Immunology Research Institute Gifu (IRIG)
Science of Immunology for Pet Health

IRIG

HANDBOOK ON

IMMUNOLOGY-BASED INNOVATIONS FOR COMPANION ANIMALS

Reference for nutritional solutions
to clinical challenges

Edited by Dr. Inge Heinzl, Klaas Krüger

Supported by EW Nutrition Pet Pioneer
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ABOUT EW NUTRITION PET PIONEER

At EW Nutrition Pet Pioneer, we are passionate advocates for pet health! Although we were established recently, we look back on decades of experience in relevant research and science.

Understanding pet owners' desire to focus on their pets' health through nutrition, we believe natural ingredients are the foundation for healthy dogs and cats. Our diverse team of scientists and pet care experts from around the world is dedicated to extending pets' healthy lifespans.

This first edition of the IRIG Companion Animal Handbook, supported by EW Nutrition Pet Pioneer, aims to equip veterinary professionals and pet nutrition experts with scientific knowledge on various health scenarios. The handbook primarily focuses on IgY technologies. With decades of research experience, IRIG is a market leader in IgY technology for animal nutrition.

The IRIG Companion Animal Handbook is not intended to discuss products; it presents only proven nutritional science.

On behalf of EW Nutrition Pet Pioneer, I would like to thank the scientific editors for compiling the content and all the experts who contributed their knowledge and expertise to the book chapters.

Klaas Krüger
Business Development Director Pet Food EW Nutrition

"we are **passionate advocates** for pet health!"

IMMUNOLOGY RESEARCH INSTITUTE IN GIFU (IRIG)

For over 30 years, the Immunology Research Institute in Gifu (IRIG) has pioneered research on chicken egg antibody (IgY) technology. An important milestone in the history of IRIG goes back to 1986 when the first research projects were conducted to explore the potential of IgY in preventing dental caries in humans and critical gastrointestinal infections in domestic animals.

First publications in various scientific journals were the outcome of these research projects, as well as key patents on the methods of production and application of specific IgY antibodies. Since 1995, IRIG focused its activities mainly on IgY application and development of products for animal farms and for use as special immunoglobulin supplements in feeds. Currently, our IgY products are used widely around the world for human and animal applications.

Research on IgY for pet care has been a continuous focus of our efforts over the last 20 years. In 2006, we published our first research paper in a professional journal, reporting on the efficacy of specific IgY in protecting dogs against canine parvovirus. This was followed by another research paper in 2011, reporting on the effectiveness of anti-gingipain IgY on the periodontal health of dogs. These publications mark the first instances in the world of using IgY to control two of the most common infections or health issues in pet dogs.

Our continuous research efforts have led to the global marketing of our oral and intestinal IgY care products in pet food diets, pet supplements, and pet care products. For more detailed information about IgY and IgY-based products, please visit **IRIG's website.**

Dr. Nguyen Van Sa
Regional Director EW Nutrition Japan

"research on IgY for pet care has been a **continuous focus"**

RESEARCH ON IGY TECHNOLOGIES

Pet owners of all kinds know the joy and companionship that a furry friend brings to their homes. With this partnership comes the responsibility to ensure their pets are healthy and energetic. A central part of this responsibility is providing appropriate nutrition, which plays a crucial role in the animals' overall health, welfare, and longevity.

Part of maintaining this health involves controlling microbial and viral challenges. IgY technology offers an innovative and powerful approach to pet food, providing passive, topical immunity against specific pathogens. By targeting specific pathogens, IgY can help prevent or mitigate infections such as gastrointestinal diseases, common in pets.

Immunology Research Institute in Gifu (IRIG) pioneered the field of IgY from 1986 onwards and is supported by EW Nutrition. Today, EW Nutrition's research network includes centers and production sites ranging from biotechnology research at EW Nutrition Innovation GmbH & Co. KG in Cologne, Germany, to precision fermentation at EW Biotech GmbH in Leuna, Germany, and IgY research at IRIG Japan.

This investment in corporate research is unique in the field of animal and pet nutrition, ensuring that EW Nutrition lives up to its promising slogan: "Functional Innovations backed by Science."

On behalf of the global research team, I thank the scientific editors for consolidating the information on IgY technology and all the scientists who work daily to provide comprehensive, science-based solutions for animal nutrition.

Dr. Andreas K. Michels
Director of Global Research and Development, EW Nutrition Innovations

"IgY technology offers **an innovative and powerful approach** to pet food"

VETERINARIANS MANAGING HEALTH & WELLBEING

The shift from pet to family member has significantly changed interactions within the human-animal family unit, bringing veterinarians into close contact with the entire family. The pets' critical influence on the family's health and well-being is now more pronounced than ever, providing all members a sense of security and confidence. Previously, the veterinarian focused on diagnosing, preventing, and treating various health issues affecting the family pet. Today, they have increased their range of services to provide more holistic pet care and additionally support the well-being of cats and dogs with nutritional, physical, and behavioral therapies. Furthermore, their responsibility now encompasses the concept of "One Health" – supporting the health and well-being of the entire family (both human and animal) and the environment in which they live.

Veterinarians actively share this social responsibility with the medical profession, primarily focusing on preventive health, the prudent use of antimicrobial therapies, and the application of appropriate and effective therapeutic alternatives. Due to the close contact between humans and companion animals, there is a daily risk of sharing resistant bacteria that may directly affect humans or the transfer of resistance mechanisms. Companion animal veterinarians have proactively enhanced their focus on preventive measures. They provide more holistic pet care and support the family's well-being to minimize preventable diseases through nutritional advice and physical and behavioral therapy. Simultaneously, they seek and implement effective and safe alternatives for managing infectious and non-infectious diseases. The "Handbook on companion animal immunology-based innovations" will introduce an alternative, natural method – IgY – in more detail. The use of avian immunoglobulins provides veterinarians with a proven safe and effective management alternative of numerous infectious diseases that threaten the well-being of the human-animal family unit.

Merideth Parke
Global Application Manager Swine at EW Nutrition

"veterinarians have proactively enhanced their focus on **preventive measures.**"

CURRENT SCIENTIFIC UNDERSTANDING ON THE INTESTINAL AND ORAL MICROBIOME IN COMPANION ANIMALS

Teresa Schmidt, DrMedVet, PhD / Jan S Suchodolski DrMedVet, PhD, DACVM

The oral and gastrointestinal microbiome is a miscellaneous community of bacteria, protozoa, fungi, viruses, and archaea, creating a complex ecosystem and interacting with their host *(1, 2)*. It is estimated that the gastrointestinal tract alone inhabits about 100 trillion microbial cells, thereby exceeding the number of mammalian host cells *(3)*. The genome size of the microbiota is larger than the mammal genome *(4)*. Bacteria make up the vast majority of this community in the gastrointestinal tract and fulfill essential immune and metabolic functions *(1, 5)*.

The microbiome has a large metabolic capacity and microbes produce a variety of different microbiota-derived metabolites. Some of these are directly produced by intestinal bacteria, while others are metabolic by-products of dietary (e.g., fiber, protein, fat) or host (e.g., bile acids) substrates. Important examples include vitamins, short-chain fatty acids (SCFAs), and secondary bile acids *(6)*. Those metabolites are summarized as metabolome and can contribute to the health or disease status of their host. The metabolites are substantial factors that can also act as signaling molecules. They are not only important for the bacteria themselves, but also for modulating the host's physiological processes. Some of the metabolic functions of the intestinal bacteria are well understood, e.g., bile acids conversion and SCFAs synthesis from dietary fiber *(1).* Many more remain to be elucidated. The intestinal microbiome represents a key organ that is in a complex multidirectional crosstalk with the other body organs via metabolic, humoral, endocrine, and immune signaling pathways *(7-9)*.

Importance of a healthy microbiome

The microbiome and its interaction with the host is are strongly implicated in the health and disease status *(3).* The complex microbial ecosystem functions as a significant metabolic organ by converting nutrients or xenobiotics and generating its own bacteria-derived metabolites *(1)*. A balanced microbiome is crucial for the health of companion animals as it aids in digestion, supports immune function, helps prevent infections with pathogens, and contributes to overall well-being *(Table 1).* Some of the major beneficial gut microbiome- related metabolic pathways include the SCFAs, bile acid, and indole metabolism *(1).* SCFAs are exclusively produced by bacteria during the fermentation of dietary carbohydrates. They serve as local and systemic energy sources, provide anti-inflammatory properties, are important for the intestinal integrity, and regulate the gut motility *(10).* Therefore, the addition of various fibers or other substrates to stimulate the production of beneficial SCFAs is an active area of research in the pet food industry. The amino acid tryptophan is another dietary component with implications on the host immune system, partly mediated through the gut microbiome.

Tryptophan from the diet is metabolized to indole by intestinal bacteria. Indole metabolites have anti-inflammatory properties and maintain gut barrier function *(11).* Primary bile acids, produced by the liver, are secreted in the gut and are mainly reabsorbed in the ileum via circulation *(12).* A minor proportion of these bile acids escape reabsorption and then enter the colon, where they are converted into secondary bile acids by the bacterium ***Peptoacetobacter*** (formerly ***Clostridium***) hiranonis *(13)*. This bacterium is the main converter of primary to secondary bile acids in dogs and cats. Secondary bile acids are involved in the glucose and lipid homeostasis and also have also anti-inflammatory properties *(14).* They play an important role in maintaining the balance of the normal microbiome by suppressing the growth of potential pathogens *(15)*. A decreased abundance of the bacterium resulting in a lower bile acid conversion is strongly associated with an imbalanced gut microbiome in companion animals *(13, 16).*

In the mouth, a plaque biofilm on the mucosal surfaces and teeth aids the resistance of commensal bacteria against mechanical irritation or invasion of external microbes *(17-20).* The biofilm prevents pathogens from colonization, but overgrowing of the biofilm can induce dysbiosis and contribute to diseases *(17, 21).* In a healthy state, the host shows an immune tolerance to the oral microbiome, enabling a symbiotic relationship with mutual benefits *(20, 22).*

Potential consequences of an abnormal microbiome

The microbiome closely interacts with the environment of the oral cavity and the gastrointestinal tract (mucosa, saliva/mucus layer, and immune system). Alterations in this microenvironment impact the microbial equilibrium, which can serve as an early marker for diseases. These alterations of the commensal microbiota are summarized as dysbiosis and are commonly characterized by a reduced bacterial diversity *(23).* Changes in the microbial community are additionally accompanied by functional changes in of the bacteria-derived metabolome *(24, 25).* Therefore, regardless of whether dysbiosis is primary (idiopathic) or a consequence of an altered mucosal environment (e.g., inflammation, mucosal remodeling due to epithelial inflammation, degradation of protective the epithelial mucus layer), the altered function of the microbiome can exacerbate the pathological conditions, leading to further inflammation and mucosal damage.

The dysbiosis pattern areis variable based on the inducing cause. One major driver of dysbiosis is when increased dietary substrate is available in the intestinal lumen, which can be preferentially utilized by some bacteria, leading to overgrowth and therefore dysbiosis. This can be either in healthy animals consumeing diets with poor digestibility, or in diseased animals with exocrine pancreatic insufficiency (EPI), or in animals with chronic mucosal inflammation (chronic inflammatory enteropathy, IBD) leading to maldigestion and malabsorption. Another major driver of dysbiosis is the use of antibiotics.

