachai, V., Wu, C.-Y., Zhang, F., Sugano, K. and Chan, F.K.L. (2019). Scientific frontiers in faecal microbiota transplantation: joint document of Asia-Pacific Association of Gastroenterology (APAGE) and Asia-Pacific Society for Digestive Endoscopy (APSDE). *Gut*, 69(1), pp.83–91. doi:10.1136/gutjnl-2019-319407.

50: Liu, X., Li, Y., Wu, K., Shi, Y. and Chen, M. (2021). Fecal Microbiota Transplantation as Therapy for Treatment of Active Ulcerative Colitis: A Systematic Review and Meta-Analysis. *Gastroenterology Research and Practice*, 2021, pp.1–13. doi:10.1155/2021/6612970.

51: Hassouneh, R. and Bajaj, J.S. (2021). Gut Microbiota Modulation and Fecal Transplantation: An Overview on Innovative Strategies for Hepatic Encephalopathy Treatment. *Journal of Clinical Medicine*, 10(2), p.330. doi:10.3390/jcm10020330.

52: Olmedo, M., Reigadas, E., Valerio, M., Vázquez-Cuesta, S., Pajares, J.A., Matilla, A., Muñoz, P. and Bouza, E. (2019). Is it reasonable to perform Fecal Microbiota Transplantation for recurrent Clostridium difficile Infection in patients with liver cirrhosis? *Revista Española de Quimioterapia*, [online] 32(2), pp.205-207. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6441988/ [Accessed 23 May 2022].

53: Thaiss, Christoph A., Zeevi, D., Levy, M., Zilberman-Schapira, G., Suez, J., Tengeler, Anouk C., Abramson, L., Katz, Meirav N., Korem, T., Zmora, N., Kuperman, Y., Biton, I., Gilad, S., Harmelin, A., Shapiro, H., Halpern, Z., Segal, E. and Elinav, E. (2014). Transkingdom Control of Microbiota Diurnal Oscillations Promotes Metabolic Homeostasis. *Cell*, [online] 159(3), pp.514–529. doi:10.1016/j.cell.2014.09.048.

54: Bokoliya, S.C., Dorsett, Y., Panier, H. and Zhou, Y. (2021). Procedures for Fecal Microbiota Transplantation in Murine Microbiome Studies. *Frontiers in Cellular and Infection Microbiology*, 11, p.711055. doi:10.3389/fcimb.2021.711055.

55: Encyclopedia Britannica. (n.d.). *Principle of competitive exclusion | biology*. [online] Available at: https://www.britannica.com/science/principle-of-competitive-exclusion.

56: Wang, W.-W., Zhang, Y., Huang, X.-B., You, N., Zheng, L. and Li, J. (2017). Fecal microbiota transplantation prevents hepatic encephalopathy in rats with carbon tetrachloride-induced acute hepatic dysfunction. *World Journal of Gastroenterology*, 23(38), pp.6983–6994. doi:10.3748/wjg.v23.i38.6983.

57: Bajaj, J.S., Ridlon, J.M., Hylemon, P.B., Thacker, L.R., Heuman, D.M., Smith, S., Sikaroodi, M. and Gillevet, P.M. (2012). Linkage of gut microbiome with cognition in hepatic encephalopathy. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 302(1), pp.G168–G175. doi:10.1152/ajpgi.00190.2011.

58: Tuniyazi, M., Hu, X., Fu, Y. and Zhang, N. (2022). Canine Fecal Microbiota Transplantation: Current Application and Possible Mechanisms. *Veterinary Sciences*, [online] 9(8), p.396. doi:10.3390/vetsci9080396.

59: Ferreira, M. da F., Salavati Schmitz, S., Schoenebeck, J.J., Clements, D.N., Campbell, S.M., Gaylor, D.E., Mellanby, R.J., Gow, A.G. and Salavati, M. (2019). Lactulose drives a reversible reduction and qualitative modulation of the faecal microbiota diversity in healthy dogs. *Scientific Reports*, [online] 9(1), p.13350. doi:10.1038/s41598-019-50090-7.

