Immunology Research Institute Gifu (IRIG)

IRIG

HANDBOOK ON

IMMUNOLOGY-BASED INNOVATIONS FOR LIVESTOCK ANIMALS

Reference for nutritional solutions to clinical challenges

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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 has been a continuous focus of our efforts for more than 30 years. In 1992, we published research papers about the efficacy of IgY against E. coli infection in piglets and calves. Our continuous research efforts have led to the global marketing of IgY-based products supporting young livestock animals in critical periods, such as the first days after birth or weaning.

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 young animals' support in critical phases has been a **continuous focus"**

IMMUNITY: THE FOUNDATION FOR SUSTAINABLE LIVESTOCK PRODUCTION

Immunity is a key topic of animal health and a critical driver for the sustainability of livestock production systems. As the demand for effective and sustainable solutions continues to grow, the use of egg yolk immunoglobulins (IgY) has emerged as an innovative and promising tool for the livestock industry.

This book explores the applications of IgY in production animals, particularly addressing significant challenges such as minimizing the impact of infectious diseases on productivity. With a global emphasis on reducing antibiotic usage to combat antimicrobial resistance, IgY offers an effective and sustainable alternative that aligns with this goal.

IgY: A Field-Ready Solution

IgY has proven highly effective in preventing and mitigating gastrointestinal infections in livestock. Its high specificity against common pathogens in swine and ruminants, combined with its ability to improve gut health without adverse effects or environmental harm, makes it an innovative solution. Additionally, the cost-effective production of IgY from egg yolks ensures accessibility for farms of all sizes. The book highlights diverse applications of IgY, such as managing post-weaning diarrhea in piglets and improving health strategies in ruminants. By serving as a passive immunotherapy, IgY complements vaccination programs and biosecurity measures, enabling a sustainable transition in livestock systems with reduced reliance on traditional antimicrobials.

A Collaborative Effort for Sustainable Progress

This work represents a collaborative effort to integrate recent scientific findings with practical experiences. It is a valuable resource for veterinarians, researchers, engineers, and agricultural professionals. Beyond presenting data and evidence, the book aims to inspire further research and encourage dialogue about the pivotal role of IgY in the future of animal health and sustainable livestock production.

On behalf of the authors and contributors, we express our heartfelt gratitude to everyone involved in this project. We hope this book serves as both a practical guide and a source of inspiration for those committed to advancing livestock health and welfare sustainably.

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IgY:

produced by birds, functional in mammals

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Immuno**g**lobulins of the egg **y**olk 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.

