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The relationship between coprophagy and autoimmune disease

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1. List of abbreviations

anti-CCP	Anti-Cyclic Citrullinated Peptide
AID	autoimmune Disease
AT	Autoimmune Thyroiditis
BP	Bullous Pemphigoid
DLE	Discoid Lupus Erythematosus
FMT	Fecal Microbiota Transplantation
GAD65	Glutamic Acid Decarboxylase 65
GALT	Gut-Associated Lymphoid Tissue
GI	Gastrointestinal
GWAS	Genome-wide Association Studies
IA2	insulinoma Antigen 2
IBD	inflammatory Bowel Disease
IMHA	Immune-Mediated Hemolytic Anemia
MG	Myasthenia Gravis
MHC	Major Histocompatibility Complex
MUO	Meningoencephalomyelitis
PF	Pemphigus Foliaceus
PV	Pemphigus Vulgaris
RA	Rheumatoid Arthritis
SCFA	Short-Chain Fatty Acids
SIgAD	Selective IgA Deficiency
SLE	Systemic Lupus Erythematosus
T1DM	Type 1 Diabetes Mellitus
Th	Helper T

1. Introduction

Autoimmune diseases, a diverse group of conditions characterized by the body's immune system attacking its own healthy tissues, pose significant health challenges across the canine population. Coprophagy, the consumption of feces, is a behavior observed in dogs that has intrigued veterinarians and pet owners alike. These two seemingly unrelated aspects of canine health, autoimmune diseases, and coprophagy, are the subjects of this research, which seeks to uncover potential connections between them.

Autoimmune diseases in dogs encompass a range of conditions such as immune-mediated hemolytic anemia (IMHA), systemic lupus erythematosus (SLE), and more. These diseases can lead to chronic health issues, reduced quality of life, and even life-threatening complications. Coprophagy, on the other hand, is a behavior wherein dogs consume feces, whether their own, that of other animals.This behavior has raised questions regarding its origins, potential health implications, and its association with autoimmune conditions.

Understanding the motivations behind coprophagy is a challenge. Some propose it as a survival instinct, dating back to a time when dogs scavenged for food, while others attribute it to behavioral and psychological factors [44]. Research has pointed to potential links between the gut microbiome, the immune system, and the development of autoimmune conditions. [33] The gut, which houses trillions of microorganisms, plays a crucial role in immune regulation. The microbiome's influence on autoimmune diseases is an emerging area of study, both in veterinary and human medicine.

This thesis aims to explore the distinctive correlation between autoimmune diseases and coprophagy. We distributed questionnaires to dog owners to try to find possible associations between autoimmune conditions in dogs and their tendency for consuming feces.

2. Literature review

3.1 Biological background on autoimmune disease

The immune system is responsible for the protection of the body against foreign organisms and the repair of damaged tissues. The adaptive immune system goes through a maturation process in which immune cells, T and B-cells, are going through selection. Cells that react against their own-tissue are eliminated and cells that tolerate their own-tissue remain. This regulatory process is crucial for the correct function of the immune system, however for those individuals in which regulation is poor, an autoimmune disease (AID) might develop resulting from immune response against self-cells.[1]

Autoimmune disease development depends on a combination of genetic background, predispositions, gender and environmental factors. Genome-wide Association Studies (GWAS) were able to identify multiple gene loci that are associated with autoimmunity. A study using mouse models revealed that there is also a presence of loci that suppress the phenotype of autoimmunity. They concluded that the risk of an individual developing an autoimmune disease depends on the summation of susceptibility and resistance loci.[2]

Immune imbalance can represent in both overactive or underactive immune responses which can lead to autoimmune disease and immunodeficiencies.

The Major Histocompatibility Complex (MHC) locus is closely associated with autoimmunity, although the mechanism is still mostly unknown, MHC is thought to increase autoimmune disease by raising antigen presentation in the periphery which results in increased T-cell activation. [3]
Selective IgA deficiency (SIgAD) is also believed to be a common reason for developing autoimmune diseases. In humans it was shown that patients with SIgAD were more likely to develop systemic lupus erythematosus, hyperthyroidism, hypothyroidism, type 1 diabetes mellitus etc.[4] Although the exact mechanism of SIgAD is unclear yet, there is a hypothesis that IgA plays a protective role against autoimmunity by interacting with the Fc portion of the IgA receptor. This interaction deactivates immune response pathways that contain the immunoreceptor tyrosine-based activation motif, achieved through partial phosphorylation. [5]

Despite the genetic contribution to the development of autoimmune disease, it was found that environmental factors play an even bigger role. Studies with monozygotic and dizygotic twins regarding the prevalence of autoimmune disease indicate that heredity accounts for only about 30% of the risk of developing autoimmune disease while environmental factors account for the remaining 70%. [3]

External environmental factors play an important role in the development of AID. Sterile tissue damage, hormones, diet, chemical toxins and infections can increase the susceptibility of an individual to particular diseases. Sex hormones, for example, either obtained naturally or artificially, can interact directly with some receptors of the immune cells thus promoting or inhibiting immune responses. Drugs such as procainamide and hydralazine have the potential to trigger the production of autoantibodies and lead to lupus-like disorders in individuals. Penicillamine has been linked to the development of myasthenia gravis (MG).[3] Chronic inflammation can play a role in the development of autoimmune diseases by triggering a cascade of immune responses and cytokines release that ultimately lead to self-directed immune attacks. T cell immune response, more specifically Th1, Th2 and Th17 may be responsible for these processes.[6] Prolonged inflammation can disrupt the delicate balance of the immune system, leading to immune dysregulation. Recently a strong connection was shown between abnormal inflammatory responses and numerous chronic diseases, with a particular focus on autoimmune conditions. These conditions encompass diseases like rheumatoid arthritis (RA), inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), gout, and diabetes.[7]

3.2 Autoimmune diseases in dogs

Systemic autoimmune disease:

Systemic lupus erythematosus (SLE)

SLE was originally recognized in human patients and its cause remains unknown. It is characterized by producing autoantibodies that are not specific to a certain organ and bind self-molecules in the nuclei of cells. These are called antinuclear antibodies (ANA). ANAs are present in the circulation and bind to nuclear antigens like double stranded DNA and histones. Thus forming immune complexes that deposit in kidney glomeruli and in small blood vessels.[8] Vasiculitis develops because of the attraction and fixation of Neutrophil granulocytes which is characteristic of a type III hypersensitivity reaction. These Neutrophils release harmful enzymes within the affected tissues that contain immune complexes, resulting in inflammation and tissue necrosis.[8]

Canines with SLE can express clinical signs such as lameness, swollen joints, kidney failure, skin lesion (**Fig. 1**) and hemolytic anemia.[9] In dogs there is a genetic predisposition and is found more often in Collies, German shepherds and Shetland sheepdogs and is less common but still might be seen in Irish setters and Poodles.[10]