60: Gweon, T.-G., Kim, J., Lim, C.-H., Park, J.M., Lee, D.-G., Lee, I.S., Cho, Y.-S., Kim, S.W. and Choi, M.-G. (2016). Fecal Microbiota Transplantation Using Upper Gastrointestinal Tract for the Treatment of Refractory or Severe Complicated *Clostridium difficile* Infection in Elderly Patients in Poor Medical Condition: The First Study in an Asian Country. *Gastroenterology Research and Practice*, [online] 2016, pp.1–6. doi:10.1155/2016/2687605.

61: Bajaj, J.S., Kassam, Z., Fagan, A., Gavis, E.A., Liu, E., Cox, I.J., Kheradman, R., Heuman, D., Wang, J., Gurry, T., Williams, R., Sikaroodi, M., Fuchs, M., Alm, E., John, B., Thacker, L.R., Riva, A., Smith, M., Taylor-Robinson, S.D. and Gillevet, P.M. (2017). Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. *Hepatology*, 66(6), pp.1727–1738. doi:10.1002/hep.29306.

62: Freitag, T.L., Hartikainen, A., Jouhten, H., Sahl, C., Meri, S., Anttila, V.-J., Mattila, E., Arkkila, P., Jalanka, J. and Satokari, R. (2019). Minor Effect of Antibiotic Pre-treatment on the Engraftment of Donor Microbiota in Fecal Transplantation in Mice. *Frontiers in Microbiology*, 10, p.2685. doi:10.3389/fmicb.2019.02685.

63: Park, S.-Y. and Seo, G.S. (2021). Fecal Microbiota Transplantation: Is It Safe? *Clinical Endoscopy*, [online] 54(2), pp.157–160. doi:10.5946/ce.2021.072.

64: Kumariya, R., Garsa, A.K., Rajput, Y.S., Sood, S.K., Akhtar, N. and Patel, S. (2019). Bacteriocins: Classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. *Microbial Pathogenesis*, 128, pp.171–177. doi:10.1016/j.micpath.2019.01.002.

65: Heilbronner, S., Krismer, B., Brötz-Oesterhelt, H. and Peschel, A. (2021). The microbiome-shaping roles of bacteriocins. *Nature Reviews Microbiology*, [online] 19(11), pp.726–739. doi:10.1038/s41579-021-00569-w.

66: Xie, W.-R., Yang, X.-Y., Xia, H.H.-X. and He, X.-X. (2018). Fecal Microbiota Transplantation for Treating Hepatic Encephalopathy: Experimental and Clinical Evidence and Possible Underlying Mechanisms. *Journal of Exploratory Research in Pharmacology*, 3(4), pp.105–110. doi:10.14218/jerp.2018.00017.

67: Sharma, A., Punekar, P. and Jain, A. (2018). A study of thyroid dysfunction in cirrhosis of liver and correlation with severity of liver disease. *Indian Journal of Endocrinology and Metabolism*, 22(5), p.645. doi:10.4103/ijem.ijem_25_18.

68: Ohara, T. (2019). Identification of the microbial diversity after fecal microbiota transplantation therapy for chronic intractable constipation using 16s rRNA amplicon sequencing. *PLOS ONE*, 14(3), p.e0214085. doi:10.1371/journal.pone.0214085.

69: R. Hausmann (2021). *The case of Bella, a french bulldog with HE*.

Figure sources

Figure 1: own drawing based on text of following source: Sigurdarson, J.J., Svane, S. and Karring, H. (2018). The molecular processes of urea hydrolysis in relation to ammonia emissions from agriculture. *Reviews in Environmental Science and Bio/Technology*, 17(2), pp.241–258. doi:10.1007/s11157-018-9466-1.

Figure 2: Cruzat, V., Macedo Rogero, M., Noel Keane, K., Curi, R. and Newsholme, P. (2018). Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. *Nutrients*, [online] 10(11), p.1564. doi:10.3390/nu10111564.