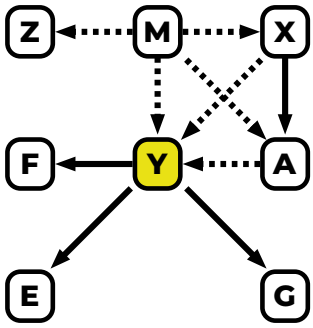


Figure 1 - The central position of IgY in immunoglobulin evolution

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).***

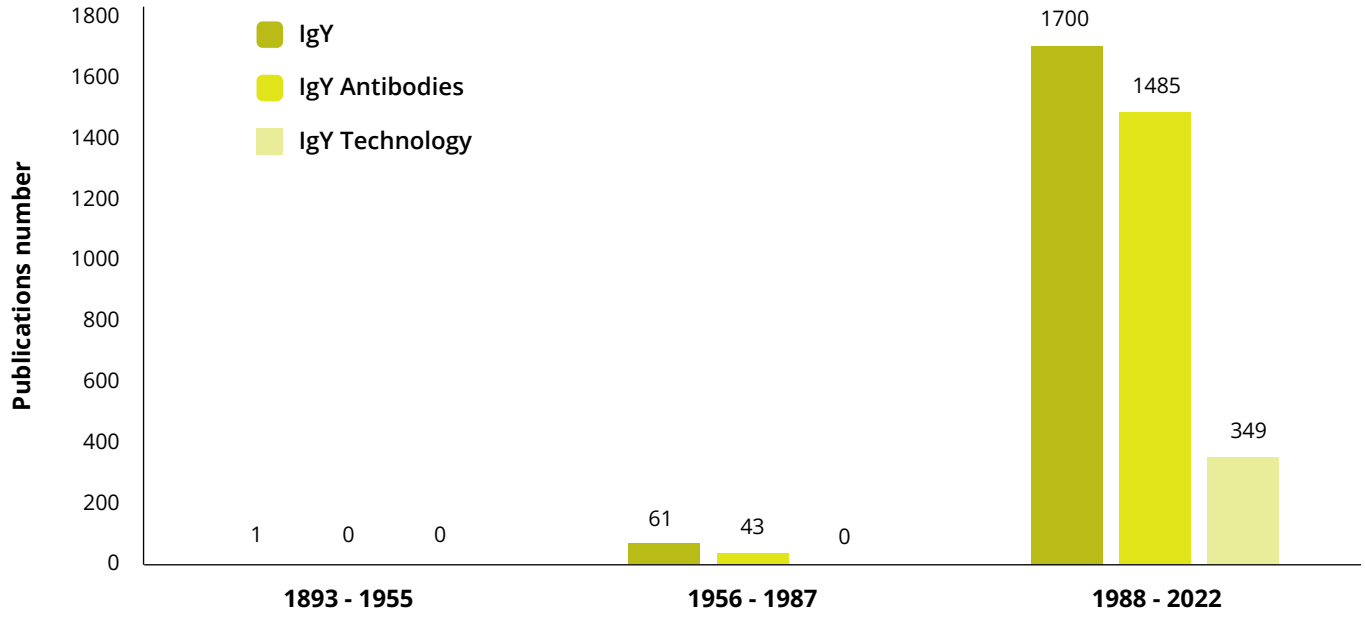


Figure 2 - Evolution of publications on avian IgY Antibodies

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).***

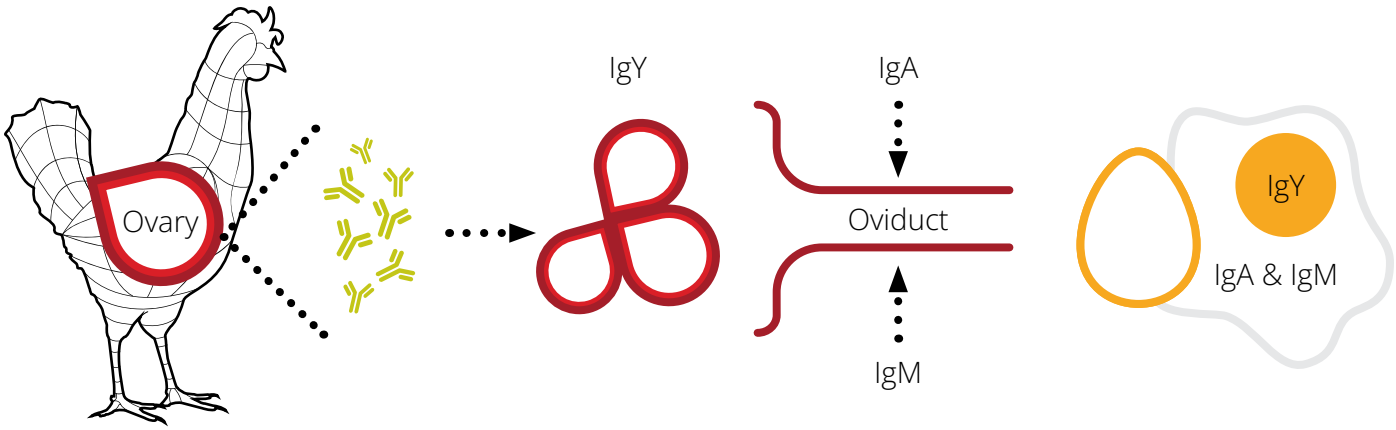


Figure 4 - Maternal antibody (MA) transfer from the hen to the egg

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)*.

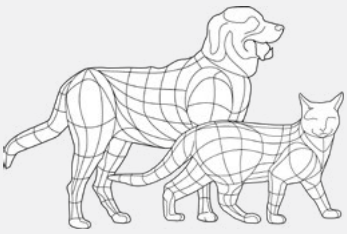
CHARACTERISTICS OF AVIAN IGY

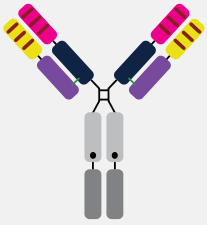
Different possibilities exist for characterizing immunoglobulins. In addition to their structure and function, stability and safety are essential properties.

IgY's molecular structure and physical properties

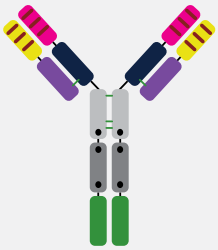
Phylogenetic studies have shown that IgY is similar to mammalian IgG and IgE ***(Figure 5)***. Regarding its function, IgY is the equivalent of mammalian IgG, but their molecular structures show some profound differences.

Structure of mammalian IgG and IgE



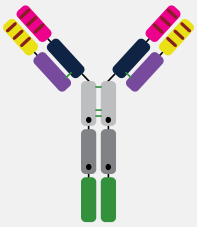


Mammalian IgG



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.

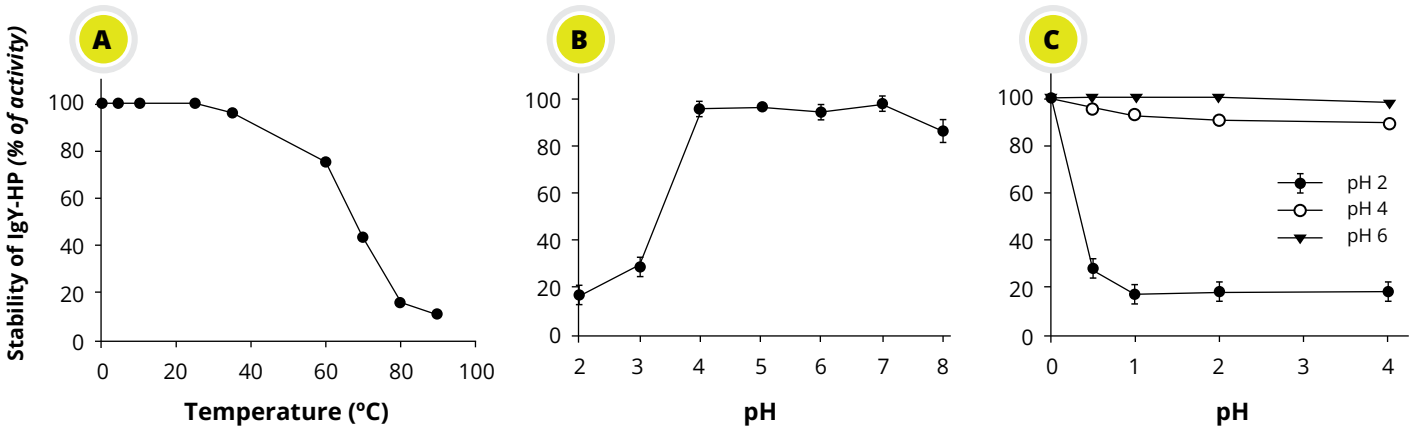


Figure 6 - Effect of heat, pH, and pepsin on the stability of IgY
A. *various temperatures for 10 min*
B. *various pHs for 4 h (B)*
C. *pepsin (15 µl/ml) at pH 2, 4, and 6 for 0.5, 1, 2, and 4 h*