Gastrointestinal disorders that are associated with dysbiosis are acute uncomplicated diarrhea, acute hemorrhagic diarrhea syndrome (AHDS), and chronic inflammatory enteropathies *(26).* It is important to note that in acute intestinal diseases, the microbiome shifts are typically mild and self-limiting. Antibiotic-induced dysbiosis also reverts itself to normal in the majority of animals, but some animals may have persistent dysbiosis. Dysbiosis associated with chronic inflammatory enteropathy is typically long-lasting over many months to even years, likely due to the chronic remodeling of the epithelium. Oral dysbiosis can have a negative effect on the oral health, promoting gingivitis and periodontal diseases in cats and dogs *(27-30).*

An impaired metabolome can have a significant impact on the entire host physiology, with systemic changes reaching beyond local effects on the oral cavity and the gut. Intestinal dysbiosis has been linked to chronic kidney disease (CKD) *(31)*, heart disease *(32-34),* diabetes mellitus *(35)*, obesity *(36),* neurologic disorders *(37),* and behavior alterations (canine anxiety and aggression) *(38).* Shifts in the oral microbiome causing gingivitis and periodontal diseases were linked to systemic inflammation, kidney disease, endocarditis, parenchymal liver diseases, and cognitive decline *(39-43).*

Important bacterial species relevant for health in dogs and cats

Microbiome research has become a rapidly evolving scientific field. Despite progress, characterizing the microbiome accurately remains challenging because of its complexity. Slightmall alterations in the microenvironment (pH, nutrients, metabolites) can affect bacterial gene expression, inducing altered metabolic functions even within the same species *(1).* Most of the conducted studies in companion animals focus on the gastrointestinal flora, but recent investigations also target also other body locations *(44).*

The oral and intestinal microbiome of companion animals is highly diverse *(1, 45).* Technical advanced molecular methods enable a more holistic characterization of the microbial ecosystem on multiple body sites of humans and animals alike *(44).* Traditional, frequently used cultural-based approaches underestimate the multiplicity of the microbiome *(1).* These approaches focus on the limited number of bacteria that are culturable (estimated a small of all existing bacteria), miss other important microorganisms (e.g., fungi, protozoa, archaea), and are likely to create a bias towards potential culturable pathogens *(1, 46, 47).* Culture independent methods such as 16S rRNA gene sequencing or metagenomic shotgun sequencing allow more accurate characterizing of the microbiome *(1).* The latter allows a better characterization of microbes on the species level or even strain level *(1).* High-throughput sequencing and complex bioinformatics enable deeper insights into the host microbiome *(44).* However, it needs to be highlighted that next-generation sequencing approaches are useful as discovery tools, but recent data shows that they lack reproducibility and that there is a large variation in reported data between different laboratories due to variation in methodology (i.e., DNA extraction, different sequences, different bioinformatics pipelines, etc.).

This makes comparison across studies extremely difficult. Therefore, new approaches focus on the use of targeted assays such as qPCR, which has high reproducibility for individual core bacterial taxa of interest, allowing a better definition of a normal microbiota.

An example is the dysbiosis index (DI), which is a quantitative PCR-based assay that can be used to assess shifts in the feline *(48)* or canine *(49)* fecal microbiome in individual patients. It is currently the only analytically validated assay to assess the fecal microbiome and has been used in various published clinical studies. The DI quantifies the fecal abundance of seven core taxa as well as the total bacterial abundance. The DI provides reference intervals for these bacterial groups and additionally calculates a single number that expresses the extent of intestinal dysbiosis. The DI correlates negatively with species richness, i.e., a higher DI indicates lower microbial diversity, and correlates DNA shotgun sequencing. The DI is helpful to demonstratein demonstrating how much the entire microbiome is shifted *(50)*.

Intestinal bacteria

Generally, ten predominant phyla inhabit the gut in companion animals, with Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria being the most common ones *(3).* The gastrointestinal tract is divided into three main compartments, the stomach, the small intestine and the large intestine, each of them with different anatomy and functions *(44)*. The conditions for microbiota vary between these compartments, resulting in a unique microbial colonization (oxygen level, pH, antimicrobial compounds, and intestinal motility) *(1).* The stomach provides an unfavorable acid environment for bacteria, with only Heliobacter spp. and a small amount of lactic acid bacteria being present in dogs *(51)*. The small intestine inhabits aerobic and anaerobic microbes, with *Enterobacteriaceae, Clostridiales, Bacteroidales,* and *Lactobacillales* as predominant bacterial groups *(52)*.

The microbiome in the large intestine mainly harbors strict or facultative anaerobic, with Firmicutes, Fusobacteria, and Bacteroidetes as predominant phyla *(53, 54).* The majority of the intestinal microbiome research is based on the analysis of non-invasive and easily accessible fecal samples, resulting in limitations when it comes to mucosa- adherent or entero- invasive bacteria and conclusions about the anterior gastrointestinal compartment *(1)*.

Predominant phyla in fecal samples are Firmicutes, Bacteroides, or Prevotella *(54-56)*. A few core bacterial taxa in canine and feline fecal samples are associated with a healthy microbiome and are also included in the DI. *Faecalibacterium, Turicibacter, Clostridium hiranonis, Blautia* and *Fusobacterium* are linked to a healthy microbiome in dogs *(49)*. *Faecalibacterium, Turicibacter, Clostridium hiranonis, Bifidobacterium,* and *Bacterioides* are health- promoting bacterial taxa in cats *(48)*. These core microbiota facilitate a healthy host metabolism by being involved in crucial metabolic processes in the gut *(Table 1).* *Faecalibacterium* and *Bifidobacterium* are involved in the SCFAs synthesis. *Peptoacetobacter hiranonis*, the main bile acid converter in cats and dogs, inhibits the growth of potential pathogens, such as *E. coli, C. difficile,* and *C. perfringens*. While many external and internal factors seem to be neglectable, the age of dogs and being on an extremely high -fat and high- protein homemade diet (Feeding a Bones and Raw Food; BARF) can impact the intestinal core microbiome *(57, 58)*.

Oral bacteria

The oral cavity is the beginning of the digestive tract, consisting of different anatomic structures, such as the tongue, teeth, muscles, mucosal surfaces, and salivary glands *(59)*. The main function of the mouth is to aiding in digestion by crushing and moisturizing food. However, tongue and teeth also serve as tools for drinking, grooming, wound care, and environment exploration *(59)*. The conditions in the mouth (pH, oxygen level, mucosal surface) and the increased exposure to the animal's environment shape the oral microbiome *(60)*. Until recently, investigating the oral microbiome of companion animals was not in the focus of the research and is, therefore, still in its infancy *(2)*. The limited existing studies are based on small sample sizes and mainly culture-dependent methods *(2).* The canine and feline oral microbiome in a physiological state is predominantly characterized by aerobic or facultative anaerobic bacteria *(61, 62).*

The canine oral core microbiome consists of 67 species present in healthy dogs *(45)*. *Proteobacteria, Bacteroidota, Fusobacteria,* and *Firmicutes* are frequently reported as predominant phyla across multiple studies *(45, 63-66)*. Healthy dogs demonstrate a higher species richness and evenness compared to diseased populations *(45)*.

The core oral microbiome in healthy dogs is mostly conserved across different regions of the world *(63)*. However, the individual canine oral microbiome can be affected by diet, age, and the location of oral sample collection, with saliva samples showing divergent results *(63, 67-69).* A small number of studies identified bacteria associated with oral health, such as bacteria of the genus *Pasteurella, Corynebacterium,* and the species *Bergeyella zoohelcum (27, 45).*

The feline oral microbiome has been investigated by only a limited number of studies. A large-scale study revealed a highly diverse feline oral microbiome, with a total of 8,344 different bacterial species detected *(2).* An individual cat inhabit around 606 microbes in their mouth, with bacteria and archaea being the most common ones *(2).* On average, the feline oral microbiome is divided into around 10-20 different phyla, with *Proteobacteria, Bacteroidetes, Firmicutes, Spirochaetes* being the most common ones across different studies *(66, 70, 71).* The core microbiome in cats appears to be highly conserved *(71).* Healthy cats show a high diversity, richness and evenness of their oral microbiome *(62, 71).* Factors that can affect the feline oral microbiome are diet, age and living environment, whereas an effect of oral sample collection location seems to be negligible *(17, 71-74)*. Higher numbers of aerobic bacteria are associated with oral health in cats, e.g., species such as *Xanthomonadaceae sp., Moraxella sp., Pseudoclavibacter sp., Bergeyella zoohelcum, Flavobacterium sp.* and *Flavobacteriaceae sp (62).*

Important bacterial species relevant for disease in dogs and cats

Intestinal bacteria, viruses and protozoa

In the recent decades, the gastrointestinal microbiome has received increased attention due to its crucial role in influencing host health and disease. While many studies focused on identifying certain disease-related enteropathogens in companion animals, it is now well-established that more holistic alterations in the intestinal microbiome are correlated with acute and chronic gastrointestinal diseases *(3).*

It is assumed that a decrease in beneficial anaerobic bacteria and also their metabolic functions, such as lowered SCFAs synthesis and/or reduced anti-inflammatory peptides (e.g., *Faecalibacterium*), and the depletion of secondary bile acids (*Peptoacetobacter hiranonis*), may be clinically more relevant than the overgrowth of opportunistic pathobionts ***(Table 1)*** ***(1, 3, 49)***.

However, an increase of facultative anaerobic bacteria of the family *Enterobacteriaceae* is considered as a marker of dysbiosis ***(26, 75).*** One species of *Enterobacteriaceae* is *E. coli,* a well-known intestinal core bacterium occurring in low abundance in healthy companion animals. In cats and dogs with chronic enteropathy, *E. coli,* shows a significantly increased abundance ***(48, 76)***. Under certain circumstances, it can evolve into a pathobiont. Structural changes and damage of the intestinal barrier (leaky gut) or a weak immune system of the host can facilitate its pathogenic effects. As a gram-negative bacterium, the outer cell membrane of *E. coli,* is composed of lipopolysaccharides (LPS) as a major component. LPS can act as a potent endotoxin, which strongly stimulates the immune system and can promote a systemic inflammatory response in a disease state. Translocated in the blood (endotoxemia) LPS can initiate severe consequences for the host by inducing a life-threatening septic shock ***(77, 78)***.