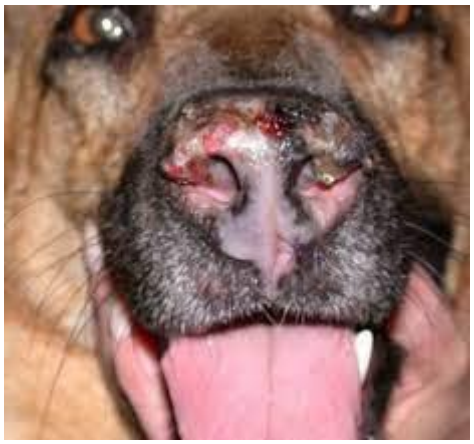

Fig. 1 lesions on nasal planum of SLE patient
(https://vetskinandear.com.au/canine-discoid-lupus-erythematosus-treatment/)

Autoimmune diseases of the hematologic system

Immune-mediated hemolytic anemia (IMHA)

IMAH is a frequent cause of anemia in dogs and is the most common autoimmune disease in dogs. It is more likely to develope in middle-aged females.[11] In this disease occurs the production of autoantibodies that bind to erythrocytes and stimulate a type II hypersensitivity. IgM is more likely to fixate the complement C1qrs and the main result will be lysis of the red blood cells extracellularly, but if IgG is produced the destruction of erythrocytes will be intracellularly, erythrocytes that have been opsonized with IgG antibodies are eliminated from circulation and broken down by macrophages in the spleen and liver. Thus reducing the hematocrit level (**fig. 2**)[12] . That is why splenomegaly is often associated with IMHA.

There is a breed predisposition for this disease including Cocker spaniels, Irish setters, Miniature dachshunds, Old English sheepdogs, Vizslas, Samoyeds and Scottish terriers.[13] In primary IMHA, also known as idiopathic, the cause is unknown and the immune system mistakenly targets and destroys the body's own red blood cells. While secondary IMHA occurs as a result of an underlying condition or trigger, such as infections, neoplasia, drug reactions for example amoxicillin,[14] can metabolize into small molecules that act like haptens that bind red blood cells and initiate antibody production. That is why it is important to take the patient's history to determine if the anemia is primary or secondary.[15]

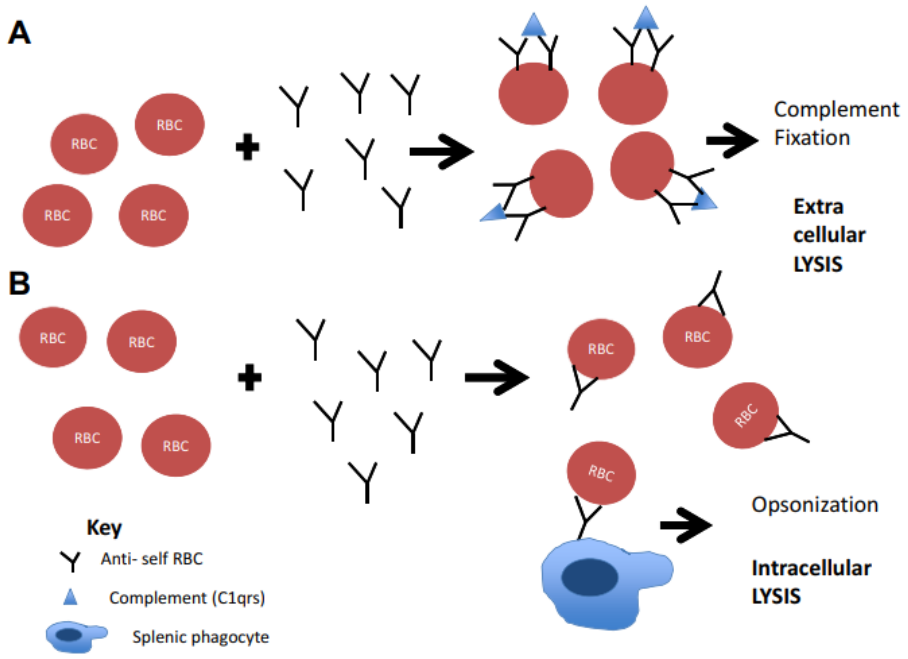

Fig 2: A- IgM pathway leading extracellular lysis and B- IgG pathway leading to intracellular lysis. (Gershwin, L. J. Current and Newly Emerging Autoimmune Diseases 2018)

Autoimmune diseases of the endocrine system

Autoimmune thyroiditis (AT)

It is the most common endocrine autoimmune disease in dogs and in humans as well. Some purebred dog breeds are more likely to have hypothyroidism including Doberman Pinschers, Golden Retrievers, Old English sheepdogs, Beagles, and others. It commonly manifest in middle-aged dogs. There is also a sex predilection, intact females are more likely to have AT. [16] In this disease the tolerance to thyroid protein is lost which causes thyroid follicular cells to be destroyed resulting in hypothyroidism. Primary hypothyroidism is considered an immune-mediated disease because the thyroid gland is infiltrated by lymphocytes. Autoantibodies against thyroglobulin are produced which is the precursor to T3 and T4. The second target is thyroid peroxidase protein which is needed for the synthesis of thyroid hormones.[8]

Various hypotheses have been suggested regarding the initial stimulus for the immune response, and it appears that genetic factors may render individuals more susceptible to

these factors. Potential triggers, including viral and bacterial infections, excessive iodide intake, or contact with specific environmental contaminants, have been put forward.[17]

Dogs with hypothyroidism show the following clinical signs, lethargy, weight gain, and dermatologic changes like pruritic seborrhea and alopecia.[9]

Diabetes mellitus

Type 1 diabetes mellitus (T1DM) in dogs can be immune-mediated or idiopathic. In case of immune-mediated, the immune system attacks and destroys the beta cells in the pancreas that produce insulin. As a result, the dog's body cannot regulate blood sugar levels, leading to hyperglycemia, which can cause a range of clinical signs and complications.