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 pigs while retaining much of its original biological activity ***(Figure 7).***

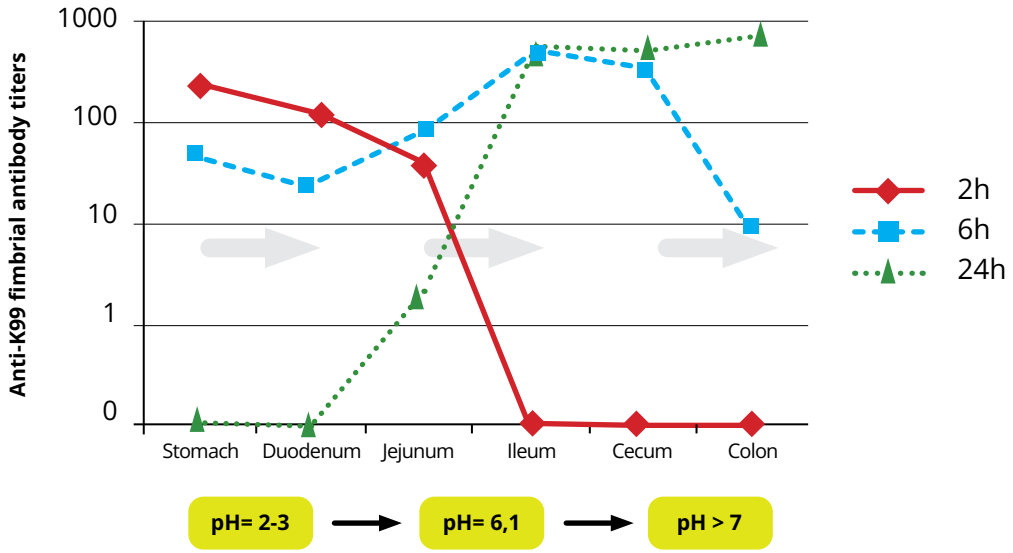


Figure 7 - In vivo passage of IgY in the gastrointestinal tract of pigs. Anti-K99 fimbriae antibody titers of IgY in the gastrointestinal tract of pigs after 2, 6, and 24 hours post-administration *(adapted from Yokoyama et al. 1993)*

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.

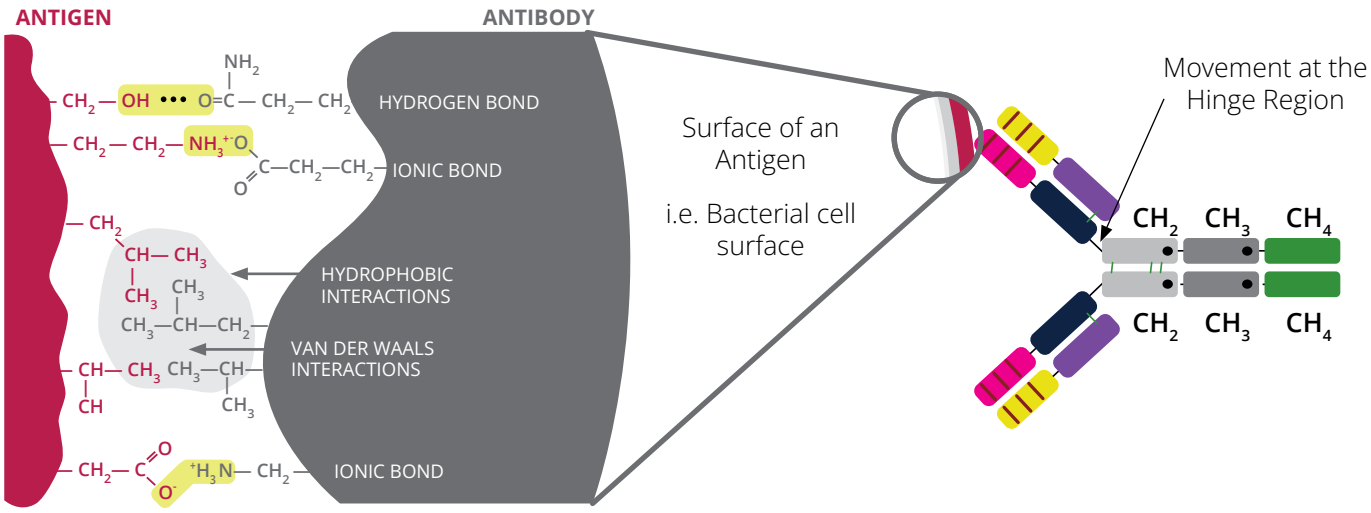


Figure 9 - The noncovalent interactions that form the basis of antigen-antibody (Ag-Ab) binding
(adapted from Goldsby et al., 2000)