Certain canine gastrointestinal diseases might be associated with more specific enteropathogens. Sudden overgrowth of *C. perfringens* strains that encode pore-forming netE and netF toxins are linked to AHDS and result in severe intestinal mucosal damage ***(79).*** The pathogenic role of *C. difficile* in canine and feline gastrointestinal diseases is a matter of debate. It is present mostly in dogs with dysbiosis and decreases the abundance of *P. hiranonis*, but it does not require specific treatment, as most animals will respond to standard treatments for chronic enteropathy ***(26, 80)***. *Campylobacter jejuni* is significant in the etiopathogenesis of chronic or intermittent canine diarrhea ***(81)***. *Granulomatous colitis* is associated with mucosa-invasive *E. coli* ***(82).*** Some diseases, such as EPI, are characterized by maldigestion of dietary substrates causing significant changes in the microbial community ***(83).***

Infectious gastroenteritis in companion animals can be also caused by viral or protozoal pathogenic agents. *Canine parvovirus* (CPV) and *feline panleukopenia virus* (FPV) are the most common causes of viral-induced acute enteritis. The parvoviruses are highly contagious and mainly affect young and inadequately vaccinated animals. Replication in the intestinal crypts and lymphoid organs causes significant damage to the intestinal mucosa. Both species show signs of lethargy, anorexia, vomiting, and diarrhea, accompanied by fever, dehydration, and leucopenia. The leucopenia can promote secondary bacterial infections, and a disrupted intestinal barrier can facilitate bacteremia increasing the risk of sepsis. Adult dogs are usually immune to the virus, whereas adult cats can still develop severe clinical signs ***(84)***. Recent literature suggests potential long-term consequences, with an increased risk of dogs developing chronic gastrointestinal disease after surviving an infection during youth ***(85, 86).*** Other viruses that are suggested to be associated with gastrointestinal diseases are coronavirus and circovirus. However, a clear pathogenic role for both viruses is not well-established. It remains a matter of debate whether they are clinically relevant and can be considered causative agents for gastrointestinal symptoms.

Intestinal protozoal infections are frequent in cats and dogs. Animals living in crowded conditions (shelter, kennels) or poor sanitation have a higher risk of infection. Juvenile naïve animals not previously exposed to the infectious agent may develop clinical signs. Adult infections usually remain subclinical unless they are associated with co-infections of other pathogens. *Giardia spp.* are common protozoa that, especially in younger companion animals, may cause chronic small intestinal diarrhea.

They infect the surface of the enterocytes in the duodenum of dogs and the ileum of cats. Damage is induced due to various mechanisms, e.g., enterotoxins, altering enterocytic function, inducing inflammation and dysbiosis *(84).* It is assumed that a treatment of *Giardia spp.* can be sufficient to control clinical signs occurring during co-infection *(87).* However, it is important to note that not all animals tested positive for *Giardia spp.* will develop clinical signs.

Oral bacteria

Conditions in the mouth are dynamic and shift rapidly *(19).* Only the best adaptable microbiota can colonize this environment *(20).* Oral microbiota are frequently exposed to external intruders and compete with these potential pathogens *(88).* Failing of the commensal microbiome in defending their niche results in dysbiosis. Dysbiosis is characterized by an imbalanced microbiome and an increased abundance of pathogens *(89).* Factors like inflammation, poor diet, and dental hygiene pave the way for oral dysbiosis *(2, 20).* Nowadays, it is commonly assumed that most dental diseases result from complex interactions among various microbes rather than changes in individual bacterial taxa *(29).* Nevertheless, certain microbial profiles and specific core microbiota may be associated with oral diseases *(2).* Interpreting the limited conducted feline and canine studies is impeded due to small sample sizes and the common application of antibiotic or immune suppressive therapy in oral diseases.

Canine periodontal disease might be associated with shifts in der oral microbiome. Periodontitis was characterized by a decrease in health-related aerobic bacteria and also an increase in potential pathogenic species such as *Porphyromonas spp., Fusobacterium spp.,* and *Prevotella spp. (27, 90)*. *Peptostreptococcus, Actinomyces, Peptostreptococcaceae,* and *Firmicutes* were predominant genera and phylum in periodontitis, gingivitis, and older dogs *(28, 63)*. As Firmicutes are part of the core microbiome of healthy dogs, increased abundance might be caused by an altered microenvironment in oral diseases (e.g. unhealthy teeth status) rather than being causative for the health decline *(27)*. Canine chronic ulcerative stomatitis, another oral disease, causes mucosal lesions, which seem to be inhabited by potential pathogens as *Porphyromonas cangingivalis, Porphyromonas gingivicanis*, two canine species related to *Porphyromonas. Gingivalis,* and a *Tannerella forsythia*-like phylotype *(90).* Dogs with oral tumors showed an increased abundance of Porphyromonas cangingivalis in their oral microbiome *(45, 91)*. Changes in the oral microbiome could even be linked to diseases of external body organs. Dogs affected by mammary tumors had a higher abundance of *Treponema* and *Bacteroides*, which might be a relevant risk factor for developing the disease *(92)*. The cognitive decline might be also linked to age-related oral dysbiosis *(43)*.

An association between periodontitis and the oral microbiome has also been identified in cats. As in dogs, cats with periodontitis showed a decrease in health-related phyla and further an increase of specific phyla, such as *Firmicutes, Synergistetes* and *Spirochetes (29, 30)*. These changes might also be related to functional alterations within the microbial communities *(5).* *Feline gingivitis* may be associated with an increase of Spirochetes in an early disease stage *(93).* *Feline chronic gingivostomatitis* (FCGS) still has an unknown pathogenesis, but disturbances of the oral microbiome might be a contributing factor *(74).*

Recent studies offer contradictory findings about the role of the oral microbiome in this disease. In some studies, FCGS cats showed a less diverse microbiome *(62, 94).* Dysbiosis was associated with a depletion of beneficial bacteria instead of an increase in pathogenic microbiota *(74).*

However, some microbiota, mainly obligate or facultative anaerobic, were enhanced in cats with FCGS ***(62)***. *Porphyromonas, Treponemas, Fusobacterium, Peptostreptococcus,* and *Fretibacterium* were more abundant in the mouths of affected cats and, therefore, might be considered as potential pathogens of FCGS ***(62, 70, 95).*** Another study detected *P. multocida* subsp. multocida as a predominant species in the FCGS oral flora ***(94).*** A slightly higher abundance of Porphyromonas was identified in a sub-group of cats affected by the feline odontoclastic resorptive lesion (FORL) ***(96).*** The subgroup further showed a lower microbial diversity with a significant decreased of a bacterium that has the potential to affect the mineral balance in the mouth ***(96, 97).*** An increase in proinflammatory markers in the saliva was also demonstrated in the subgroup (98). The oral microbiome of FIV-infected cats showed an increased relative abundance of *Fusobacteria* and *Actinobacteria* ***(99).***

Since oral microbiome research in companion animals is still in its infancy, little is known about specific pathobionts. However, evidence from human medicine emphasizes *Porphyromonas gingivalis* and *Treponema spp.* as oral pathobionts in periodontitis, species related to commonly reported potential oral pathogens in cats and dogs ***(100, 101).*** *Porphyromonas gingivalis* is considered to be part of the natural human oral flora. However, the bacterium carries several virulence factors, such as LPS and the proteolytic enzyme gingipain. These key factors are highly destructive to the periodontium and can negatively affect the host's immune response. Therefore, an increased abundance of *Porphyromonas gingivalis* can lead to severe damage in the oral cavity ***(101, 102).*** A recent study has shown that translocation of the oral pathobiont *Porphyromonas gingivalis* into the gut enhances immune cell differentiation in the intestinal lymphoid tissue. These immune cells can migrate to the oral cavity and contribute to the aggravation of periodontitis. These findings highlight the critical role of the gut-mouth axis in health and disease ***(103).***

Modulating the intestinal microbiome

Due to the large number of bacteria in the intestine, it is challenging to induce large shifts in the microbiota. Dysbiosis, especially in chronic intestinal conditions affecting the mucosal epithelium, often requires multi-modal therapy and long-term therapy. Initial treatment focuses on dietary changes, potentially combined with probiotics, prebiotics, and fecal microbiota transplantation (FMT). Antibiotics are nowadays reserved as a last resort if standard therapies fail ***(104).*** Dysbiosis can persist for months to years, even in clinical remission due to ongoing mucosal changes in chronic inflammatory enteropathies. Nutritional therapy can improve clinical signs and may help shift the microbiome towards a more normal state. Anti-inflammatory therapy with corticosteroids has been shown to help. FMT can quickly normalize the microbiome, but long-term success depends on the underlying disease.

Diet can have a major impact on clinical remission and partly the microbiome. Highly digestible, hydrolyzed protein, fiber-enriched, and novel protein diets can induce clinical remission in animals with chronic enteropathy. These digestible diets reduce bacterial proliferation by lowering undigested nutrients. Fiber-enriched diets and those with high digestibility may partially normalize the microbiome over time.

Prebiotics are indigestible carbohydrates that foster beneficial bacteria growth. They can be soluble or insoluble, fermentable or non-fermentable. Fermentable prebiotics convert to short-chain fatty acids with multiple benefits. Additional fiber supplementation, like psyllium husk, may be helpful.

Probiotics are live bacteria that benefit the host. Only a few commercial products have been evaluated in clinical studies showing microbiota normalization in various studies.

Antibiotics such as tylosin or metronidazole can improve clinical signs in chronic gastrointestinal diseases but may cause relapse and dysbiosis. Antibiotics are recommended only after dietary and anti-inflammatory treatments fail or if systemic inflammation is present ***(104).***

Fecal Microbiota Transplantation involves transferring stool from a healthy donor into a recipient's gut. Its success varies with the underlying disease. In chronic enteropathies, FMT may improve fecal scores quickly, but relapses can occur if underlying pathology persists. For antibiotic-induced dysbiosis or acute diarrhea without underlying disease, FMT often leads to prolonged normalization of clinical signs.

Immunoglobulin Y (IgY),
administered as a dietary supplement, can be a useful option to selectively modulate the microbiome in companion animals. They could directly address potential pathobionts or their produced toxins, thereby **ameliorating gastrointestinal signs.**

Furthermore, IgY could serve as an alternative to antibiotics, circumventing their negative effects (e.g., dysbiosis). Additionally, this approach could target non-bacterial agents (e.g., viruses, protozoa) that promote gastrointestinal diseases in cats and dogs.

Table 1 - Some of the major beneficial gut microbiome related metabolic pathways

Source	Bacteria involved	Microbial metabolite (s)	Effects in host	
			Beneficial (in normal concentrations)	*Potentially deleterious (in abnormal concentrations)*
Carbohydrates from diet	Various (e.g. *Faecalibacterium, Turicibacter, Blautia*)	Fermentation to short-chain fatty acids (SCFA)	Anti-inflammatory Improve intestinal barrier function Regulate intestinal motility Local and systemic energy source	Abnormal SCFA ratio can activate virulence factors of enteropathogens
Primary bile acids (BA) from the liver	*Peptoacetobacter hiranonis*	Conversion to secondary bile acids	Anti-inflammatory Inhibits the growth of transient and potential pathogens	Increase of primary BA can cause secretory diarrhea
Tryptophan from diet	Various (eg, *Bifidobacterium spp*)	Indole metabolites	Anti-inflammatory; maintain intestinal barrier function	increased concentrations cytotoxic; indoxyl sulfate acts as uremic toxin
Tyrosine and phenylalanine from diet	Various	P-cresol		Acts as uremic toxin and leads to progression of chronic kidney disease

IgY:

produced by birds, functional in mammals

Shofiqur Rahman
Immunology Research Institute in Gifu, Japan (IRIG)

Immunoglobulins of the egg yolk or IgY are immunoglobulins produced by hens and initially intended for the chicks as a care package for the first days after hatching. Fortunately, IgY also benefits mammals and can help protect other animals or humans against pathogens, allergies, etc.

For a long time, rabbits and mice have been the common mammalian species that produce poly- and monoclonal antibodies. However, the production of antibodies by hens has many advantages. The antibodies are deposited in high concentrations in the eggs; their collection and separation are noninvasive and easier than separating IgG from the serum of mice or rabbits.