Although the existence of circulating antibodies against insulin and beta cell antigens has been confirmed, the exact contribution of autoimmunity to the loss of beta cells is still a subject of ongoing research. The infiltration of lymphocytes into the islets has been observed, and antibodies targeting islet cells, insulin, proinsulin, intracellular glutamic acid decarboxylase 65 (GAD65), and insulinoma antigen 2 (IA2) have been detected in dogs with diabetes. In humans with type 1 diabetes, the presence of antibodies targeting GAD65 and IA2 typically precedes the onset of hyperglycemia or clinical symptoms.[18]

There is a genetic predisposition, mostly manifested in purebred female dog breeds. Breeds with a high odds ratio for development of diabetes mellitus include Samoyed (3.36), Giant schnauzer (4.78), Fox terrier (3.02), and Miniature poodle (1.79) [19]

The clinical signs of T1DM in dogs are similar to those of other types of diabetes mellitus and can include polyuria/polydipsia, increased appetite, weight loss, and lethargy. Over time, the condition can lead to more severe symptoms such as vomiting, diarrhea, and dehydration. In severe cases, dogs can develop ketoacidosis, a life-threatening condition characterized by the accumulation of ketones in the blood. [8]

Hypoadrenocorticism (Addison’s disease)

Hypoadrenocorticism, also known as Addison's disease, is a condition in which the adrenal glands do not produce enough steroid hormones, including cortisol and

aldosterone. This can result in a range of clinical signs and potentially life-threatening complications if left untreated.

Hypoadrenocorticism is frequently a result of immune-mediated damage to the adrenal glands, leading to a reduction in the production of mineralocorticoids and glucocorticoids. However it can also be caused by drugs that cause adrenocortical necrosis like mitotane, enzyme inhibition like trilostane or because of neoplastic or fungal disease that cause infiltrative processes.[20]

Genetic factors also play a role, and there is a genetic predisposition to hypoadrenocorticism. The condition is more prevalent in specific breeds, including Portuguese water dogs, Great Danes, standard poodles, West Highland White terriers, and Nova Scotia duck tolling retrievers.[21]

The clinical signs of hypoadrenocorticism in dogs can be non-specific and may include weakness, vomiting, diarrhea, lethargy, and decreased appetite. In severe cases, dogs may develop shock, collapse, and even death. [8]

Autoimmune diseases of the skin

Discoid lupus erythematosus (DLE)

Discoid lupus erythematosus (DLE) is an autoimmune disease that primarily affects the skin and mucous membranes in dogs. It is a chronic condition that can cause discomfort and can be challenging to manage. The lesions are similar to SLE but in the absence of a positive ANA and without involvement of other body systems.[8]

The precise cause of DLE remains uncertain. However, the presence of antibodies and complement (anti-IgG and anti-C3 reveals) at the junction between the dermal and epidermal layers indicates an immune-mediated mechanism as the likely pathogenesis. Using immunofluorescence, the detection of a fluorescent band along the dermal-epidermal junction can also be used for diagnosis. [22]

The clinical signs of DLE in dogs typically include scaling, crusting, and ulceration of the skin around the nose and face. Other signs may include depigmentation, scarring, and

thickening of the affected areas. In some cases, dogs may also develop lesions on other parts of the body.[22]

Pemphigus vulgaris (PV)

Pemphigus vulgaris (PV) is an autoimmune disease that affects the skin and mucous membranes in dogs. It is a rare but serious condition that can cause significant discomfort and potentially life-threatening complications.

PV is caused by the body's immune system attacking the connections between skin cells, leading to blister formation and ulceration. The exact mechanisms that trigger this autoimmune response are not well understood but it is possible that the formation of vesicles is driven by the interaction between IgG antibodies and the desmoglein antigens present in the keratinocyte glycocalyx, followed by complement fixation. [23]

The clinical signs of PV in dogs typically include the development of painful blisters and ulcers on the skin and mucous membranes. These can occur anywhere on the body, but are most commonly found on the face, paws, and oral cavity (Fig 3). In some cases, dogs may also develop fever, lethargy, and decreased appetite.[9]

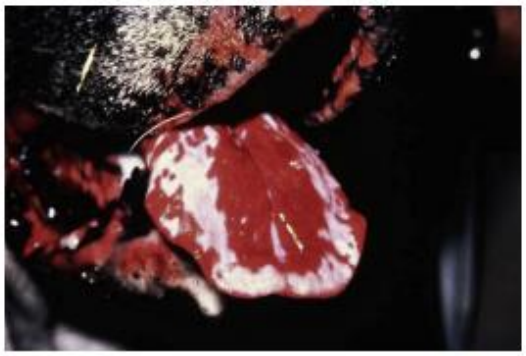

Fig. 3.: Severe ulceration of the oral mucosa in a canine patient with pemphigus vulgris.
(Gershwin, L. J. (2018). Current and Newly Emergin Autoimmune Diseases.)

Pemphigus foliaceus

Pemphigus foliaceus (PF) is a common autoimmune skin disease that affects dogs. It is caused by an attack on the desmoglein-1 protein of the skin cells by the immune system, leading to the formation of blisters and pustules on the skin. The pathogenesis is akin to what is described for pemphigus vulgaris, but the lesions are situated at a more superficial level within the epidermis.[9]

Clinical signs of PF in dogs include crusts, erosions, and pustules on the skin, particularly on the face, ears, feet, and groin. The disease can also cause itching and pain, and in severe cases, lead to fever, lethargy, and anorexia.[24]

Bullous pemphigoid

Bullous pemphigoid (BP) is a relatively common autoimmune diseases in dogs that affect the skin. It is characterized by the formation of blisters and ulcers. In this disease autoantibodies are attacking collagen XVII epitopes. As a consequence, lesions are generally located on nonmoucosal skin. There is an uncommon form of this disease-mucous membrane (cicatricial) pemphigoid. In which there are autoantibodies directed against basement membrane proteins. The presence of IgG deposits at the basement membrane zone (BMZ) is a common characteristic of canine BP. While circulating anti-BMZ IgG autoantibodies have been detected in certain cases of canine BP, the precise skin protein that these autoantibodies target has not yet been determined.[25]

Clinical signs of BP in dogs can include erosions, ulcers, and blisters on the skin, particularly on the abdomen, groin, and axillary regions. The disease can also cause itching and pain, and in severe cases, lead to fever, lethargy, and anorexia.[26]

Joints diseases

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an immune-mediated inflammatory disorder that is characterized by chronic joint inflammation, pain, and mobility impairment. The synovial fluid accumulates numerous polymorphonuclear cells, and within the joint, there is the development of pannus tissue and a substantial infiltration of lymphocytes into the synovial membrane, leading to the creation of germinal centers.

The etiology is still unclear, some evidence suggest that it can have genetic background, in humans, risk factors like smoking may contribute to the development of the disease as well as certain infectious agents such as *Escherichia coli*, and *Epstein-Barr virus*. In addition to dysbiosis was recognized in humans in the early stage of the disease.[27] In RA elevated levels of immune complexes and IgM rheumatoid factors are detected in both sera and synovial fluids. In cases of severe erosive disease, there is an increase in IgA rheumatoid factors. Moreover, heightened levels of anti-collagen II antibodies are observed, which play a role in the formation of immune complexes within the joints.[28]

Clinical signs include lameness, joint inflammation, stiffness, and pain, varying in intensity. Radiographs play a pivotal role in detecting alterations within joints, including erosions, reduction in joint space, and bone growth. Blood assessments, including rheumatoid factor and anti-cyclic citrullinated peptide (anti-CCP) antibodies, serve to validate immune participation and eliminate alternate possibilities.[29]

Neuromuscular Autoimmune Disease

Myasthenia gravis

Myasthenia gravis (MG) is an autoimmune disease that affects the neuromuscular junction, the area where the nerve endings connect with the muscles they control. In a healthy neuromuscular junction, nerve impulses stimulate the release of acetylcholine, a neurotransmitter that binds to receptors on the muscle fibers, causing them to contract.