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.***

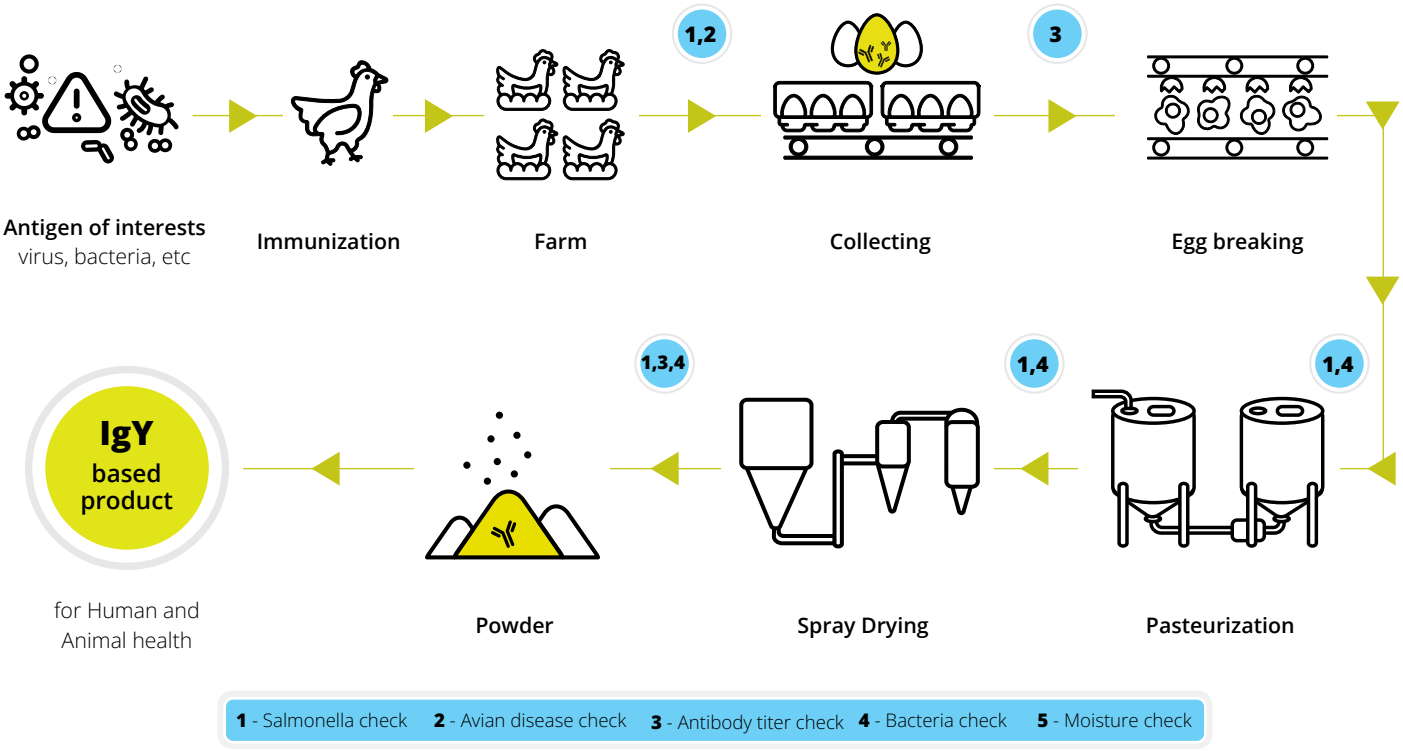


Figure 10 - Snapshots of processing steps of hyperimmunized eggs into IgY products and following quality control

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 LIVESTOCK

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 IgY to control and prevent diseases in production animals, specifically focusing on globally occurring diseases for which the antibodies have been scientifically documented. Sometimes, the antibodies have not been explicitly tested on farm animals. However, if the antibodies are the same or the epitopes IgY was created against, they should also work.

Use of IgY in Pigs and Piglets

Viral infections

Rotaviral Enteritis

Characteristics: Rotaviruses mainly affect nursing and post-weaned piglets. The transmission is fecal-oral. In acute outbreaks, the incubation time is 18 h – 3 days. Without complications, the disease shows high morbidity and low mortality.

After being swallowed, the virus targets intestinal epithelial cells on the tips of the small intestinal villi and damages or destroys them. The destruction of villous epithelium and a villous fusion leads to impaired intestinal function and reduced disaccharidase activity. The accumulation of disaccharides in the intestine results in an osmotic movement of fluid into the intestinal lumen, along with malabsorption and diarrhea. It is often accompanied by *E. coli* or *C. perfringens* coinfections, drastically altering the disease outcome.

Pathogen/s: *Rotavirus Type A, B, C, E, or H* (***Iowa State University***), with A being the most common in suckling and weaning piglets (***Ferrari et al., 2022***). Rotaviruses are double-stranded RNA, non-enveloped viruses of the Reoviridae family.

IgY: Specific IgY was already tested in a neonatal gnotobiotic piglet disease model (***Vega et al., 2012***), in calves (***Kuroki et al., 1994***; K***uroki et al., 1997***; ***Vega et al., 2011***; ***Vega et al., 2015***), in cats (***Hiraga et al., 1990***), and in mice (***Kuroki et al., 1993***).

Transmissible Gastroenteritis (TGE)

Characteristics: TGE is an acute, rapidly spreading disease in pigs of all ages. Mortalities are about 100% in piglets younger than 2 weeks, with mortality decreasing with age. Pigs older than 4 weeks often survive.

The disease is characterized by diarrhea, vomiting, rapid dehydration, shivering, and marked thirst. The pigs weaken rapidly and usually die within 1 to 2 days. An endemic form of TGE occurs in herds with partial immunity or concurrent porcine respiratory coronavirus (PRCV) infection, resulting in milder symptoms and significantly reduced mortality.

Pathogen/s: TGE virus (TGEV), a member of the Coronaviridae.

IgY: Specific IgY was already tested in piglets (***Zuo et al., 2008***; ***Nguyen, 2009***) and showed increasing survival rates.