Figure 3: www.pathologyoutlines.com. (n.d.). *Hepatic encephalopathy*. [online] Available at: https://www.pathologyoutlines.com/topic/cnshepatic.html [Accessed 22 April 2022]

Figure 4: Ng, S.C., Kamm, M.A., Yeoh, Y.K., Chan, P.K.S., Zuo, T., Tang, W., Sood, A., Andoh, A., Ohmiya, N., Zhou, Y., Ooi, C.J., Mahachai, V., Wu, C.-Y., Zhang, F., Sugano, K. and Chan, F.K.L. (2019). Scientific frontiers in faecal microbiota transplantation: joint document of Asia-Pacific Association of Gastroenterology (APAGE) and Asia-Pacific Society for Digestive Endoscopy (APSDE). *Gut*, 69(1), pp.83–91. doi:10.1136/gutjnl-2019-319407.

Figure 5: Ohara, T. (2019). Identification of the microbial diversity after fecal microbiota transplantation therapy for chronic intractable constipation using 16s rRNA amplicon sequencing. *PLOS ONE*, 14(3), p.e0214085. doi:10.1371/journal.pone.0214085.

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Appendix 1

SYNLAB vet
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Tierhalter:	**HAUSMANN**	Tiername / Rasse:	**BELLA, Franzoesische Bulldo**		
Tierart:	**Hund**	Geschlecht:	**weiblich**	Entnahme:	**18.10.2021**
Alter:	**1**	Geburtsdatum:	**01.01.2020**	Eingang:	**19.10.2021 08:08**
Vorbericht:				Untersuchungsbeginn:	**19.10.2021**
Material:	**1x EDTA-Blut, 1x EDTA-Plasma gefroren, 1x Serum**			Endbefund:	**21.10.2021 14:13**
Barcode:	**2416244234**	Labor-ID:	**XK 1422 3034**		

Untersuchung:	Ergebnis:	Einheit:	Referenzbereich:	Grafik:	Methode:
Notfallprobe	bearbeitet				3
Geriatrie-ScreeningHund	.				
Blutbild, groß	.				
Leukozyten	27.6 ▲	G/l	4.5 - 12.0		FCM
Erythrozyten	6.83	T/l	5.9 - 8.5		FCM
Hämoglobin	144	g/l	142 - 202		FCM
Hämatokrit	0.46	l/l	0.45 - 0.64		RECH
MCV	67.4 ▼	fl	67.5 - 81.9		FCM
HBE (MCH)	21.1 ▼	pg	22.4 - 25.8		RECH
MCHC	31.3	g/dl	29.5 - 35.8		RECH
Thrombozyten	277	G/l	110 - 580		FCM
CHr (Retikulozytenhämoglobin)	1.55	fmol/l	1.36 - 1.69		FCM
Retikulozyten abs.	61	G/l	< 121		RECH
Das Blutbild wurde kontrolliert.					
Differentialblutbild					
Neutrophile	75	%	43 - 76		FCM
Stabkernige	0	%	< 1		MIK
Lymphozyten	12 ▼	%	14 - 46		FCM
Monozyten	7	%	0 - 8		FCM
Eosinophile	4	%	0 - 14		FCM
Bitte beachten: geänderter Referenzbereich					
Basophile	1	%	0 - 1		FCM
Sonstige Zellen	2 ▲	%	0 - 1		MIK
Bitte beachten: geänderter Referenzbereich					
Neutrophile abs.	20700 ▲	/µl	2500 - 8100		RECH
Stabkernige abs.	0	/µl	0		RECH
Lymphozyten abs.	3312	/µl	1000 - 3900		RECH
Monozyten abs.	1932 ▲	/µl	< 600		RECH
Eosinophile abs.	1104 ▲	/µl	< 1100		RECH
Basophile abs.	276 ▲	/µl	0 - 120		RECH
Bitte beachten: geänderter Referenzbereich					
Sonstige Zellen abs.	552	/µl			RECH
Leber					
Alkalische Phosphatase	224 ▲	U/l	< 128		PHO 1
AST (GOT)	97 ▲	U/l	< 62		PHO 1
ALT (GPT)	397 ▲	U/l	< 118		PHO 1
γ-GT	<5	U/l	< 11		PHO 1
GLDH	13.9 ▲	U/l	< 10.5		PHO 1
Bilirubin gesamt	5.13	µmol/l	< 5.20		PHO 1
Pankreas					
alpha-Amylase	407	U/l	311 - 1142		PHO 1

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Tierhalter: **HAUSMANN** Tiername / Rasse: **BELLA, Franzoesische Bulldo**