However, in MG, the body's immune system produces autoantibodies that attack and destroy the acetylcholine receptors on the muscle fibers These antibodies bind to the receptors (**Fig. 4**), and elicit a type II hypersensitivity mechanism, that results in complement-mediated destruction of the receptors either blocking or interfering with the binding of acetylcholine, leading to decreased transmission of nerve impulses to the muscle fibers. [30]

The resulting muscle weakness and fatigue are commonly observed in the muscles of the eyes, face, throat, neck, and limbs. In severe cases, the respiratory muscles can also be affected, leading to respiratory distress and even failure.[8]

The underlying cause of MG is still not well understood, but it is believed to be a combination of genetic and environmental factors. Certain breeds of dogs, such Newfoundland and the Great Dane may be more susceptible to developing MG.[31]

Patients with Myasthenia gravis commonly exhibit clinical signs of neuromuscular weakness, which can manifest acutely and progress to an eventual inability to stand. Additionally, canine patients with MG may experience regurgitation of food and water due to a weakened esophageal muscle, leading to a condition known as megaesophagus.[30]

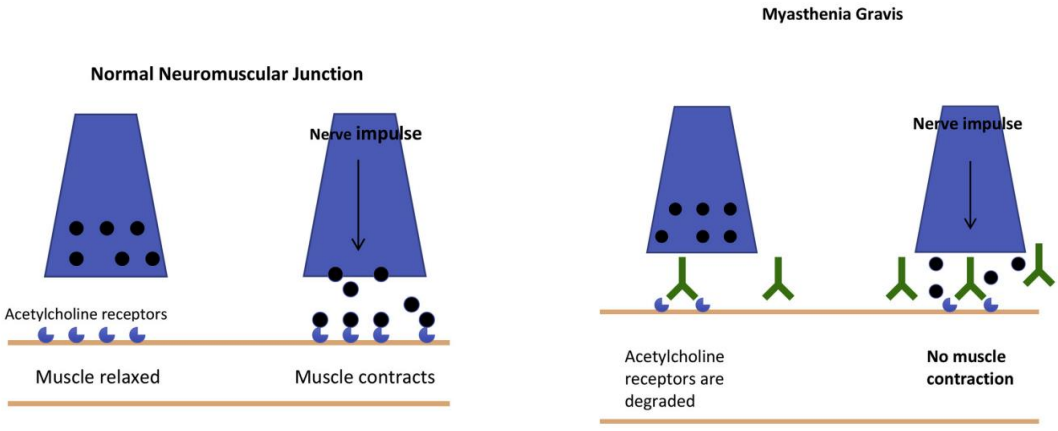

Fig 4- Normal neuromuscular junction and neuromoscular junction in MG, in which aoutoantibodies damage the receptors and interfer with the binding of Ach to the receptors.
(Gershwin, L. J. (2018). Current and Newly Emerging Autoimmune Diseases.)

Meningoencephalomyelitis

The literature on MUO in dogs describes three distinct diseases that are commonly grouped together as noninfectious MUO: granulomatous MUO, necrotizing encephalitis, and meningoencephalitis of unknown origin.[32]

Meningoencephalomyelitis (MUO) of unknown origin, is a frequent occurrence in certain dogs breeds, including Chihuahuas, West Highland white terriers, and Dachshunds, appear to be predisposed to MUO.[33]

This disease is believed to be an immune-mediated disorder that bears resemblance to experimental autoimmune encephalitis, a condition that is artificially induced in rodents. MUO is also considered to be a promising model for multiple sclerosis in human patients. Clinical signs of MUO in dogs may present as symptoms of central nervous system disorders, including vestibular problems and seizures.[33]

3.3 The gut microbiome of dogs

The gastrointestinal (GI) tract is home to a complex ecosystem known as the gut microbiome, which includes bacteria, archaea, viruses, and eukaryotic organisms. Among these, bacteria are the most abundant and play a crucial role in digestion by fermenting dietary fibers. Beyond digestion, the gut microbiome also has important metabolic and immune functions. It helps protect the host against harmful pathogens and educates the immune system. As such, the gut microbiome is considered a vital metabolic and immune organ.

The primary inhabitants of the gut microbiome are largely strict or facultative anaerobic bacteria, particularly in the densely populated large intestine. In dogs and cats, the dominant phyla include *Firmicutes*, *Fusobacteria*, and *Bacteroidetes*.[34]

Variations in bacterial populations exist in different regions of the gastrointestinal tract due to differences in physiological conditions. The stomach, small intestine, and large intestine exhibit differences in oxygen levels, pH, antimicrobial substances, and intestinal motility. In dogs, the stomach contains only a few types of bacteria that can withstand the

acidic environment, primarily Helicobacter spp. and to a lesser extent, lactic acid bacteria. The small intestine harbors a mixture of aerobic and anaerobic bacteria, while the large intestine is densely populated with mainly anaerobic bacteria.[34]

The majority of studies have focused on analyzing the fecal microbiome, as it is the most easily obtainable sample type in clinical settings. However, analyzing fecal samples may not provide a comprehensive understanding of the potential existence of bacteria that are adherent to the mucosa or invasive to the intestine, as well as the composition and quantity of the microbiota in the small intestine. Despite the use of advanced molecular methods to characterize the intestinal microbiota, describing all of the bacteria present in this highly diverse ecosystem remains a challenge.

As mentioned before the 3 main phyla *Firmicutes*, such as *Clostridia* and *Bacilli*, *Bacteroidetes*, and *Fusobacterium*.