Porcine Epidemic Diarrhea (PED)

Characteristics: PED is an infectious intestinal disease that affects piglets more severely than adult pigs due to a lack of specific antibodies. Morbidity is about 100% in all ages, with mortalities of 50% in piglets within 2 weeks of birth and 1-3% in aged animals. Transmission occurs via different ways (aerosol, fecal-oral, fecal-nasal, breast milk, sexual intercourse, contaminated vehicles/equipment, farm workers).

In general, the animals show symptoms such as vomiting, liquid diarrhea, dehydration, anorexia, and weight loss. Newborn piglets suffer from deadly watery diarrhea.

Pathogen/s: Porcine Epidemic Diarrhea Virus (PEDV), a member of the Coronaviridae.

IgY: Specific IgY was tested in piglets (*Kweon et al., 2000*; *Nguyen, 2009*), showing reduced mortality.

Porcine Circovirus-Associated Disease (PCVAD or PCVD)

Characteristics: Transmission of the responsible viruses can be horizontal (oronasal - aerosols, ejaculate) or vertical (transplacental). Latently and acute-infected pigs shed the pathogens in high amounts via all body fluids and excrements. PCVAD comprises several diseases:

1. Post-weaning multisystemic wasting syndrome (PMWS) occurs mainly in farms with strong hygienic deficiencies. The piglets and grower/finisher pigs become stunted, the lymph nodes are enlarged, and the animals develop respiratory symptoms, including sneezing, dyspnea, rhinitis, or conjunctivitis with swollen eyelids. They also suffer from diarrhea and anemia and show icteric skin and rough coats (*Messner and Fink, 2024*).

2. Porcine dermatitis nephropathy syndrome (PDNS) mainly affects grower/finisher pigs. It is characterized by skin lesions (round or irregular, red-violet flecks (maculae), papules, and plaques that can propagate over the whole body (ecchymoses). Due to dirt and dust, they look grey to black. Besides the skin alterations, the animals show anorexia, fever, loss of weight, dyspnea, and subcutaneous edema (*The Pig Site, 2018*).

3. Porcine respiratory disease complex (PRDC) mainly occurs in finishers of 15 to 20 weeks of age. The disease results from the interplay of PCV with other viruses (Influenza-A virus, PRRS virus) or bacteria (e.g., Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, Haemophilus parasuis). The viral primary infection causes damage to the lung tissue and weakens the animal. Immunosuppression enables secondary infections. Clinical signs depend on the pathogens involved. They usually comprise fever, apathy, pain reactions (arched back), inappetence and resulting weight loss, dyspnea with cough, pneumonia, myocarditis, and/or pericarditis. Other clinical pictures can also occur (e.g., pleuropneumonia if Actinobacillus pleuropneumoniae are involved or meningitis with streptococcus suis).

4. Reproductive failure results from transplacental infections, mainly in start-up herds with a high proportion of unvaccinated or PCV2-susceptible gilts. Clinical signs are delayed farrowing, mummified fetuses, and stillborn piglets.

5. acute pulmonary edema

6. porcine dermatitis

7. granulomatous enteritis (*Cino-Ozuna et al., 2011*; *Segalés et al., 2005*; *Chae, 2005*).

Pathogens: Porcine circovirus Type 2 (PCV2), belonging to the family of Circoviridae. As a non-enveloped virus, it is highly resistant to dryness and many disinfectants.

IgY: *Wuhan Huaynang Animal Pharmaceutical Co Ltd (2012)* holds a patent for producing IgY against porcine circovirus-resistant type 2 and tested it in vitro and in mice.

Bacterial infections

Salmonellosis

Characteristics: Infectious disease of the small and large intestine, causing inflammation and necrosis and resulting in diarrhea. It can affect all ages but is most common in weaned and growing-finishing pigs.

The pigs are febrile, show reduced feed intake and liquid yellow feces, possibly with shreds of necrotic debris. Diarrhea in individual animals usually lasts for 3-7 days. Salmonellosis causes increased mortality and growth retardation.

The clinical manifestation typically involves a systemic disease accompanied by septicemia, often linked to pneumonia, and is predominantly caused by the *S. Choleraesuis serovar. S. Typhimurium* and *S. 1,4,[5],12:i*: cause enteric disease characterized by diarrhea.

Attention: Salmonella-contaminated pork products pose a risk to human health. Salmonellosis is a notifiable disease!

Pathogens: *Salmonella enterica serotype Choleraesuis* (adapted to pigs), *Salmonella Typhimurium* (generalist)

IgY: Anti-*Salmonella Typhimurium* IgY has already been developed by *Li et al., 2016*, *Yokoyama et al., 1998b*, and developed and tested in vitro by *Lee et al., 2002*. Specific IgY against *Salmonella typhimurium* was tested in calves *(Yokoyama et al., 1998)* and in mice *(Yokoyama et al., 1998b)*.

Enterotoxigenic *Escherichia coli* (ETEC) Infection

Characteristics: ETEC strains colonize the small intestine (jejunum and ileum). With their fimbriae, they attach to the absorptive epithelial cells and cause infection. Once established, they produce enterotoxins that disrupt the intestinal barrier, causing significant inflammation. Infection by ETEC typically results in severe, watery diarrhea with dehydration, acidosis, and death. The level of intestinal colonization determines whether a disease will result from infection.

Neonatal colibacillosis manifests in profuse, watery scour within three days of age, with severe and rapid dehydration. Vomiting is not usually observed. In some cases, death can occur quickly, even in some piglets, before diarrhea becomes apparent. Often, the disease occurs in the whole litter and spreads from one litter to the other within a house.