Barcode: **2416244234** Labor-ID: **XK 1422 3034**

Untersuchung:	Ergebnis:	Einheit:	Referenzbereich:	Grafik:	Methode:
DGGR-Lipase	50	U/l	< 127		PHO 1
Bewertung DGGR-Lipase: 127 - 213 U/l = Graubereich im Hinblick auf eine Pankreatitis. > 213 U/l = Hinweis auf Pankreatitis Eine Diagnosestellung erfolgt unter Berücksichtigung der klinischen, labordiagnostischen und ggf. sonographischen Befunde.					
Muskulatur					
CK (NAC)	160	U/l	< 338		PHO 1
Fettstoffwechsel					
Cholesterin	1.97 ▼	mmol/l	4.30 - 10.50		PHO 1
Triglyzeride	0.39 ▼	mmol/l	0.40 - 2.80		PHO 1
Niere					
Kreatinin	57.5	µmol/l	< 141		PHO 1
Harnstoff	2.33 ▼	mmol/l	3.2 - 11.5		PHO 1
Bitte beachten: geänderter Referenzbereich					
Elektrolyte					
Natrium	150	mmol/l	144 - 152		ISE 1
Kalium	4.1	mmol/l	4.1 - 5.7		ISE 1
Bitte beachten: geänderter Referenzbereich					
Natrium/Kalium-Quotient	37 ▲		26 - 35		RECH
Calcium	2.44	mmol/l	2.28 - 2.85		PHO 1
Bitte beachten: geänderter Referenzbereich					
Calcium (korr. auf Albumin)	2.68	mmol/l			RECH
Hypalbuminämien führen zu falsch niedrigen Calcium-Werten. Bei erniedrigtem Albumin ist deshalb eine rechnerische Korrektur des Calcium-Wertes notwendig.					
Magnesium	0.89	mmol/l	0.70 - 1.07		PHO 1
Chlorid	109	mmol/l	106 - 117		ISE 1
Phosphat	1.97	mmol/l	0.82 - 2.00		PHO 1
Kohlenhydratstoffwechsel					
Glukose	4.88	mmol/l	3.9 - 6.7		PHO 1
Fruktosamine	169	µmol/l	160 - 350		PHO 1
Bitte beachten: geänderter Referenzbereich					
Proteinstoffwechsel					
Albumin	25.2 ▼	g/l	28.1 - 39.4		PHO 1
Globuline	36.8	g/l	25.7 - 42.2		RECH
Gesamteiweiß	62.0	g/l	57 - 76		PHO 1
BNP canin (canines Nt-proBNP)	<250	pmol/l	< 900		EIA 2, 3
Interpretation: <900 pmol/l: Die Wahrscheinlichkeit, dass die klinischen Symptome (d. h. respiratorische Symptome und/oder Leistungsintoleranz) durch eine Herzinsuffizienz bedingt sind, ist gering. 900-1800 pmol/l: Eine Nt-proBNP Konzentration in diesem Bereich erlaubt keine Unterscheidung der klinischen Symptome in kardiale und nichtkardiale Ursachen. Zur Eingrenzung der Ursache werden weitere diagnostische Schritte empfohlen. >1800 pmol/l: Die Wahrscheinlichkeit, dass die klinischen Symptome, (d.h. respiratorische Symptome und/oder Leistungsintoleranz) durch eine Herzinsuffizienz bedingt sind, ist hoch. Eine weitere kardiologische Aufarbeitung des Patienten wird empfohlen.					
TSH canin	0.04	ng/ml	0.03 - 0.40		CLIA 1
T4 gesamt	16.99 ▼	nmol/l	19.3 - 57.9		CLIA 1, 3
Interpretation: Widersprüchliche Ergebnisse: Der TSH-Wert spricht für eine euthyreote Stoffwechsellage, die peripheren Schilddrüsenhormone sind jedoch erniedrigt. Mögliche Ursachen: - ca. 30% der klinisch manifesten Hypothyreosen zeigen normale TSH-Werte - Nicht-thyreoidale Erkrankungen (Euthyroid sick syndrome) - Medikamente (z.B. Corticosteroide, Phenobarbital,Sulfonamide)					