As a functional foodstuff in human nutrition, IgY is safe and can predictably exert its activity throughout the entire length of the alimentary tract. While IgY may not exert total microbial eradication, it may significantly reduce infectious pathogen load to a point where the host's immunity can finish the job of immunological protection.

HISTORY OF IMMUNOGLOBULINS IN YOLK (IgY)

In 1893, the first scientific report about the transmission of maternal antibodies of the hen to the egg was published by ***Felix Klemperer***. He showed that the egg yolk of hens immunized against tetanus protected mice when infected with a tetanus bouillon culture. ***G.A. Leslie and L.W. Clem (1969)*** suggested the name Immunoglobulin Y for these antibodies. Other synonymous names are Chicken IgG, Egg Yolk IgG, and 7S-IgG. Since the 1980s, IgY has been frequently studied due to the revolution of overall technology, and in the 1990s, the term 'IgY technology' was introduced to describe a procedure to produce polyclonal antibodies of the Y class (IgY).

In 1995, ***Warr et al.*** reported that IgY is a key isotype in antibody evolution. IgY was thought to have diverged from an ancestral IgM, and it was a widely held belief that an IgM gene duplication event led to the formation of IgY. IgY is also thought to be the precursor of IgG and IgE.

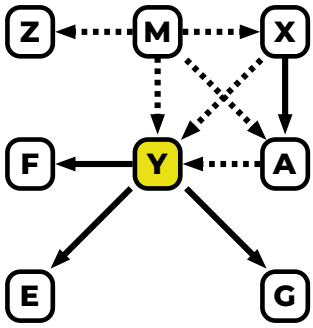


Figure 1 - The central position of IgY in immunoglobulin evolution

Solid arrows indicate an orthologous relationship between isotypes. Broken arrows connect isotypes that have a putative orthologous relationship, not yet verified. The broken arrow that relates IgA to IgY refers to an ancestral form of IgA. (adapted from Zhang et al., 2017)

At some point during the evolution of the mammalian lineage, IgY underwent a gene duplication event and diversified into IgE and IgG. Thus, it was proposed that IgM gave rise to the mucosal antibody IgX and then to IgA, which, on their part, gave rise to IgY and the serum antibodies IgG and IgE. These relationships are depicted in ***Figure 1***, highlighting the central role of IgY.

Physicochemical and antigenic evidence obtained during the past three decades has indicated that IgY occurs throughout the vertebrate classes Amphibia, Reptilia, and Aves. The diverse capabilities of IgY in so many species make it clear that molecular genetic studies of this molecule will broadly contribute to our understanding of Ig evolution. Looking back on evolution will advance our knowledge of mammalian antibody function.

In 1996, the European Centre for the Validation of Alternative Methods (ECVAM) to animal testing strongly recommended avian antibodies as alternatives to mammalian ones (Schade et al., 1996). In parallel, in 1999, the IgY technology was approved as an alternative method for supporting animal welfare by the Veterinary Office of the Swiss Government.

The field is more than 120 years old. However, in recent decades, significant advances in research and development areas such as genetics, biochemistry, bioengineering, and bioprocessing have prompted new approaches to this old technology.

The first standardized laboratory practice of IgY technology, the 'IgY Laboratory Manual', was reported in 2001 ***(Schade et al., 2001)***. During the years 2002-2006, the project 'Multidisciplinary Hen Egg Research' was started through a Cooperative Organization Science and Technology action (COST 923) in the European Union framework for the versatile utilization of eggs. ***Huopalahti et al.*** summarized in 2007 one project in which the biomedical use of IgY became the focus of the action plan, and a Chinese version of an IgY monograph was published in 2011 by Zhang et al.

A survey of the NCBI database using different search terms, namely 'IgY Technology', 'IgY Antibodies', and 'IgY', covered a timeline from 1893 to 2022. This survey, analyzing three time periods (1893-1955, 1956-1987, and 1988-2022), showed a progressive increase in avian IgY publications since the 1980s ***(Figure 2).***

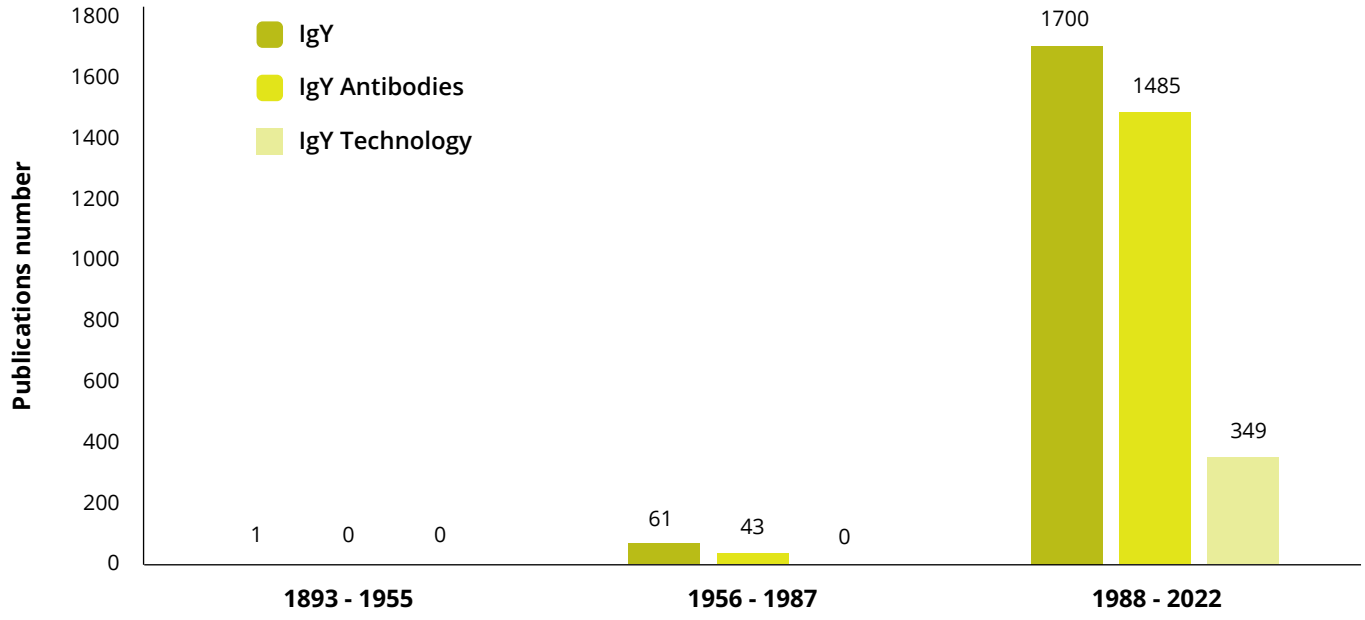


Figure 2 - Evolution of publications on avian IgY Antibodies

A search of publications on avian IgY Antibodies and IgY Technology was performed on the NCBI database with different search terms over a time window from 1893 to 2022. The search terms were "IgY Technology", "IgY Antibodies", and "IgY", and the total number of publications for each term were 1762, 1528, and 349, respectively (June 4, 2022).

CHICKEN IMMUNITY AND IMMUNE SYSTEM

The avian immune system is a system of biological structures and cellular processes that protect birds from disease. Like other (animal/human) immune systems, it is divided into non-specific or innate and specific or acquired immunity ***(Figure 3)***.

Non-specific immunity does not distinguish between invaders but responds to characteristics common to many types of pathogens. It includes two barriers. The first barrier (e.g., skin, mucosas, low pH in the stomach) prevents pathogens or harmful substances from entering the organism. The second barrier consists of a humoral and a cellular component. The humoral component is based on plasma proteins available in body fluids such as blood and lymph. Examples are the complement system and cytokines such as Interferon and the fever-causing Interleukin-1. The cellular component implies phagocytic cells consisting of monocytes/macrophages, granulocytes (i.e., neutrophils, eosinophils, basophils, and mast cells), and dendritic cells.

In the case of **specific or acquired immunity**, two different kinds of white blood cells (lymphocytes), bursa-derived (B cells) and thymus-derived (T cells) lymphocytes, carry out the immune response on the cellular side. The humoral part is done by antibodies (immunoglobulins) circulating in the blood and binding foreign antigens to inactivate them. ***Figure 3*** and ***Table 1*** show the three barriers of the immune defense and the different elements of the avian immune system, respectively.

Non-specific Defense	Specific Defense
1. Barrier: Mechanical, that means everything which prevents the pathogen from entering the body (e.g. skin, mucosa, acid pH in the stomach)	***3. Barrier:*** Specific defense with the production of antibodies (immunoglobulins) and memory cells for a possible confrontation with the pathogen later.
2. Barrier: Cells and systems in the body, which override general characteristics of pathogens (e.g. lipopolysaccharides in the membrane of bacteria, double-strain RNA in some viruses	

Figure 3 - The three "steps" of immune defense

Table 1 - Elements of the avian immune defense (IgY antibodies as biotherapeutics in biomedicine)

Organs/Tissues	Cellular Elements	Humoral Elements
Primary lymphoid organs: Bursa of Fabricius Thymus ***Secondary lymphoid organs:*** Spleen Bone marrow Harderian gland Pineal gland Mucosa-associated lymphoid tissue (MALT) Lymphoid nodules	Lymphocites T-cells B-cells Macrophages	Immunoglobulins (IgY, IgA, IgM) Complement Cytokines

Immunoglobulins in chickens and mammals

Avians and mammals' specific or adaptive immune systems are based on immunoglobulins. All birds, including chickens, ostriches, quails, turkeys, ducks, and geese, produce three types of immunoglobulins (IgA, lgM, and lgY) *(Härtle et al., 2014)*, and mammals five (IgA, IgD, IgE, IgG, and lgM) *(Benedict et al., 1963*; *Leslie & Chem, 1969)*. A basic comparison of the immunoglobulin classes or isotypes between avian and mammals is shown in ***Table 2***.

Table 2 - Comparison of the immunoglobulin classes between avians and mammals

Avians	Mammals
IgM (10%) Homolog proposed by Chen et al. (1982) **IgY (75%)** **IgA (15%)** Homolog proposed by Burns and Maxwell (1981)	**IgM (19%)** **IgD (1%)** **IgG (70-75%)** **IgA (10-15%)** **IgE (0.001%)**

TRANSFER OF IGY FROM HEN TO CHICK

The transfer of maternal antibodies, also known as passive immunity, is the natural transfer of immunoglobulins from the mother to the progeny.

In birds, maternal antibodies are passed from hyper-immunized or naturally infected hens to the progeny through the egg. This passive immunity has a relatively short survival in the host, commonly 1-2 weeks, in any case, less than 4 weeks, and it should protect the young chicks during the first few weeks of life when their immune system is not fully developed to react adequately to an early challenge. The relevant antibodies (IgY, IgA, and IgM) are deposited in the egg yolk and albumin.

Transport of IgY from maternal serum to the offspring *(Ferreira Júnior et al., 2018)* is a unique process comprising two steps.

Maternal antibodies transfer from the hen to the egg

Maternal antibodies are first transferred from circulating maternal blood to the yolks of maturing oocytes in ovarian follicles, analog to the cross-placental transfer of antibodies in mammals. The passage of IgY into the ova is regulated by the follicular epithelium, which goes through morphologic changes as the ova grows. This epithelium becomes flatter and thinner in the larger ovum, allowing the passage of a high amount of IgY. The transfer of IgY through the ovarian follicular epithelium reaches its maximum 3-4 days before ovulation. Due to the development of the vitelline membrane between the ovum and the follicular epithelium of the ovary in preparation for ovulation, it starts to decrease. Since a single hen usually has several ova in different stages of development, the amounts of IgY transferred to the different ova differ.