Post-weaning diarrhea (PWD) is one of the leading causes of morbidity and mortality in newly weaned pigs suffering from weaning stress and resulting dysbiosis. The infection can increase the susceptibility of weaned pigs to other enteric infectious diseases, possibly resulting in diarrhea or sudden death.

Pathogens: Gram-negative enterotoxigenic *Escherichia coli* (ETEC) strains that express the fimbriae F4, F5, F6, and F41, the common causes of neonatal colibacillosis, and F4 (K88), F18, the leading causes of post-weaning diarrhea.

IgY: Specific IgY against ETEC was tested in piglets (*Alustiza et al., 2016*, *Zúñiga et al., 1997*, *Wang et al., 2019*, *Yokoyama et al., 1992*, *Yokoyama et al., 1997*).

Necrotic Enteritis

Characteristics: The disease mainly occurs in piglets during the first 2 weeks after birth. There are peracute, acute, or subacute to chronic forms. The ß-toxin produced by the pathogen plays a decisive role in the pathogenesis of the disease. Clinical symptoms include red-brownish diarrhea, sometimes containing blood, and severe disturbances in the organism with mortality rates up to 100%.

Pathogens: *Clostridium perfringens* A&C, a soil and fecal-borne organism that forms spores (sporulate) and can survive very long, even in inhospitable environments.

IgY: Specific IgY against *Clostridium perfringens* antigens was successfully tested in broilers as prevention from necrotic enteritis (*Goo et al., 2023*, *Khalf et al., 2016*) and anti-clostridial IgY in broilers experimentally infected with necrotic enteritis (*Abadeen et al., 2022*).

IgY against clostridia spores was tested in vitro in a mouse model (*Pizarro-Guajardo, 2017*), and *Guerra Alves et al. (2024)* produced specific IgY against *C. perfringens* type C beta-toxin.

Clostridoides difficile Enteritis or Neonatal necrotic enterotyphlocolitis

Characteristics: The disease occurs mainly in neonatal piglets at 1-7 days and is caused by the toxins of *C. difficile*. Transmission takes place by carrier sows and due to survival in the environment. The infected piglets suffer from acute enteritis with fever, intestinal cramps, and mucosanguinolent diarrhea. The disease can be observed in approximately one-third of the affected litter. Sometimes, individual piglets have dyspnea, abdominal distention, and scrotal edema.

Postmortem, moderate lesions to severe mesocolonic edema, with yellowish, pasty to watery contents, can be observed. In the case of severe systemic disease, ascites and hydrothorax can be seen.

Pathogen: *Clostridioides difficile* (before 2016, *Clostridium difficile*), an aerobic, gram-positive, spore-forming rod; more oxygen-sensitive than *Clostridium perfringens. C. difficile* proliferates in the cecum and the spiral colon. It produces two major toxins (A&B) that are involved in lesion production.

IgY: Anti-spore IgY was tested in vitro in two mouse models (*Pizarro-Guajardo, 2017*), and colonization factor (CF)-specific egg yolk antibodies (IgY) were tested in Syrian hamsters (*Mulvey et al., 2011*)

Campylobacteriosis

Characteristics: The disease mainly affects piglets. The pathogens are situated in the small and the large intestine of the animals.

The animals do not always show symptoms. Possible symptoms are watery diarrhea with mucous and occasional blood, dehydration, fever, and loss of body condition. Campylobacters (especially *C. jejuni*) are a common cause of foodborne illness in humans.

Poor hygiene, such as dirty and wet floors, continuous flow without health breaks, and secondary infections contribute to the development of the disease.

Pathogens: *Campylobacter spp. (Campylobacter coli, Campylobacter jejuni, Campylobacter hyointestinalis, Campylobacter mucosalis)* with *Campylobacter coli* being the most common in pigs

IgY: Anti-*Campylobacter jejuni* IgY was tested in broilers (***Soltani et al., 2025***; ***Hermans et al.,2014***). Anti-*Campylobacter jejuni* IgY was produced by ***Thibodeau et al. (2017).*** IgY against five *C. jejuni* colonization-associated proteins or CAPs (CadF, FlaA, MOMP, FlpA, and CmeC) were tested in vitro and significantly reduced adherence of *C. jejuni* to chicken hepatocellular carcinoma (LMH) cells (***Al-Adwani et al., 2013***).

Leptospirosis

Characteristics: Transmission of leptospirosis happens via infected urine or contaminated water (ponds, lakes, slow-moving water sources), bite wounds, or skin damage from other infected animals, such as rodents, or from the sow to the piglets.

Depending on the species, the serogroups, and the serovars, the animals show different symptoms, including dullness, anorexia, diarrhea, jaundice, red-colored urine, but also weakness of the hindquarters, meningitis, or tremor if the bacteria enter nervous tissue. The pathogens infect the fallopian tubes and seminal vesicles; in pregnant sows, they enter the pregnant uterus, attack the fetus, and cause abortion 10 days to 4 weeks post-infection. Abortions, stillbirths, together with fever and milk loss, and also high neonatal mortality are typical signs of leptospirosis in breeding herds.

Pathogen/s: *Leptospira* spp (e.g., *L. interrogans, L. weilii*), gram-negative bacteria belonging to the Leptospiraceae family. The pathogens enter the organism via skin and mucosa. Leptospirae are sensitive to antibiotics (e.g., penicillin, streptomycin), most disinfectants, and drying.