Appendix 2

SYNLAB

DAkkS Deutsche Akkreditierungsstelle D-PL-14016-01-00

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Tierhalter:	**HAUSMANN**	Tiername / Rasse:	**BELLA, FRZ BD**		
Tierart:	**Hund**	Geschlecht:	**weiblich**	Entnahme:	**29.10.2021**
Alter:	**1**	Geburtsdatum:	**01.01.2020**	Eingang:	**30.10.2021 06:52**
Vorbericht:				Untersuchungsbeginn:	**30.10.2021**
Material:	**1x EDTA-Blut, 1x EDTA-Plasma gefroren, 1x Serum**			Endbefund:	**30.10.2021 12:39**
Barcode:	**2416243936**	Labor-ID:	**XK 1436 3166**		

Untersuchung:	Ergebnis:	Einheit:	Referenzbereich:	Grafik:	Methode:
Individuelles Praxis-Profil 1	.				
Bearbeitung Abrechnung	bearbeitet				
Geriatrie-ScreeningHund	.				
Blutbild, groß	.				
Leukozyten	27.7 ▲	G/l	4.5 - 12.0		FCM
Erythrozyten	6.03	T/l	5.9 - 8.5		FCM
Hämoglobin	127 ▼	g/l	142 - 202		FCM
Hämatokrit	0.41 ▼	l/l	0.45 - 0.64		RECH
MCV	68.2	fl	67.5 - 81.9		FCM
HBE (MCH)	21.1 ▼	pg	22.4 - 25.8		RECH
MCHC	30.9	g/dl	29.5 - 35.8		RECH
Thrombozyten	259	G/l	110 - 580		FCM
CHr (Retikulozytenhämoglobin)	1.53	fmol/l	1.36 - 1.69		FCM
Retikulozyten abs.	96	G/l	< 121		RECH
Segmentkernige	82 ▲	%	55 - 75		MIK
Stabkernige	6 ▲	%	< 1		MIK
Lymphozyten	5 ▼	%	13 - 30		MIK
Monozyten	7 ▲	%	0 - 5		MIK
Eosinophile	0	%	0 - 6		MIK
Basophile	0	%	0 - 1		MIK
Sonstige Zellen	0	%	0 - 1		MIK
Bitte beachten: geänderter Referenzbereich					
Segmentkernige abs.	22714 ▲	/µl	3000 - 9000		RECH
Stabkernige abs.	1662 ▲	/µl	0		RECH
Lymphozyten abs.	1385	/µl	1000 - 3900		RECH
Monozyten abs.	1939 ▲	/µl	< 600		RECH
Eosinophile abs.	0	/µl	< 1100		RECH
Basophile abs.	0	/µl	0 - 120		RECH
Bitte beachten: geänderter Referenzbereich					
Sonstige Zellen abs.	0	/µl			RECH
Das Differentialblutbild wurde manuell überprüft.					
Leber					
Alkalische Phosphatase	137 ▲	U/l	< 128		PHO 1
AST (GOT)	78 ▲	U/l	< 62		PHO 1
ALT (GPT)	245 ▲	U/l	< 118		PHO 1
γ-GT	<5	U/l	< 11		PHO 1
GLDH	8.2	U/l	< 10.5		PHO 1
Bilirubin gesamt	5.13	µmol/l	< 5.20		PHO 1
LDH	79	U/l	< 497		PHO 1
Pankreas					
alpha-Amylase	267 ▼	U/l	311 - 1142		PHO 1

SYNLAB vet

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Barcode:	**2416243936**	Labor-ID:	**XK 1436 3166**