As IgA and IgM are transferred by mucosal secretion in the oviduct, specifically in the magnum, they are mainly found in the albumen *(Rose et al., 1974)*. ***(Figure 4).***

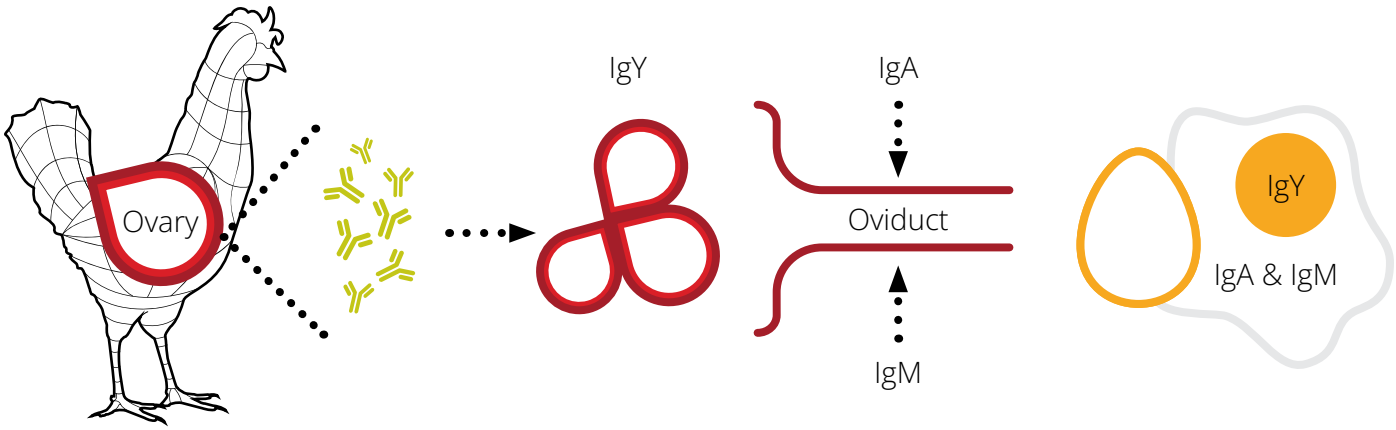


Figure 4 - Maternal antibody (MA) transfer from the hen to the egg

Transfer from the egg to the chick

In the second step, the IgY is transferred from the egg yolk across the avian yolk sac to the offspring via embryonic circulation (Linden & Roth, 1978; Tressler & Roth, 1987). The transfer starts from day 7 of embryonic development and reaches its maximum rate 3-4 days before hatch.

This second transfer step relies on the IgY Fc receptor, FcRY *(West et al., 2004);* the relevant receptor involved in IgY transport from the hen to the ovum is unknown. The FcRY binds IgY at pH≤6.5 and releases it at pH≥7, allowing a receptor-ligand association inside intracellular vesicles and the discharge in the blood of the chicks *(He & Bjorkmann, 2011)*.

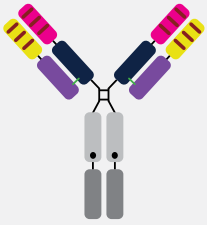
CHARACTERISTICS OF AVIAN IGY

Different possibilities exist for characterizing immunoglobulins. In addition to their structure and function, stability and safety are essential properties.

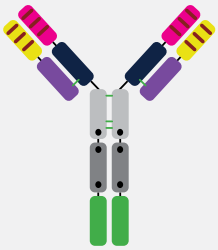
IgY's molecular structure and physical properties

Phylogenetic studies have shown that IgY is similar to mammalian IgG and IgE ***(Figure 5)***. Regarding its function, IgY is the equivalent of mammalian IgG, but their molecular structures show some profound differences.

Structure of mammalian IgG and IgE

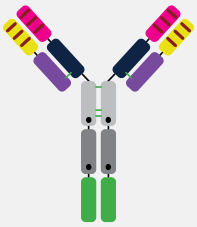


Mammalian IgG



Mammalian IgE

Structure of chickens IgY



Avian IgY

Figure 5 - Structural comparison between mammalian IgG, IgE, and avian IgY.
Adapted from Abbas et al., 2019 and Steinberg, 2021

The general structure of the IgY molecule is the same as that of the IgG molecule, with two heavy (H) chains and two light (L) chains. However, IgY has a molecular mass of 180 kDa and is larger than mammalian IgG (150 kDa).

The greater molecular mass of IgY compared to IgG is due to a higher molecular mass (67–70 kDa) of the H chain in IgY than the H chain in mammals (50 kDa) and an increased number of heavy-chain constant domains and carbohydrate chains ***(Warr et al., 1995)***. IgG has 3 C regions (CH1–CH3), while IgY has 4 C regions (CH1–CH4), and the presence of one additional C region with its two corresponding carbohydrate chains logically results in a greater molecular mass of IgY compared with IgG.

Other structural differences include the hinged region of IgY being much less flexible than that of mammalian IgG. Due to its different structure, it has also been suggested that IgY is a more hydrophobic molecule than IgG ***(Davalos-Pantoja et al., 2000).***

Physicochemical stability of IgY

IgY is proteinaceous and, therefore, sensitive to heat, pH, and pepsin ***(Figure 6)***, properties that pose real challenges when orally applied for gastrointestinal issues. Therefore, the effects of heat, atmospheric pressure, pH, pepsin, and gut passage on IgY stability were studied extensively.

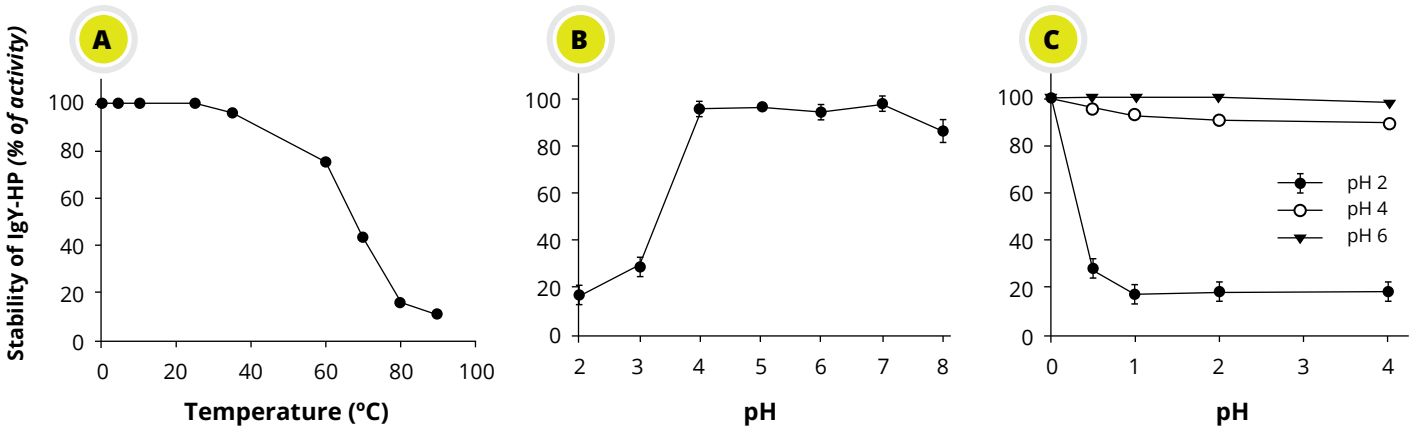


Figure 6 - Effect of heat, pH, and pepsin on the stability of IgY
A. *various temperatures for 10 min*
B. *various pHs for 4 h (B)*
C. *pepsin (15 µl/ml) at pH 2, 4, and 6 for 0.5, 1, 2, and 4 h*

In general, it can be said that the binding activity of IgY to an antigen decreases:

1. with increasing temperature and heating time. IgY is stable at temperatures between 30°C and 70°C. Heating for 15 min at 70°C or higher decreases its activity *(Shimizu et al., 1988*; *1992*), and IgY is significantly denatured when treated at temperatures higher than 80°C *(Chang et al., 1999)* or with pressures higher than 4,000 kg per cm^2 *(Shimizu et al., 1994).*

2. In the presence of pepsin. IgY was quite resistant to trypsin and chymotrypsin inactivation but was degraded by pepsin *(Hatta et al., 1993).* The stability of IgY against pepsin appeared to be highly dependent on pH and the enzyme/ substrate ratio. At pH 5 or higher, IgY resisted pepsin and retained its antigen-binding and cell-agglutinating activities. However, at pH 4.5 or below, both activities were lost. IgY digested with pepsin at pH 4 retained 91% and 63% of its activity after 1-hour and 4-hour incubation, respectively.

However, researchers have also conducted investigations showing the in vivo passage and efficacy of IgY in the gastrointestinal tract of piglets *(Yokoyama et al., 1993)* and calves *(Ikemori et al., 1996)*. Results indicated that IgY as powder was transported as immunologically functional molecules from the stomach down to the small intestine of calves while retaining much of its original biological activity ***(Figure 7).***

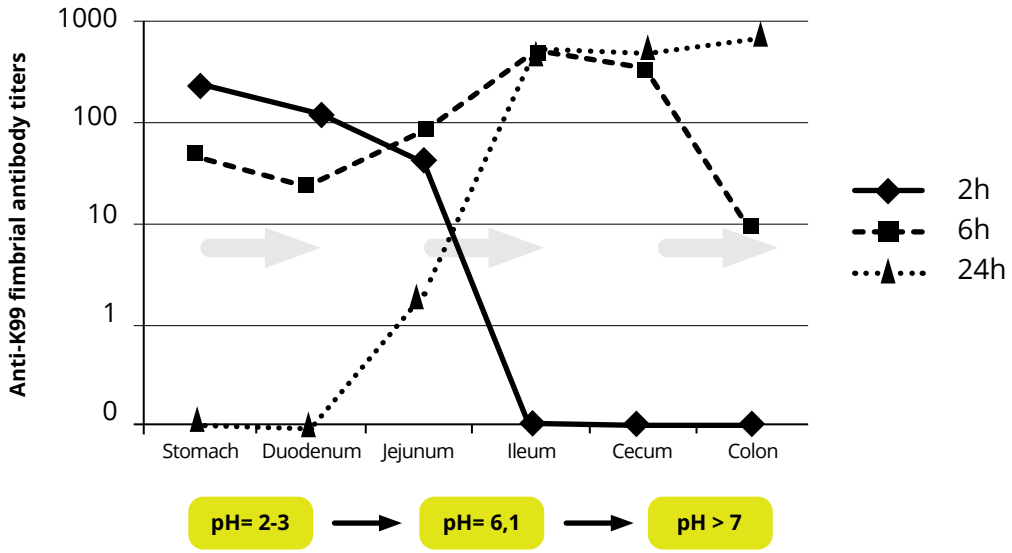


Figure 7 - In vivo passage of IgY in the gastrointestinal tract of pigs. Anti-K99 fimbriae antibody titers of IgY in the gastrointestinal tract of pigs after 2, 6, and 24 hours post-administration *(adapted from Yokoyama et al. 1993)*

Storage stability of IgY

IgY is naturally protected by the yolk granules. Under specified conditions, IgY's stability during storage is reasonably good. Dried IgY preparations should be kept in cool, dry, and dark places. They can be stored without significant loss of antibody activity for two years and longer at room temperature (15-25°C).