IgY: Leptospira-specific IgY was tested in hamsters ***(Lv et al., 2024).***

Ileitis/Proliferative enteropathy (PE)

Characteristics: The disease is characterized by the thickening of the intestinal mucosa due to the proliferation of epithelial cells in the intestinal crypts (***McOrist et al., 1995***). This proliferation is provoked by an infection with intracytoplasmatic *L. intracellularis* and their replication. Lesions mainly occur in the ileum but also possibly in the jejunum and the colon. The most important clinical signs are mild to severe diarrhea and weight loss. Often, the animals suffer from subclinical diarrhea with no diarrhea, fewer lesions accompanied by commensurate retardation of growth.

There are two critical clinical-pathological forms of proliferative enteropathy: the uncomplicated chronic porcine intestinal adenomatosis characterized by marked corrugated thickening. This chronic disease can be complicated by opportunistic bacteria such as Clostridia and further process to necrotic enteritis with fibrinogenic exudate adherent to thickened corrugated mucosa and the acute form associated with bloody diarrhea, high mortality, mucosal proliferation, and hemorrhage.

Highly digestible diets and vaccinations can help to keep this disease in check.

Pathogen: *Lawsonia intracellularis*, an anaerobic obligate intracellular gram-negative infecting the small and infrequently also the large intestine of pigs (***Karuppannan and Opriessnig, 2018***)

IgY: Anti-LI Igy was produced and tested in vitro. The immunoglobulins recognized whole-cell LI and LI flagellin proteins (***Asawakarn et al., 2007***)

Parasites

Cryptosporidiosis

Characteristics: *Cryptosporidium* spp are opportunistic, worldwide found parasites and cause diarrhea in animals and humans, with pigs one of the most important potential hosts.

Cryptosporidium mainly infects slightly older pigs (8-21 days old). Infected pigs can show diarrhea, vomiting, dehydration, reduced daily gain, and worse feed conversion. However, sometimes, no symptoms are seen. The parasites mainly live in the intestinal tract and the gallbladder.

Factors such as rats and mice, contaminated water sources, poor hygiene, and dirty pens contribute to disease spreading (***Chen et al., 2023***).

Pathogen: *Cryptosporidium* spp; in pigs, 13 different species/ genotypes have been isolated (***Chen et al., 2023***); Wang et al., ***2021***, 2022). The most common species in swine are *Cryptosporidium suis, Cryptosporidium scrofarum* (pig genotype II), and *Cryptosporidium parvum*.

IgY: Anti Cryptosporidium IgY has been tested in calves (***Nozaki et al., 2019***) and scid mice (***Kobayashi et al., 2004***). ***Farhang et al. (2016)*** produced IgY against *Cryptosporidium parvum* oocysts.

Coccidiosis

Characteristics: The disease mainly attacks piglets of 5 to 15 days of age, and a typical picture of a catarrhal to necrotic enteritis together with villi atrophy and fusion is shown. In older piglets, the disease often progresses without symptoms.

Infected suckling piglets excrete thin, mushy, later liquid, foul-smelling, yellowish (rarely gray) feces that do not contain blood. Diarrhea usually persists for 3 to 7 days. Although the piglets are heavily soiled with feces and appear apathetic, they continue to suck milk. High morbidity but low mortality.

Pathogen: *Cystoisospora suis* has worldwide distribution and occurs commonly in piglet production with a prevalence of up to 80% and higher. Excretion of oocysts typically increases in piglets up to an age of 2-3 weeks, then decreases. Due to quick sporulation, the densities of sporulated oocysts in piglet boxes are very high.

IgY: IgY produced against Encurin, which is involved in signal transduction and gamete fusion during the fertilization process in the pathogens, led to up to 100% inhibition of the development of sexual stages and oocyst formation (***Feix et al., 2022***).

Strongyloidiasis

Characteristics: The parasite can infect all age groups; clinical signs, however, are usually confined to nursery piglets. Only the female threadworms parasitize. They burrow into the wall of the piglets' small intestine and are present in the tunnels in the epithelium at the base of the villi. Transmission occurs via larvae penetrating the skin and migrating to the lungs or via infective larvae in the sow's milk. Possibly dormant larvae in the sow's udder can be given to several litters.

Light and moderate infections usually do not cause symptoms. Piglets up to 3 months with intense infections show diarrhea, anemia, and emaciation, with piglets younger than 2 weeks possibly dying. Infection induces strong immunity; hence, older pigs usually do not have clinical signs.

Pathogen: *Strongyloides ransomi* (pig threadworm) is a nematode parasite of pigs found worldwide. It is of importance in subtropical and tropical climates.

IgY: Specific anti-*Strongyloides venezuelensis* IgY antibodies were produced and tested in vitro to detect blood-circulating Strongyloides in humans (***De Faria et al., 2019***)

Giardiasis

Characteristics: Usually no symptoms. Prevalence might be higher in weaners than in piglets or adult pigs.

Pathogens: *Giardia lamblia*, parasites occurring in the small intestine

IgY: Anti-*Giardia lamblia* (*G. lamblia*) IgY was tested in mice. It improved body weight gain and reduced the output of cysts (***Selim et al., 2017***)

Use of IgY in Calves and Small Ruminants

In general, all the diseases occurring in calves can also occur in sheep and goats. Only if the disease explicitly occurs in lambs or goat kids will it be mentioned.

Viral Infections

Rotaviral Enteritis

Characteristics: The disease usually occurs in calves/ lambs and goat kids between 4 days to 3 weeks / 2 – 14 days of age. Clinical disease is rare in calves older than one month; however, periodic asymptotic re-infection and shedding in older cows and calves is possible.

The animals suffer from pale yellow diarrhea, sometimes containing mucous and blood flecks and lasting for 4-8 days. The animals are dull, don't want to drink, become dehydrated, and are prone to secondary infections.