The presence of *Fusobacterium* in dogs is typically associated with health. Specifically, *Fusobacterium mortiferum* and *Fusobacterium perfoetens* are often observed in the GI tract of dogs. However, antibiotic treatment and GI diseases severely impact the abundance of *Fusobacteria* in dogs, and their recovery is slower compared to other phyla. Therefore, specific food ingredients that can increase the abundance of *Fusobacteria* may be a therapeutic target in dogs.[35]

The *Proteobacteria* and *Actinobacteria* phyla are also commonly found in the gut microbiome, particularly in the small intestine, and are typically present in smaller numbers in fecal samples. For instance, *Enterobacteriaceae*, a family of facultative anaerobic bacteria that includes *Escherichia coli,* are able to thrive in the small intestine due to their ability to use the oxygen available there. While *Enterobacteriaceae* are part of the normal gut microbiome in small numbers, an increase in their abundance in fecal samples is a hallmark of dysbiosis and is associated with various diseases, both within and outside of the gastrointestinal tract.[35]

The maintenance of a well-balanced gut microbiome has a positive impact on the overall health of the host by regulating the immune system, protecting against intestinal pathogens, and supplying essential nutrients and vitamins. Bacteria ferment dietary

carbohydrates into short-chain fatty acids (SCFA) which have multiple benefits, including providing energy for epithelial cells, regulating intestinal motility, and possessing anti-inflammatory properties.[35]

Dysbiosis, the disturbance of the gut microbiome, is linked to various localized or systemic diseases, including dogs. The hallmark of dysbiosis is the rise of Enterobacteriaceae, a family of facultative anaerobic bacteria. The gut microbiota's composition affects immune function, antibody production, and the differentiation of intestinal Th cells. Dysbiosis may also cause intestinal inflammation in various diseases, resulting from the disrupted metabolism of bile acid or decreased production of anti-inflammatory molecules like SCFAs and indoles. The gut microbiome plays a vital role in regulating health, and its disruption can lead to various health issues.[35]

It is crucial to highlight that intestinal bacteria are merely a single component of a complex interplay that exists among the intestinal epithelial cells, the mucus layer within the intestine, the host immune system, and the luminal environment. The microbiota's makeup is partially influenced by various factors, such as diet, medication like antibiotics and chemotherapeutics, inflammation in the gut, structural modifications in the intestine, and other similar factors.

3.4 The gut and the immune system

The gut plays a crucial role in the immune system and in maintaining homeostasis. It serves as a barrier that separates the body from the external environment and is constantly exposed to a large number of foreign antigens, including food and microbial components. Therefore, the gut immune system has developed several mechanisms to tolerate harmless antigens while responding to harmful ones.

The gut-associated lymphoid tissue (GALT) is a collection of lymphoid tissue that includes Peyer's patches, mesenteric lymph nodes, and isolated lymphoid follicles. The GALT is responsible for the sampling and recognition of luminal antigens and the initiation of immune responses to pathogens.

In addition to the GALT, the gut is also populated by the gut microbiota which is involved in the regulation of various aspects of the immune system. Including the development and function of immune cells. Gut microbiome shapes the maturation of immune cells, such as T and B cells, as well as the production of immune-modulatory molecules. It is involved in the establishment of immune tolerance, which prevents harmful immune responses to harmless antigens.

The gut microbiota achieves this by interacting with gut epithelial cells, which act as the first line of defense against luminal antigens. It regulates the integrity of the intestinal epithelial barrier and stimulates the production of antimicrobial peptides and mucus, which help to prevent the invasion of harmful pathogens. The gut microbiota also modulates the production of cytokines, such as IL-10 and TGF-β, which have immunosuppressive properties.[36]

The composition of the gut microbiota has significant effects on immune function, including the local production of antibodies. While gut microbes are separated by the inner mucous layer and glycocalyx from direct contact with enterocytes, intestinal dendritic cells can extend their dendrites into the intestinal lumen and sample the microbiota. Most of the invading bacteria are killed by macrophages, while some are presented to B cells which produce IgA. The IgA is secreted into the lumen, binding to bacteria and activating targeted bacterial destruction.[37]

The differentiation of intestinal helper T (Th) cell precursors into Treg or Th17 cells depends on signals received from the microbiota. In homeostasis, the production of Treg cells is favored and that of Th17 cells is suppressed, resulting in minimal inflammation within the intestinal wall. In the absence of Treg cells, uncontrolled effector T cells can respond to microbial antigens and trigger inflammation.[38]

The relationship between the gut microbiome and autoimmune diseases is a topic of ongoing research, and while the exact mechanisms are not yet fully understood, there is growing evidence that the composition and function of the gut microbiome can have significant effects on the development and progression of autoimmune diseases.

There is evidence to suggest that dysbiosis, or an imbalance in the gut microbiome, may contribute to the development of autoimmune diseases by promoting inflammation and altering immune function.[36]

There is also indication to suggest that the gut microbiome may play a role in regulating immune function in autoimmune diseases. For example, it has been shown that certain gut bacteria can stimulate the production of regulatory T cells, which help to suppress autoimmune responses.[36]

Rheumatoid arthritis (RA) has been associated with the gut microbiome. Research in humans has revealed that individuals with RA often have a higher abundance of *Prevotella* bacterial species in their stool.[39] Notably, *P. copri* has been identified as a contributor to its proinflammatory impact. To investigate the inflammatory influence of this bacterium, studies were conducted in mice. It was observed that in SKG mice, which are predisposed to arthritis, the presence of this bacterium initiates a Th17 immune response. [40]

In human inflammatory bowel disease (IBD), through metagenomic sequencing utilizing Illumina technology, it has been demonstrated that Crohn's disease patients exhibit a distinct gut microbiota composition compared to both healthy individuals and those with ulcerative colitis.[41]

In the case of IMHA, a study on dogs that used gene sequencing to profile the fecal microbiome, found an association with alterations in the composition of fecal microbiota. These changes are characterized by a decrease in the relative abundance of the *Treponema* spp., an increase in the relative abundance of pathobionts like *Clostridium septicum* and *Escherichia coli*, and an overall increase in microbial diversity.[42]

In multiple sclerosis , it has been suggested that changes in the gut microbiota may contribute to the breakdown of the blood-brain barrier, leading to an autoimmune attack on the nervous system.[43]

In a human study, individuals diagnosed with systemic lupus erythematosus (SLE), research has indicated a reduced *Firmicutes/Bacteroidetes* ratio and notable variations in the abundance of specific genera. Notably, there was a significant enrichment of genera

including *Rhodococcus, Eggerthella, Klebsiella, Prevotella, Eubacterium,* and *Flavonifractor* among SLE patients. Conversely, the genera *Dialister* and *Pseudobutyrivibrio* were found to be decreased in individuals with SLE.[44]

Research done for humans with myasthenia gravis revealed a notable distinction in the microbial composition between individuals with MG and those categorized as HC (healthy control) subjects. This discovery indicates that dysbiosis within the gut microbiota might be linked to neuromuscular junction diseases, representing a significant advancement in our understanding of how the gut microbiome influences the nervous system. [45]

While more research is needed to fully understand the relationship between the gut microbiome and autoimmune diseases, these findings suggest that targeting the gut microbiome may be a promising avenue for the prevention and treatment of autoimmune diseases.