COMPARISON OF IGY AND IGG

The following differences between IgG and IgY mean a clear advantage for IgY regarding the usage of this technology in many areas of research, such as diagnostics *(Erhard et al., 2000)*, antibiotic-alternative therapy *(Carlander et al., 2000)*, and xenotransplantation *(Fryer et al., 1999).*

General characteristics

To be effective in medicine and research, IgY must show several essential characteristics. Among these are its possible interactions with the mammalian immune system and the producible amounts. Other characteristics are its stability in the organism or during storage, possible purifications, etc.

Table 3 summarizes the overall comparison of mammalian IgG and chicken IgY characteristics.

Table 3 - Comparison of selected characteristics of IgG and IgY

Parameters	IgG	IgY
Species	Mammals	Birds, reptiles, amphibians, and lungfish
Sites of generation	Lymph nodes, spleen, and bone marrow	B. of Fabricius, spleen, bone marrow (not birds)
Antibody subclasses	IgG_1, IgG_2, IgG_3 and IgG_4	IgY
Source of antibodies	Serum	Serum and egg
Antibody collection	Invasive, painful	Meets 3R principle of animal welfare (eggs)
Average antibody levels/animal	5 mg/mL of blood, blood collection up to 40 mL/month	50-100 mg/egg yolk
Monthly antibody yield/animal	200 mg/rabbit/month	1,400 - 2,800 mg/chicken/ month
Immune response to mammalian conserved antigens	Weak	Strong
Antibody avidity	High	3-4 times higher compared to IgG
Molecular weight (kDa)	150	180
pH stability	2.0-11.0	3.5-11.0

Higher efficacy of IgY

Concerning the use of IgY in immune therapy, prophylaxis, or diagnostics, the most relevant criterion is efficacy. IgY shows a higher efficacy than IgG because of:

1. Its customized production: IgY is tailor-made and is specific against gut/infected area pathogens (compared to nonspecific IgG). IgY can be produced against individual, specific pathogens.

2. The genetic selection theory states repeated hyperimmunization creates more potent antibody molecules. The antibodies become well-trained and equipped to protect the organism against imminent pathogens.

3. Its molecular structure: IgY is much bigger than IgG. A bigger size means more surface area, faster settlement, and a better approximation to the pathogen, which has been proven in in-vitro studies.

4. Its protease resistance: IgY is resistant to the pancreatic enzymes trypsin and chymotrypsin and is only sensitive to pepsin and papain. In comparison, IgG is degraded by all these enzymes.

5. Its maternal antibody transfer mechanism: IgY must be genetically strong. Milk-based IgG is provided daily to the baby for a longer time (a few days to years). Yolk-based IgY, however, is provided only once to the chick before hatching.

6. IgY enhances the uptake of IgG from colostrum. Calves fed colostrum containing egg yolk had higher TP, ALB, and IgG levels and increased GGT activity ***(Quezada-Tristan et al., 2014).***

7. Its lower "pickiness": concerning the species, hyper-immunized IgY is less "picky" and can be used in piglets, calves, sheep, and many more animals. In contrast, if, e.g., bovine colostrum is applied to piglets or other animals, it will be less effective because dairy cows commonly are not vaccinated against non-dairy-relevant pathogens. High-quality colostrum is an excellent source of IgG, but the quality of colostrum is very variable and depends on the pathogens with which the cow has been confronted.

8. Its higher affinity: compared to mammalian IgG, chicken IgY has a 3-5 times higher affinity and reacts more quickly to the same antigens, as demonstrated in competition assays by ***Stuart et al. (1988)***, ***Ikemori et al. (1993),*** and ***Lemamy et al. (1999).***

THE PRODUCTION OF IGY

The primary animal for IgY production is the avian species chicken. IgY production includes:
1) antigen of interest
2) immunization
3) immunization routes (nose, eye, breast muscle (best for chickens))
4) hyperimmunized egg collection
5) egg breaking
6) pasteurization of egg liquid
7) spray drying
8) IgY powder production

Choosing the best antigen

Immunization is controllable, but many parameters must be considered. The nature and dose of the antigen, the type of the used adjuvant, the route of administration, characteristics of the chicken (e.g., keeping conditions, age, breed, effect on egg laying capacity), and overall immunization schedule all influence the immunization result, which is the antibody and the titer.

Different types of antigens, such as nucleic acids, proteins, lipids, and carbohydrates, are used to produce IgY[1]. In addition, to elicit an immune humoral response, immunization is done with recombinant proteins[2] or peptides[3]. Both complex antigens (e.g., whole viruses, bacteria, and parasites[4]) and individual biomolecules (e.g., large proteins[5] or small peptides conjugated to a suitable carrier protein, such as keyhole limpet hemocyanin (KLH)[6]) have been used to stimulate the development of specific IgY in hens. The antigen dose may be critical since too much or too little antigen can lead to an undesirable immune response *(Schade et al., 2001)*.

Immunization

To produce specific IgY antibodies, hens are immunized with the target antigen. These antibodies, particularly the Fab domain of IgY, lack a hinge region, making them less flexible but able to bind to a wide range of antigenic epitopes, including proteins, carbohydrates, nucleic acids, and fimbriae. Even a small amount of antigen (in the milligram or microgram range) can trigger a sufficient IgY response, with antibody levels remaining high for several weeks to months.

[1] *Zhen et al., 2011*
[2] *Nasiri et al., 2016*
[3] *Hodek et al., 2015*
[4] *Grando et al., 2017; Amro et al., 2018; Lopes et al., 2019; de Faria et al., 2019; da Silva et al., 2020*
[5] *Skottrup et al., 2019; Lu et al., 2020*
[6] *Grzywa et al., 2014; Łupicka-Słowik et al., 2014*

The interaction between antigens and antibodies is considered non-covalent, similar to the "lock and key" fit of enzyme-substrate interactions, and does not permanently alter either the antigen (Ag) or the antibody (Ab) ***(Figure 9)***.

IgY products can have different qualities depending on the production conditions. Standardized products, mandatory for consistent results, contain defined titers of the individual antibody fractions, whereas non-standardized products can vary. To produce a specific antibody, the hens must be challenged by the individual, respective specific antigen.

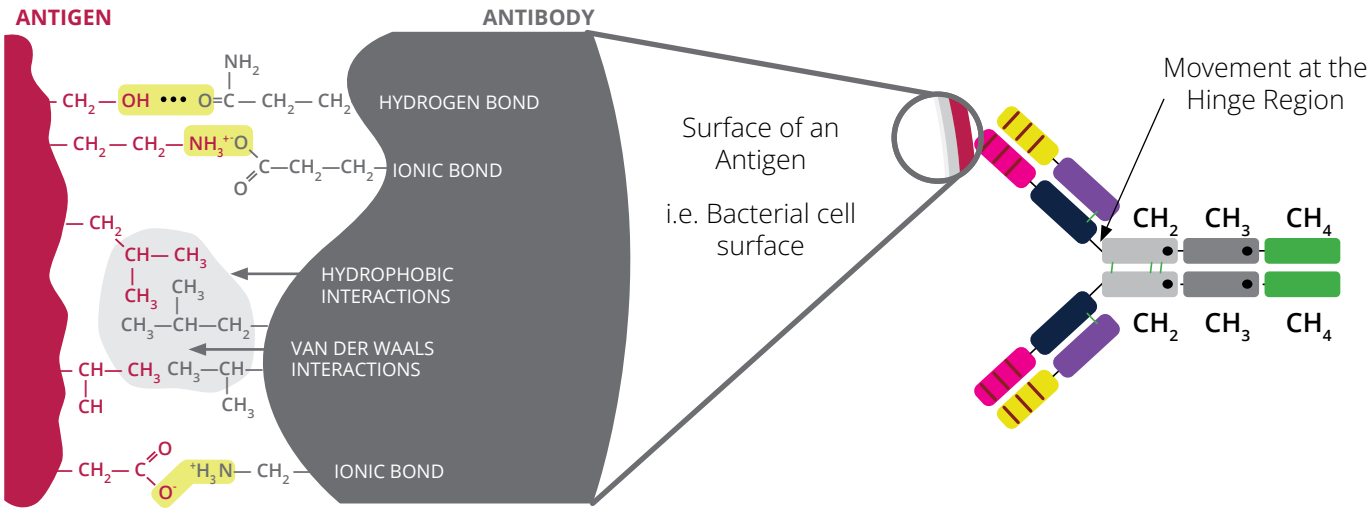


Figure 9 - The noncovalent interactions that form the basis of antigen-antibody (Ag-Ab) binding
(adapted from Goldsby et al., 2000)

Processing of hyperimmunized eggs into egg (IgY) products

After collecting and cleaning the eggs, they get broken, the eggshell removed, and either the whole egg is used for further processing or the egg white and yolk get separated. The whole egg, as well as the egg yolk and the egg white, are filtered and pasteurized.

Then, the fractions are directly packaged (for liquid products) or spray-dried (in the case of powders). Snapshots of different processing steps of hyperimmunized eggs into egg (IgY) products are shown in ***Figure 10.***

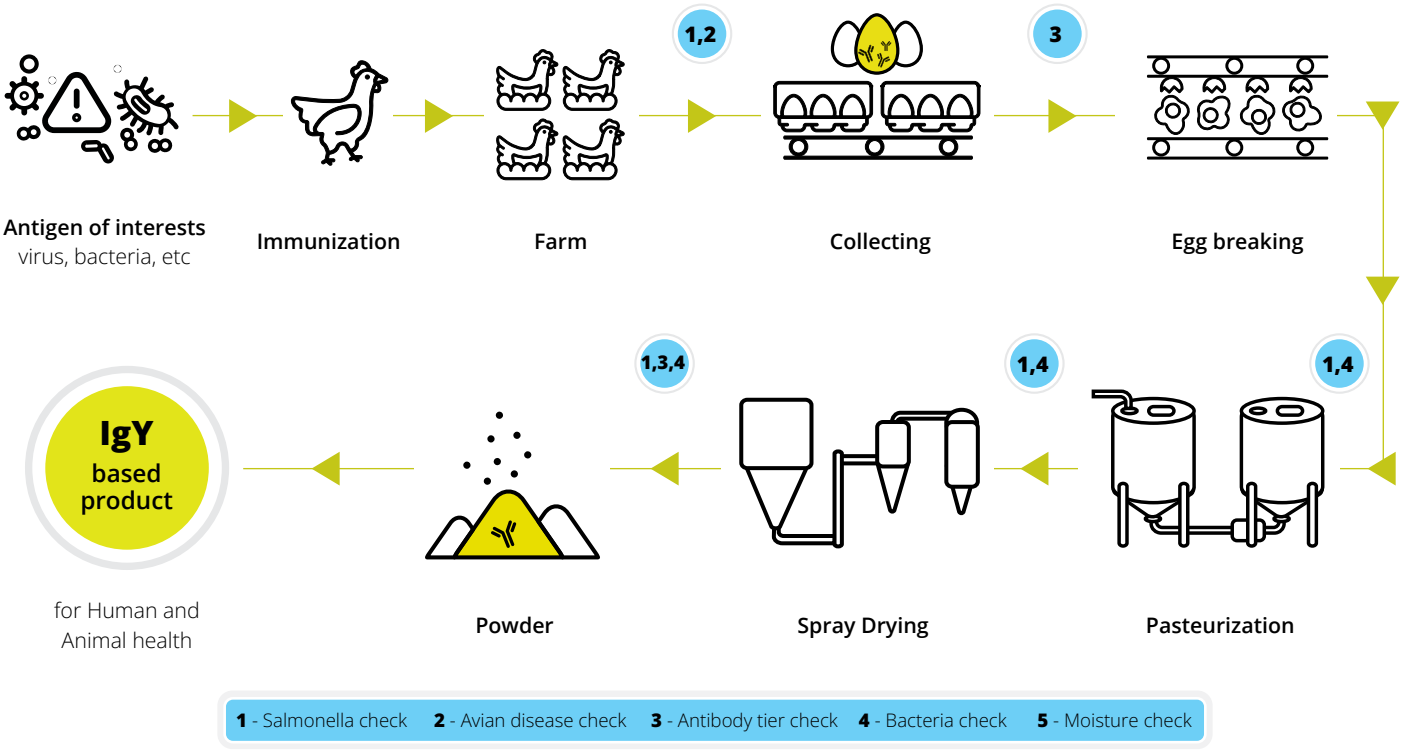


Figure 10 - Snapshots of processing steps of hyperimmunized eggs into IgY products and following quality control