Untersuchung:	Ergebnis:	Einheit:	Referenzbereich:	Grafik:	Methode:
DGGR-Lipase	76	U/l	< 127		PHO 1
Bewertung DGGR-Lipase: 127 - 213 U/l = Graubereich im Hinblick auf eine Pankreatitis. > 213 U/l = Hinweis auf Pankreatitis Eine Diagnosestellung erfolgt unter Berücksichtigung der klinischen, labordiagnostischen und ggf. sonographischen Befunde.					
Muskulatur					
CK (NAC)	186	U/l	< 338		PHO 1
Fettstoffwechsel					
Cholesterin	2.72 ▼	mmol/l	4.30 - 10.50		PHO 1
Triglyzeride	0.39 ▼	mmol/l	0.40 - 2.80		PHO 1
Niere					
Kreatinin	18.6	µmol/l	< 141		PHO 1
Harnstoff	2.33 ▼	mmol/l	3.2 - 11.5		PHO 1
Bitte beachten: geänderter Referenzbereich					
Ammoniak	213 ▲	µmol/l	< 59		PHO 1
Bei der Befundbewertung ist zu beachten, daß die Konzentration von Ammoniak infolge Zersetzung von Harnstoff laufend ansteigt. Eine valide Aussage setzt daher eine Zentrifugation der EDTA-Blutprobe innerhalb von 15 min sowie das unmittelbar anschließende Einfrieren des abgetrennten Plasmas voraus.					
Elektrolyte					
Natrium	151	mmol/l	144 - 152		ISE 1
Kalium	4.6	mmol/l	4.1 - 5.7		ISE 1
Bitte beachten: geänderter Referenzbereich					
Natrium/Kalium-Quotient	33		26 - 35		RECH
Calcium	2.29	mmol/l	2.28 - 2.85		PHO 1
Bitte beachten: geänderter Referenzbereich					
Calcium (korr. auf Albumin)	2.54	mmol/l			RECH
Hypalbuminämien führen zu falsch niedrigen Calcium-Werten. Bei erniedrigtem Albumin ist deshalb eine rechnerische Korrektur des Calcium-Wertes notwendig.					
Magnesium	0.99	mmol/l	0.70 - 1.07		PHO 1
Chlorid	116	mmol/l	106 - 117		ISE 1
Phosphat	1.84	mmol/l	0.82 - 2.00		PHO 1
Kohlenhydratstoffwechsel					
Glukose	5.27	mmol/l	3.9 - 6.7		PHO 1
Fruktosamine	173	µmol/l	160 - 350		PHO 1
Bitte beachten: geänderter Referenzbereich					
Proteinstoffwechsel					
Albumin	24.9 ▼	g/l	28.1 - 39.4		PHO 1
Albumin/Globulin Quotient	0.8		0.7 - 1.7		RECH
Globuline	32.1	g/l	25.7 - 42.2		RECH
Gesamteiweiß	57.0	g/l	57 - 76		PHO 1
T4 gesamt	7.18 ▼	nmol/l	19.3 - 57.9		CLIA 1 3
Hinweis: Die zusätzliche Bestimmung von TSH und/oder fT4 ist zur Absicherung der Diagnose einer Hypothyreose zu empfehlen. Zu beachten ist, dass nicht-thyreoidale Erkrankungen und Medikamente (z.B. Corticosteroide, Phenobarbital, Sulfonamide) die peripheren Schilddrüsenhormone supprimieren können.					

Mit kollegialen Grüßen - validiert durch: Tierärztin Anne-Christin Matschos

Rechnung an: Einsender

Untersuchung bei SYNLAB vet a) Augsburg / k) Leverkusen, h) Hamburg, l) Leipzig, n) Berlin, /1) Untersuchung im SYNLAB-Verbund, / 2) Untersuchung extern / 3) nicht akkreditiert

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Thesis progress report for veterinary students

Name of student: Sara Maria Luise Schäfer

Neptun code of the student: U9C06G

Name and title of the supervisor: David Sandor Kiss, PhD

Department: Department of Physiology and Biochemistry

Thesis title: Fecal microbiota transplantation as a treatment of hepatic encephalopathy in dogs

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	01	20	Correction of structure, removing some sections and bring them in order	
2.	2022	02	10	Adding transitions for each section	
3.	2022	03	04	improvements of figures	
4.	2022	04	18	Improvement of own created figure	
5.	2022	06	10	Adding abstract, conclusion, discussion	

Grade achieved at the end of the first semester: 5 (excellent)

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	09	08	Correction of some sections	
2.	2022	09	19	Correction of discussion and abstract	
3.	2022	10	4	Correction of caption of figures	
4.	2022	10	14	Correction of list of references	

5.	2022	11	08	Correction of formatting	

Grade achieved at the end of the second semester: 5 (excellent)

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: .....

Date of handing the thesis in..... .....