Fecal transplantation

Fecal microbiota transplantation (FMT) involves the transfer of fecal material from a healthy donor into the gastrointestinal tract of a recipient who has a medical condition, with the aim of influencing and modifying the recipient's intestinal microbiome. This procedure can be administered through various methods, including enema, colonoscopy, duodenoscopy, insertion via nasogastric/nasojejunal tubes, or oral ingestion using capsules. It is mostly done in the human medical field as a treatment for clostridium difficile infection with remarkable success, and with still on-going research it might be applicable also to patients with IBD. [46]

There is not a lot of experience with FMT in dogs, a few case studies have been observed for the therapy IBD, in one of them a 10-year-old toy poodle who had IBD, underwent a series of nine FMT procedures administered via enema over a six-month period. These treatments led to improvements in the dog's Clinical IBD Activity Index and fecal consistency throughout the intervention. Additionally, the recipient's fecal microbiome was observed to align more closely with that of the donor following the FMT treatments. In another case of an 8-month-old French bulldog suffering from chronic colitis and

testing positive for a C. difficile fecal culture, significant improvements in defecation frequency and the appearance and consistency of feces were observed within a short period of 2 to 3 days after receiving a single oral fecal microbiota transplantation (FMT). Encouragingly, there was no recurrence of the condition for at least 6 months following the procedure.[47]

3.5 Coprophagy

Coprophagy, also known as feces-eating, is the consumption of feces by an animal. This behavior is relatively common in dogs, although the frequency and reasons for this behavior are not well understood. There are several theories as to why dogs engage in coprophagy. Some researchers believe that coprophagy may be a result of nutritional deficiencies or imbalances in the dog's diet. Other theories suggest that coprophagy may be a survival instinct in which dogs consume feces to avoid detection by predators or to reduce the presence of parasites in their environment. According to some, coprophagy may be a result of boredom or stress in dogs.[48]

The incidence of coprophagy in dogs is not well-documented, but studies suggest that it may be relatively common. Some estimates suggesting that up to one-third of dogs may engage in this behavior at some point in their lives.[48] Certain dog breeds may be more likely to engage in coprophagy, however there is no explanation yet available for the reason.

While coprophagy itself may not be directly related to autoimmune diseases in dogs, some dogs with coprophagy may be at increased risk of contracting parasites, [49] bacterial infections, and other diseases, which may contribute to a manifestation of a disease.

4. the aim of the study

The aim of our work is to find a link between autoimmune diseases and coprophagia in dogs. Based on literature, we hypothesize that changes in healthy gut flora can lead to autoimmune diseases and fecal transplantation can alleviate symptoms of some autoimmune diseases (e.g. diabetes) in humans. Then coprophagia may also be directed towards restoring the gut flora. To find this out, we have encouraged questionnaires to be filled out and then evaluate the results to try to obtain useful information.

We started from a previous thesis [50], which had a completion rate of over a thousand. The thesis was looking for answers to the question of the possible link between coprophagia as a habit and diseases. In that paper, a negligible number (15/1025) of owners of dogs with autoimmune diseases completed the test. Statistically, the small sample size did not yield significant results (p=0,2562), but a clear trend was observed.

5. Material and methods

For this study, an online questionnaire was distributed to dog owners in English and Hebrew between August 23rd and October 20th 2023 and was filled by people from various countries.

The questionnaire could be accessed via the following link: https://fm.addxt.com/form/?vf=1FAIpQLSeUG0wAV8wesfykkKrN4iCJubveBquhp2hV8huqKwezkzjOw&nd=1, which was shared on Facebook, one of the largest social networking sites. Our target audience was dog owners who own a dog diagnosed with an autoimmune disease. We found them in the following communities: different dog lovers groups, the university Facebook group and several groups dedicated to publishing questionnaires for research purposes.

6. Questionnaire structure

The questionnaire is made of 26 questions and can be divided into a few categories:

a. Basic information about the owner:
   - Age
   - Education level

- Place of living (town/ city, with/without garden)

b. Basic information about the dog
   - Sex and neutered status
   - Place of keeping (indoors/outdoors)
   - Number of visits to the veterinarian
   - Vaccination history
   - Fitness
   - If it receives any treatments
   - Antiparasitic usage

c. Dog's diet
   - Kind of food
   - Change in feed
   - If it is on a special diet
   - number of meals per day
   - if it receives vitamin/ mineral supplements
   - if it receives and pro/pre-biotics

d. coprophagy status
   - if the dog eats feces and how often
   - what kind of feces
   - if it is related to something

e. autoimmune disease status
   - which one if any
   - treatment frequency
   - what treatments

The questionnaire was evaluated using a Fisher exact statistical significance test. Significant correlations were sought to confirm or refute our hypothesis and this method was therefore found to be appropriate.

7. Results

93 people completed the questionnaire. 29 (31%) of the dogs engaged with coprophagy at least once. 19 (20%) of the dogs had an autoimmune disease: 2 of them had Rheumatoid arthritis and 17 had an AI disease that was not listed in the options. 5 dogs (5%) were both coprophage and had an AI disease.

While 29 (31%) owners said that their dog had eaten feces at least once, a further 12 (12.9%) owners answered yes to the related questions. These responses were treated separately (+ after) but were considered coprophagic.

Of the owners, 4+5 (9.7%) indicated that their dog consumed its own feces. Of these, 4+4 were females/ sterilized females.

A total of 57 (61.3%) dog owners reported that their pets had a health problem. The number of obese dogs reported by owners was 12 (12.9%), with a dog of normal weight but eaten a diet feed due to obesity. A total of six dogs (5 obese and a normal weight) are on diets, three of them are coprophagic. Of those not on diet, 5 are coprophagous. There is one dog that is fed dry food ad libitum, this is also a coprophagous dog. In addition, the owner says that it is also suffering from autoimmune disease (rheumatoid arthritis) and is being treated for it, but we don't know what medication. Another dog is on a non-steroidal anti-inflammatory drug (NSAID) for an unclear reason. This animal is not on a diet, eats dry and canned food twice a day and also consumes feces.

The number of dogs on a diet due to allergies is ten (10.7%). Six of them are coprophages, and two of them eat their own feces. Two are on monoclonal antibody therapy. One dog is on immunotherapy (the owner does not say exactly which therapy), is not a coprophage, nor is the dog taking antibiotics and antimycotics. Of the dogs with allergies, 4 dogs are on pre- or probiotics on the vet's advice. Two of the four - which also receive vitamin supplements - are also feces eaters.

The number of animals with gastrointestinal problems is six (6.5%). Three of them are coprophages, and these three are also receiving treatment: one dog is on NSAID and monoclonal antibody, and on the vet's advice vitamin and pre/probiotic , one dog is on vitamin and pre/probiotic only, and the third dog is overweight and on NSAID.