IgY – Fields of application

Depending on the kind of antibodies (monoclonal or polyclonal, see Textbox), IgY can be used for different applications.

Binding only to one antigen epitope, monoclonal antibodies show high specificity. The fact that they are all equal (clone) means a high batch-to-batch reproducibility. Due to these characteristics, monoclonal antibodies are mainly used in precision-focused applications like diagnostic assays (ELISA, Western Blot) or targeted therapeutics (e.g., targeted cancer therapies).

Monoclonal antibodies:
- originate from one B-cell line (cell clone), tracing back to one B-lymphocyte
- are directed against one single epitope

Polyclonal antibodies:
- produced by several different B-lymphocytes
- bind to one antigen but to multiple epitopes

On the contrary, polyclonal antibodies originate from different B-lymphocytes, which bind to different epitopes of one antigen. Therefore, they show cross-reactions with similar pathogens having the same epitopes and a broader specificity. They can also be used in immunohistochemistry, immunofluorescence, Western Blot, and early diagnostics, as well as therapy such as reversing life-threatening digoxin or digitoxin toxicity and passive immunization of, e.g., young mammals.

In veterinary medicine and animal nutrition, egg immunoglobulins are used for passive immunization to protect animals, especially newborns, against pathogens. Depending on the construction of the placenta, immunoglobulins are transferred already in the womb or must be applied during the first days after birth.

Another field of application is reducing antibiotic use by preventing diseases with passive immunization or supplementing antibiotic therapies.

Use of IgY for passive immunization

Passive immunity, a method involving the transfer of ready-made antibodies from one organism to another to provide immediate protection, has a long-standing history. It was first used more than 100 years ago, as already mentioned, by ***Klemperer (1893)*** and ***Albert Calmette and his team in 1896***. This tradition of passive immunization using specific IgY antibodies has evolved and become especially valuable for controlling infections and conducting immunologic research.

Especially in young mammals, which, depending on the built-up of the placenta, are born without a functioning immune system, the transfer of maternal antibodies is essential. These antibodies help the youngest ones to overcome the first days of life when facing a new environment outside the womb with plenty of pathogenic germs. Usually, young animals get these antibodies via the colostrum from the mother. However, what can be done if the dam cannot provide colostrum (death, infection of the mammary gland…)? In this case, IgY produced against species-specific antigens can help.

Support for antibiotic reduction programs

The advantages of using chicken IgY have been recognized by many authors (e.g., ***Karlsson et al., 2004***; ***Schade et al., 2005***; ***Thirumalai et al., 2019)***. Since antibiotics are commonly used or misused for treating gastrointestinal or respiratory infections, the frequency of antibiotic-resistant organisms has increased at an alarming rate against a backdrop of decreasing numbers of new antibiotics being developed and added to the market. Therefore, we must resort to simple yet effective natural remedies, of which IgY seems to be one of the most potent and easily generated alternatives/complements for antibiotics. In a trial conducted by ***Shimizu et al. (1993),*** IgY showed a better effect than the commonly used antibiotic. So, together with other developments in recent antimicrobials and chemotherapeutic research, IgY has the potential to play a contributory role in delaying the advent of the dreaded post-antibiotic era.

USE OF IGY IN PET HEALTH

The administration of chicken egg yolk antibodies (IgY) is a promising nutritional strategy to control pathogen infections in animals and is sometimes used as an alternative to antibiotics for the treatment and control of diseases. This chapter discusses the use of oral IgY to control and prevent pet diseases, specifically focusing on globally occurring diseases for which the antibodies have been scientifically documented. Sometimes, the antibodies have not been explicitly tested in cats and dogs. However, if the antibodies are the same or the epitopes IgY was created against, they should work as well.

Use of IgY to improve oral health

Dental disease is one of the most common medical conditions seen by veterinarians in dogs and cats, possibly seriously debilitating the animals. Due to pain, the animal eats less and loses weight, resulting in a shortened life span. Prophylactic use of IgY supports healthy teeth and gums, enhancing the well-being of the companion animal. Since animals cannot communicate verbally, dental diseases often go unnoticed for months, or even years, until the discomfort results in visible signs, for example, decreased appetite and weight loss, and, in the end, a shortened lifespan.

Canine and Feline Periodontitis

Characteristics: The accumulation of dental plaque and tartar on the teeth affects teeth-surrounding structures. If left untreated for a longer time, the disease can lead to periodontal ligament and bone destruction, tooth loss, and severe health issues.

Pathogen/s: *Porphyromonas gulae, Porphyromonas gingivalis,* and *Prevotella spp* are among the key causal microorganisms initiating plaque formation. They secrete enzymes, gingipain or interpain A, which, among other functions, degrade cytokines and down-regulate the immune response by reducing inflammation *(Arastu-Kapur, 2020*; *Potempa, 2009).*

IgY: Trials showed that IgY-GP inhibits the enzyme activity, growth, and adherence of Porphyromonas gingivalis to gingival epithelial cells, preventing plaque accumulation and the cascade to clinical dental disease *(Shofiqur et al., 2011*; *Oba et al., 2018)*.

Use of IgY with intestinal dog diseases

Gastrointestinal diseases affecting our companion animals may be mild and self-resolve with limited intervention or severe, requiring treatment and possibly intensive care and hospitalization. Immunoglobulins from the egg (IgY) can bind pathogens in the gastrointestinal tract, preventing their attachment and invasion of the enterocytes.

Viral infections

Canine Parvovirus

Characteristics: Canine parvovirus infection is a potentially fatal disease in dogs. The virus targets rapidly dividing cells, thereby affecting the intestinal villi crypts and bone marrow, causing severe hemorrhagic gastroenteritis and immune suppression. In very young dogs, the heart muscle may also be damaged.

Pathogen/s: *Canine parvovirus type 2 (CPV-2),* a highly contagious DNA virus that, once shed into the environment by infected animals, can survive and remain infectious for years.

IgY: Trials show good results and the suitability of IgY to be used for therapy against canine parvovirus *(Nguyen et al., 2006)*.

Rotavirus

Characteristics: Rotavirus targets enterocytes on the tips of small intestinal villi. Infected cells die and slough into the intestinal lumen, resulting in mild or moderate villous atrophy. Symptoms include watery to mucoid diarrhea for 8-10 days, vomiting, nausea, and loss of appetite.

Pathogen/s: *Canine Rotavirus (CRV),* a double-stranded RNA, nonenveloped virus; fecal-oral transmission.

IgY: Specific IgY was already tested in cats *(Hiraga et al., 1990)*, in calves *(Kuroki et al., 1994; Kuroki et al., 1997; Vega et al., 2011*; *Vega et al., 2015)*, and in mice *(Kuroki et al., 1993)*.

Canine Morbillivirus

Characteristics: This highly contagious immunosuppressive disease affects dogs. Early symptoms resemble those of a cold and progress to bronchitis, catarrhal pneumonia, and gastroenteritis. In the later stages, neurological symptoms such as convulsions may occur. Some cases also present with severe keratosis of the nose and hardening of the footpads, known as "hard pad disease."

Due to the broad range of clinical symptoms, laboratory tests are necessary to confirm the disease. Various biological samples are used to detect the virus, primarily through PCR but also via immunochromatography, immunofluorescence, and ELISA *(Costa et al., 2019)*. Currently, there is no specific drug for treatment. Vaccinations are used, but the virus has geographical genetic variations *(Pratelli, 2011).*

Pathogen/s: *Canine morbillivirus* (formerly termed *Canine distemper virus*, CDV), belonging to the family of Paramyxoviridae and the genus Morbillivirus. They cause moderate to severe immunosuppressive, respiratory, gastrointestinal, and neurological diseases in a variety of hosts, from humans (measles) to canines, and present an exciting model to research inter-species jumping *(Quintero-Gil et al., 2019)*

IgY: Antibody-based therapy could be an efficient intervention. Specific IgY antibodies have been generated by immunizing laying hens with CDV *(Guimarães et al., 2009).*

Dengue fever

Characteristics: Small reddish dots or small wound eruptions on the skin, blood in the nostrils, eye secretions, no appetite, and an animal that appears weak; pale gums and ears when you check inside — all these symptoms, indicating a low platelet count.

Pathogen: Dengue virus (DENV) transmitted by Aedes mosquitoes

IgY: Anti-DENV2 IgY produced in goose neutralized the virus in vitro and in vivo in mice without binding to Fcγ receptors on myeloid cells and generating ADE (antibody-dependent enhancement *(Fink et al., 2017).*

Bacterial infections

E. Coli Infections

Characteristics: In dogs with compromised immune systems, such as puppies, an *E. coli* infection can cause severe diarrhea, repeated vomiting, loss of appetite, collapse or weakness, and difficulty breathing. In these cases, the infection can quickly progress to sepsis.

In adult dogs, *E. coli* is suspected to migrate from the gut to the urinary tract, leading to infections (UTIs). *E. coli* is responsible for 50-60% of UTIs *(EFSA, 2022).* Symptoms include excessive drinking, frequent urination, painful urination, blood in the urine, straining to urinate, and foul-smelling urine.

Additionally, *E. coli* can lead to the development of canine pyometra (accumulation of purulent exudate in the uterine lumen, the most prevalent reproductive disease in canines) *(Greiner et al., 2008*; *Hagman, 2018)* or prostatic abscesses. Symptoms of these conditions include lethargy, depression, anorexia, abdominal discomfort, straining to urinate or defecate, fever, discharge from the penis, vomiting, weakness, and collapse.

Pathogen/s: *E. coli spp*

IgY: IgY against several *E. coli* have been tested, e.g., in piglets *(Wang et al., 2019*; *Yokoyama et al., 1992)*, in weaned pigs *(Zúñiga et al., 1997*; *Yokoyama et al., 1997)*, and in calves *(Ikemori et al., 1992)*.