In fact, there are a total of 2 dogs that may have an unknown autoimmune disease, receiving an unknown immunotherapy treatment. One is on NSAID, and pre/probiotics as indicated by a vet, yet we have no such information on the other. Although the owners have not directly reported coprophagia, both seem to be, based on the responses. Of the two dogs with rheumatoid arthritis, the previously mentioned overweight dog has coprophagia, the other dog does not. They are not taking any supplements, but one is a coprophage. Two of the animals are 5-10 years old, and two are 10-15 years old. The breeding ratio is one female and three males (one neutered). Their main data are summarized in Table 1. Note that a significant number of the owners claimed that their dogs were autoimmune (19 dogs in total, of which a significant majority turned out to be allergic.)

1. Table: Dogs with autoimmune diseases and their main data

Age (years)	Sex	Autoimmune disase	Treatment	Coprophagia	Pro/prebiotics
5-10	neutered male	?	Immunotheraphy, NSAID	Yes	Yes
5-10	male	?	Immunotheraphy	Yes	No
10-15	female	Rheumatoid arthritis	?	Yes	No
10-15	male	Rheumatoid arthritis	?	No	No

A further 30 dogs are suffering from a disease not named by the owner or are receiving treatment. 18 of them are coprophagic.

Of the coprophagic dogs, 9 are taking pre- or probiotics as prescribed by the vet. One of them is on a continuous supplementation. A further 3 dogs are supplemented at the owner's suggestion. In the same group, 7 dogs receive vitamin and trace mineral supplementation. Three receive it at the owner's discretion, these animals do not receive pre- or probiotic supplementation. A total of four dogs were recommended vitamin

supplementation by a veterinarian, one of whom was also recommended to take a pre- or probiotic supplement, and two owners, who add a vitamin supplement on their own decision.

Of those with a medical condition, 9 are receiving a pre/probiotic supplement on the advice of their vet, two of them on a continuous basis. Of these, six dogs are also coprophage (2+4). Eight of the dog owners consider supplementing their dog with a pre- or probiotic as a supplement, four of these dogs (2+2) are coprophages. In 10 cases, the vet recommended vitamin supplementation for animals with the disease. Four of these dogs were also recommended a pre/probiotic (two dogs are taking it continuously). Three of the owners decided to add a microflora booster to the vitamin. Three owners choose to give only the vitamin to their dog, and one also receives a preparation to support the gut flora.

The statistical results are shown in table 2, figure 5. The coprophagy and breeds and coprophagy and feed tendency are not shown.

2. Table correlation between coprophagy and other datas

Correlations

Coprophagy	Breed	Significant difference - tendency: more coprophagy in mixed
Coprophagy	Age	Non-significant, tendency: coprophagy occures more often in younger dogs
Coprophagy	Sex	Significant, tendency: coprophagy more often in neutered female dogs
Coprophagy	Keeping	Non-significant, tendency: none
Coprophagy	Condition	Non-significant, tendency: none
Coprophagy	Feed	Significant, tendency: coprophagy more often in case of dry food
Coprophagy	Spec. Diet	Non-significant, tendency: more often in case of spec diet
Coprophagy	Pre / Probiotics / Vitamines	Non-significant, tendency: none
Coprophagy	Antiparasitic	Non-significant, tendency: more often when 4+

Coprophagy	Autoimmune diseases	occasions / year Non-significant, tendency: none

8. Discussion

The questionnaire was constructed by dividing the responses into 5 broad groups. As far as possible, we only asked open-ended questions for easier evaluation.

In the first group we asked for some information about the owner. This question was omitted in previous surveys. Since some of the questions assume conscious animal-keeping behavior, we thought we would get back more well-completed questionnaires. We also thought about providing a guide to help fill in the questions. This was rejected because of the changing reading habits of young people [51,52].

The second group asked for basic information about the dog. Important information was whether the dog had received antibiotic treatment, and deworming and vaccination habits were also revealing data.

One third of the dogs in the survey were mixed breeds. Of the purebreds, 5 Shih Tzu, 4 Daschund and King Charles cavalier and 3 Golden Retriever and German Shepherd dogs participated in the studies, with a further 27 breeds with one or two individuals. Thus, breed disparity could not be very well established, although statistical results showed a significant correlation between mixed breed dogs and coprophagia. Based on a previous study [53], the Shih tzu breed in Brazil was found to have a high proportion of coprophagous dogs. This is due to the fact that this breed was very popular there. In a previous study in Hungary, no breed disparity was highlighted [50]. As for autoimmune disease, it is known that in most breeds, the site of the factorial originated from a small population, which limited genetic variability [54].

The spaying, and the gender status and age of the dogs were also an issue. Autoimmune diseases are most prevalent in the population among females of reproductive age, which is attributed to the immunostimulatory effect of the hormone estrogen [55]. Coprophagia is a common phenomenon in wild female wolves. It may also persist in dogs (Hart et al., 2018). As before, the majority of autoimmune dogs in this questionnaire were male dogs

(10/15 and 4/5, respectively). Neutering was not relevant before (Streicher) and is not relevant now. In our study the distribution of males was even: 23 females, 24 males, 19 neutered females and 27 neutered males.

In terms of age, coprophagia in puppies is due to curiosity. In adult dogs, it is often due to health reasons [56]. Autoimmunity is more frequent in older age, which can be attributed to altered immune regulation (Vadasz et al., 2013). In the present study, dogs under one year of age are not included, The age distribution is shown in Table 3. Our studies showed that 50-50% of the autoimmune dogs were in the 5-10 and 10-15 year age groups, as expected. The distribution of allergies was balanced (3 dogs aged 1-5 years, 3 dogs aged 5-10 years, 4 dogs aged 10-15 years). In humans, allergic diseases are one of the most prevalent chronic diseases in people aged 15 years and older [57]

3. Table: Age distribution of the animals tested

Age	Animal	% of tested animals
0-1 year	0	0%
1-5 years	41	44%
5-10 years	29	31%
10-15 years	22	24%
15+	1	1%
Total	93	100%

It was surprising to find that while more than half of the respondents had a garden (55%), only 4 dogs lived outside in a garden or kennel. And 30% of all dogs were both outside and inside. This suggests that a significant proportion of people have a garden but do not give their dog the opportunity to live outside.

Another interesting piece of information is that, despite the awareness of dog ownership, 14% of owners do not take their dog to the vet every year, which is also reflected in the vaccination figures. The issue of rabies vaccination was directly excluded from the questionnaire, as the laws on this may vary from country to country. Excluding this, a quarter of dogs do not receive regular vaccinations and there were 8 dogs that are not treated for parasites once a year. In contrast, feeding seems to be much more conscious.

The third asks about the dog's diet. Only 10 of the dogs were fed with leftover food. The majority are on dry food (89 dogs), but 15 dogs have access to canned food. Three quarters of owners are loyal to one brand. The majority of dogs (62%9) are fed twice a day, i.e. starvation was the reason for coprophagia in two cases (one owner wrote that his dog on a diet was coprophagic because of being overweight, and another said that the dietary food had low protein content and that his dog was eating feces as a protein supplement). Vitamin and mineral supplements and pre and probiotics are used by a minority of owners, 17 dogs out of 93 using the former and 27 the latter. Table 4 shows the treatments most commonly given to dogs. Vitamin and mineral supplements and pre and probiotics are not included. Five (out of six) of the animals receiving antibiotic treatment also receive supplements for gut flora.

4. Table: treatment for the dogs

Treatment	Dogs
antibiotics, antimycotics	6
steroids	2
non-steroids	6
immunotheraphy	3
monoclonal antibody	3
Other	29

Regarding the statistical analyses, it can be said that, in terms of breed, mixed breed dogs made up the majority of the individuals studied, while other breeds seemed to be under-represented. This may be because of the environment of adopting from shelters nowadays, but we do not think that keeping purebreds is disappearing at the same time. There seems to be a trend between age and coprophagy. Younger dogs are more likely to eat feces. We don't really know how to explain this. Although this is a known phenomenon in puppies [56], dogs under 1 year of age were not included in the survey. The significant correlation between dry food feeding and coprophagia is also due to one-sided sampling. We find it significant that a positive trend was observed in both animals on special diets and dogs treated with antiparasitic drugs more than four times a year.

There is evidence that healthy dogs of normal body condition do not have dysbiosis, yet in obese or malnourished individuals the gut flora is upset. The question of which is the cause, and which is the effect is often difficult to elucidate [58,59]

Based on the questionnaire, we consider 2 cases of autoimmune disease to be plausible in addition to 2 dogs with rheumatoid arthritis, although allergy cannot be excluded there either. In 10 cases, the dog is clearly being treated for allergy. Thus, our suspicion that there is a link between canine coprophagy, and autoimmune disease has not been confirmed. At first reading this seems quite clear, but no one has confirmed it. What is certain is that several publications have come to light in relation to human fecal transplantation and autoimmune disease [60,61,62} which have confirmed the positive effect of transplantation.

We consider this important to maintain a healthy flora.

9. Summary

This thesis embarks on an exploration of coprophagy in dogs, seeking to unravel its intricacies and potential connections to autoimmune diseases. The intriguing relationship between autoimmune diseases and gut flora, observed in both humans and canines, is important for understanding coprophagy beyond a mere behavioral curiosity.

The gut, a complex ecosystem housing trillions of microorganisms, plays a crucial role in immune system regulation. In humans, studies have revealed a compelling connection between alterations in gut flora and autoimmune diseases. Particularly, fecal transplantation was shown to have a positive effect on autoimmune diseases.

In this study we consider coprophagy as a potential indicator of the delicate balance within the canine gut. While the questionnaire results did not conclusively confirm a direct link between coprophagy and autoimmune conditions, the patterns observed suggest an intricate relationship deserving further research.

We found that younger dogs were more likely to engage in coprophagy. Dogs on specialized diets, and animals receiving antiparasitic more than four times a year exhibited a higher prevalence of coprophagic behavior. Notably, suspicion arose that

these behaviors may be associated with allergic reactions. This hypothesis opens a door to understanding coprophagy not merely as a behavioral quirk but as a potential manifestation of underlying health conditions.

Drawing inspiration from human medicine, where fecal transplantation has demonstrated positive effects on autoimmune diseases, this study underscores the interconnectedness of gut health, behavior, and the immune system. The thesis emphasizes the need for continued exploration, encouraging researchers to investigate the influence of coprophagy on the canine immune system and the potential for innovative interventions inspired by the success observed in human medicine.

Összefoglalás

Szakdolgozatom a kutyák ürülékevési szokásai és az autoimmune megbetegedései között lévő összefüggések feltárására vállalkozik. Az autoimmun betegségek és a bélflóra közti kapcsolat, mind az embereknél, mind a kutyáknál ismert és jelenleg is kszámos kutatás középpontjában áll. Feltételezéseink szerint a coprophagia nem magyarázható pusztán genetikailag kódolt kíváncsi viselkedésen.

A bél egy összetett ökoszisztéma, melyen mikroorganizmusok trilliói élnek, és döntő szerepet játszik az immunrendszer szabályozásában. Embereken végzett vizsgálatok meggyőző bizonyítékokat szolgáltatnak a bélflóra változásai és az autoimmun betegségek között. A székletátültetésről kimutatták, hogy pozitív hatással lehet az autoimmun betegségek tüneteinek csökkentésére.

Jelen tanulmány feltételezése alapján a kutyák belében lévő kényes mikrobiológiai egyensúly felbomlásának következménye és egyben indikátora is lehet a coprophag viselkedés. Eredményeinkhez kérdőíves felmérést elemeztünk, mely bizonyos szempontból a tulajdonosok sokszor szubjektív megítélésén múlik. Eredményeink nem

erősítették meg egyértelműen az ürülékevés és az autoimmun betegségek közötti szignifikáns kapcsolatot, de megfigyeléseink további kutatást érdemlő, bonyolult kapcsolatra utalnak.

Megállapítottuk, hogy a fiatalabb kutyák nagyobb valószínűséggel esznek ürüléket, akárcsak a speciális étrenden tartott állatok. Az évente legalább négy parazitaellenes kezelést kapó állatok esetében is gyakrabban lehetett coprophagiát megfigyelni. Felmerült annak a gyanúja, hogy ezek a viselkedési abnormalitások allergiás reakciókkal hozhatók összefüggésbe. Ez a hipotézis felhívja a figyelmet arra, hogy ne pusztán viselkedési furcsaságként, hanem az állat egészségének változásának lehetséges megnyilvánulásaként értelmezzük.

A humán orvostudományból merítve inspirációt, ahol a székletátültetésnek pozitív hatása van az autoimmun betegségekre, ez a tanulmány hangsúlyozza, hogy a bélrendszer egészsége, az immunrendszer és az ürülékevés között érzékeny összefüggés lehet. A dolgozat kiemeli a további kutatások fontosságát. Arra ösztönzi a kutatókat, hogy tovább vizsgálják a coprophagia hatását a kutyák immunrendszerére, valamint az állatorvosokat, hogy a humán gyógyászatban megfigyelt sikereket megfontolva innovatív beavatkozások lehetőségét tegyék lehetővé az autoimmune és más immunrendszeri megbetegedések esetében.

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11. Acknowledgments

I would like to thank Dr. Lőrincz Márta, and Földi Dóra for their guidance and support during this project, I would also like to thank Dominik Gulyás for the statistical analysis and the entire department of microbiology and infectious diseases for helping me to complete my thesis.