Salmonellosis

Characteristics: Dogs are relatively resistant to Salmonellosis but often develop a subclinical form. In this case, they are infectious to other animals and humans as they shed small amounts of bacteria (10^{10}/g of feces) into the environment for about six weeks or longer. The clinical form occurs mainly in young, immune-deficient, or older animals. It is characterized by fever, vomiting, diarrhea, and anorexia.

Issue: Salmonella can be transmitted to humans. Salmonella is a notifiable disease.

Pathogen/s: *Salmonella Typhimurium, S. Newport, S. Livingstone,* and *S. Infantis predominant*.

IgY: Anti-*Salmonella Typhimurium* IgY has already been developed by *Lee et al., 2002, Li et al., 2016* and *Yokoyama et al., 1998. Bustos et al. (2021)* produced specific IgY against *S. Newport*. The application of the egg immunoglobulins either attenuated the proliferation of the pathogens, reduced their adhesion to the intestinal mucosa, or attenuated immune reactions.

Clostridiosis

Characteristics: Clostridia infection may affect the small or large intestines with mild, self-limiting disease or acute and severe hemorrhagic gastroenteritis.

Pathogen/s: *Clostridium spp*

IgY: IgY was successfully tested in mice (anti-*Clostridium difficile* spores - *Pizzarro-Guajardo, 2017),* in chicken (anti-NE alpha-toxin - *Khalf et al., 2016*; against four different *C. perfringens* recombinants (α-toxin, NE B-like toxin (NetB; EB), elongation factor-Tu (ET), and pyruvate:ferredoxin oxidoreductase - *Goo et al., 2023)*, and in humans *(Mulvey and co-workers, 2011)*.

Campylobacteriosis

Characteristics: Watery to mucoid diarrhea, straining, abdominal cramping or pain, lethargy, and fever. Diarrhea probably lasts a week or more and often relapses suddenly after the dog appears to have recovered.

One main problem is that the disease can be transmitted to humans.

Pathogen/s: *Campylobacter jejuni, Campylobacter helveticus,* and *Campylobacter upsaliensis*

IgY: Anti-C. jejuni IgY resulted in decreased cecal counts of *C. jejuni* in broilers ***(Hermans et al., 2014).***

Mycoses

Candidiasis

Characteristics: Clinical symptoms depend on the localization of the infection. Besides the skin, candidiasis can occur in the gastrointestinal tract and be transferred to other mucous membranes such as the oral cavity, pharynx, esophagus, vagina and/or prepuce, and penis (balanoposthitis). If the gastrointestinal tract is attacked, the animals suffer from chronic diarrhea. If mucous membranes are attacked, white-grey plaques are surrounded by a reddish, hyperemic border. Erosions or ulcerations may also occur in these areas. Infections of the urinary tract may be characterized by dysuria and/or hematuria.

In rare cases, the pathogens can spread systemically and lead to the formation of microabscesses. The clinical picture of systemic candidiasis varies greatly and ranges from unspecific disorders of the general condition to symptoms of failure of affected organs.

Pathogen/s: *Candida spp,* including *Candida albicans*

IgY: In vitro and in vivo effectiveness of egg yolk antibody against *Candida albicans* (anti-CA IgY) was tested by ***Ibrahim et al., 2008*** in mice where Anti-CA IgY significantly reduced the number of C. albicans and the scores of tongue lesions. Moreover, anti-CA IgY reduced the colonization of *C. albicans* in the animals' organs, indicating that anti-CA IgY protects against the oral candidiasis of experimentally infected mice and reduces the dissemination of *C. albicans.* ***Takeuchi et al. (2014)*** reduced the number of CFUs in the oral cavities of older people and identified this preparation as applicable for prophylactic use. ***Kamikawa et al. (2016)*** inhibited the adhesion of *Candida albicans* and *Candida glabrata* to denture base material with specific IgY.

Parasites

Giardiasis

Characteristics: Symptomatic and asymptomatic course of disease possible. The parasites attach themselves to the intestinal wall, and the damage leads to the symptoms of diarrhea (from soft to watery, often with a greenish tinge, occasionally containing blood) with and without vomiting.

Pathogen: *Giardia duodenalis* (other names: *G. intestinalis* or *G. lamblia*), facultative pathogen gut parasite; 8 different genotypes (A-H) in dogs, mainly C and D

IgY: Anti-Giardia lamblia IgY was tested in mice orally infected with *G. lamblia* trophozoites. The test group showed improved body weight and a significantly decreased output of cysts ***Selim et al., 2016.***

Coccidiosis

Characteristics: Intestinal tract infection: Coccidia reproduce inside intestinal cells, resulting in cell death and, with a high challenge, clinical disease presenting with diarrhea. In puppies, older dogs, and immunocompromised dogs, coccidiosis may lead to severe watery diarrhea, dehydration, abdominal distress, vomiting, and, in severe cases, death.

Pathogen/s: *Cystoisospora spp.*

IgY: IgY was produced against three sexual stage-specific proteins of Isospora suis and tested in vitro. These antibodies can help to interrupt the parasite's development and transmission to susceptible hosts *(Feix et al., 2022)*. In vivo, IgY was tested in chicken (anti-*Eimeria tenella*—*Juárez-Estrada, 2021*; *Xu et al., 2013)*, and the vaccination of breeder hens against Eimeria maxima protected the progeny *(Wallach et al., 1995)*.

Cryptosporidiosis

Characteristics: The main clinical signs are diarrhea and vomiting, weight loss, dehydration, and stomach cramps. In adult and healthy dogs, cryptosporidia in the feces is not a cause for concern. However, in puppies and immunosuppressed dogs, it can be life-threatening.

Pathogen/s: *Cryptosporidium canis, Cryptosporidium parvum* (can also cause disease in humans)

IgY: IgY against the P23 protein in *C. parvum* showed high specificity for the parasite and reduced oocyst shedding by 70% in a mouse model *(Omidian et al., 2014).*

Use of IgY to fight cat diseases

Viral infections

Rotavirus

Characteristics: Mild to severe diarrhea, vomiting, dehydration, abdominal cramps, and fever.

Pathogen/s: *Rotavirus*

IgY: Neutralization of the infectivity of the pathogenic virion by blocking its entry to host enterocytes, preventing initial infection, and suppression or minimization of intestinal colonization or cell-to-cell spread of infection resulting in down-modulated clinical symptoms in rotavirus-induced enteritis.

IgY was, besides others, successfully tested in cats *(Hiraga et al., 1990)*, in calves *(Kuroki et al., 1994; Kuroki et al., 1997; Vega et al., 2011*; *Vega et al., 2015)*, and in mice *(Kuroki et al., 1993)*.

FIV (cat HIV / cat AIDS)

Characteristics: FIV compromises the immune system by killing or damaging immune cells, making cats vulnerable to various infections, including those of the digestive tract. This can lead to symptoms such as loss of appetite, inflammation of the gums and mouth, and diarrhea. Despite this, infected cats can appear normal for many years.

Pathogen/s: Feline immunodeficiency virus (FIV)

IgY: Currently, no specific IgY is available. However, *Supeanu et al. (2016)* tested the application of non-specific IgY in cats suffering from FIV and got promising results. Weight gain and appetite, the number of white line immune cells, anemia, and social behavior improved, and the product did not show any adverse effects.

Bacterial infections

E. Coli Infections

Characteristics: *E. coli* can compromise the intestinal tract and spread to the urogenital tract. The symptoms depend on the site of infection:

- Intestinal tract: watery diarrhea with or without blood, vomiting, decreased or total loss of appetite, and depression.
- Urinary tract: increased frequency of urination with urine loss outside the litterbox, vocalization during urination, malodorous urine partly with blood, increased thirst, painful or distended abdomen, fever, decreased or complete loss of appetite.
- Pyometra: Vaginal discharge, distended or painful abdomen, increased thirst and/or urination (similar to urinary tract infection), lethargy, depression, decreased or complete loss of appetite

Pathogen/s: *E. coli spp*

IgY: IgY against several *E. coli* have been tested, e.g., in piglets ***(Wang et al., 2019***; ***Yokoyama et al., 1992)***, in weaned pigs ***(Zúñiga et al., 1997***; ***Yokoyama et al., 1997)***, and in calves ***(Ikemori et al., 1992)***.

Salmonellosis

Characteristics: Salmonellosis can manifest as different infections:

Salmonellosis can cause an acute or chronic intestinal infection or inflammation (enteritis) or a more severe infection such as septicemia (bacterial blood infection). Symptoms of acute salmonellosis in cats may include all symptoms of a gastroenteric disease (diarrhea, vomiting, mucus in the stool, abdominal pain, distended abdomen, fever, weight loss, dehydration…), conjunctivitis, swollen lymph nodes, abortion or infertility, vaginal discharge, higher heart rate, shock. In the most severe case of septicemia, the cats can show fever or hypothermia, dehydration, rapid or trouble breathing, low blood pressure, jaundice, vomiting, drooling, and pale gums.

Chronic salmonellosis can manifest in fever, weight loss, blood loss, bloody stool, other infections unrelated to the digestive system, or intermittent diarrhea lasting weeks or months. However, cats commonly show no symptoms, appear healthy, and function as a carrier.

Salmonellosis is a zoonotic disease that can be passed from cats to humans and vice versa. This fact makes salmonella a notifiable disease

Pathogen/s: *Salmonella spp*.

IgY: *Salmonella Typhimurium*-specific IgY has already been developed and tested in mice ***Li et al., 2016*** ***Yokoyama et al., 1998.***

Campylobacteriosis

Characteristics: The characteristic symptom is diarrhea. In severe cases, diarrhea is accompanied by vomiting, fever, or bloody stool. One main problem is that the disease can be transmitted to humans.

Pathogen/s: *Campylobacter jejuni, Campylobacter helveticus,* and *Campylobacter upsaliensis*

IgY: Anti-C. jejuni IgY resulted in decreased cecal counts of *C. jejuni* in broilers ***(Hermans et al., 2014).***

Mycoses

Candidiasis

Characteristics: Inflammation of the digestive system.

Pathogen/s: *Candida albicans*

IgY: In vitro and in vivo effectiveness of egg yolk antibody against *Candida albicans* (anti-CA IgY) was tested by ***Ibrahim et al., 2008*** in mice where Anti-CA IgY significantly reduced the number of *C. albicans* and the scores of tongue lesions. Moreover, anti-CA IgY reduced the colonization of *C. albicans* in the animals' organs, indicating that anti-CA IgY protects against the oral candidiasis of experimentally infected mice and reduces the dissemination of *C. albicans*. ***Takeuchi et al. (2014)*** reduced the number of CFUs in the oral cavities of older people and identified this preparation as applicable for prophylactic use. ***Kamikawa et al. (2016)*** inhibited the adhesion of *Candida albicans* and *Candida glabrata* to denture base material with specific IgY.

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Pathogen: *Giardia duodenalis* (other names: *G. intestinalis* or *G. lamblia*), facultative pathogen gut parasite; 8 different genotypes (A-H); in cats, mainly F and A

IgY: Anti-Giardia lamblia IgY has already been produced by ***Selim et al. (2016).*** They tested their IgY in mice orally infected with *G. lamblia* trophozoites. The test group showed improved body weight and a significantly decreased output of cysts.

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