Pathogens: Bovine rotavirus, a non-enveloped RNA virus from the Reoviridae family. Of the seven serogroups of rotaviruses, group A is the major cause of neonatal calf diarrhea. B and C viruses have also been identified in the field (***Geletu, 2021***). The rotaviruses frequently possess G6 and P[5]/P[11] genotypes (***Louge Uriarte, 2023***). In sheep, ovine rotaviruses of the genotypes G6, G8, and G10 were found and in goats the genotypes G3, G6, G8, and G10 ***(Papp et al., 2014).***

IgY: Specific IgY was already tested in calves (*Kuroki et al., 1994*; *Kuroki et al., 1997; Vega et al., 2011*; *Vega et al., 2015*), but also in a neonatal gnotobiotic piglet disease model (*Vega et al., 2012*), in cats (*Hiraga et al., 1990*), and in mice (*Kuroki et al., 1993*). *Özpinar et al. (1996)* tested an IgY-based product against diarrhea in calves where mainly rotavirus was identified.

Coronaviral Calf Scours / Winter Dysentery

Characteristics: In calves, coronaviral scours occur during the first 3 weeks of life but can also arise in 24-h-old colostrum-deprived animals and calves of up to 5 months. It results from a viral infection of the small and large intestines. The viruses destroy the villi and lead to severe, sometimes bloody diarrhea and high mortality rates. Continued feeding aggravates the situation as the gut can no longer absorb all nutrients, the undigested nutrients get fermented in the large intestine, and fluid accumulates, leading to bacterial overgrowth and overproduction of organic acids.

In adult cattle, the virus is associated with acute diarrhea, mainly occurring in the cold season and thus called winter dysentery. The older animals are often more affected. They might show blood in the feces, a severe milk drop, and fever.

Besides diarrhea, the bovine coronavirus can also be responsible for the bovine respiratory disease complex (BRDC), the leading cause of morbidity and mortality in 6-10-month-old beef cattle after they enter the feedlots.

The virus can also cause diarrhea in lambs and goat kids.

Pathogens: Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses and are classified into four genera: α, β, γ, and δ. For bovines, the pneumo-enteric viruses belonging to the species β-coronavirus 1 are relevant.

IgY: Anti-bovine coronavirus IgY tested in calves increased survival rate and weight gain *(Ikemori et al., 1997)*.

Bacterial Infections

Enterotoxigenic *E. coli* infection – Enteric Bacillosis

Characteristics: The bacteria are equipped to survive in the gut and are localized in the lumen and on the mucosal surface of the small intestine. Animals are mainly affected up to the age of 4 days.

The enterotoxins the bacteria produce increase the intestinal cells' secretion of ions, reduce water reabsorption, and make a non-inflammatory secretory diarrhea. The fimbriae produced by ETEC allow the binding and targeting of the heat-labile enterotoxin.

Pathogens: Enterotoxigenic **E. coli** (ETEC), producing mainly F5 (K99), F41, or F17 fimbriae to attach to the epithelial cells of the small intestine and heat-stable enterotoxin (Sta), causing fluid secretion (*Dubreuil et al., 2016*; *Bihannic, 2014*).

IgY: Specific IgY was tested in calves and led to transient diarrhea, higher survival rate, and good body weight (*Ikemori et al., 1992*).

E. coli Septicemia

Characteristics: Septicemia mainly occurs in 2 to 5-day-old calves when the bacteria enter the bloodstream, replicate, and circulate in the body. The clinical signs can vary from recumbency and suckling inability to severe diarrhea and sudden death without any symptoms. In the case of an extended sepsis period and if the invasion of bacteria is not controlled, joint arthritis and meningitis can follow. *E. coli*-induced septicemia causes high mortality worldwide.

Colostral immunity is considered the first line of defense against *E. coli* infections, so a failure of passive transfer is one of the contributing factors to the disease.

Pathogens: ETEC (enterotoxin-producing *E. Coli*), EPEC (enteropathogenic *E. coli*), and STEC (Shiga toxin-producing *E. coli*) have the potential to cause colisepticemia. For this purpose, they need characteristics (virulence factors) that enable them to adhere to and penetrate mucosal surfaces, compete for iron in the extracellular space, negotiate bactericidal plasma factors, and cause bacteremia and septicemia.

IgY: Specific IgY was tested in calves and led to transient diarrhea, higher survival rate, and good body weight (***Ikemori et al., 1992***). IgY against FanC (K99, ETEC)) was produced by ***Nasiri et al. (2016)***. Anti-EPEC IgY was produced by ***Amaral et al. (2002)***, and ***De Almeida (2003)*** developed IgY against bundle-forming pilus A (BfpA), one of the virulent factors for EPEC pathogenesis. ***Ma et al. (2020)*** achieved selective killing of STEC with IgY-conjugated chitosan nanoparticles in the GIT.

Watery Mouth Disease in Sheep

Characteristics: The disease is most common in neonatal lambs aged 6 to 48 h, especially in intensive indoor situations and due to failure of passive transfer. The lambs ingest large numbers of *E. coli* from the environment (ewe's fleece when they search the udder, soiled bedding) during the first hours of life. In older lambs, the acid conditions of the stomach destroy the bacteria, but in newborn lambs, the pH is still neutral. The bacteria multiply in the intestines, and when they die, endotoxins are released. Suppose the amount of endotoxin exceeds the detoxification capacity of the liver. In that case, the lamb suffers from endotoxemia and shows the typical signs of watery mouth disease, starting with dullness and feed refusal and ending up with collapse and profuse salivation. Further symptoms are a swollen, air-filled gut and diarrhea. The lambs die within hours.

Morbidity: 24%, mortality: 83% (***Farm Health Online, 2018***)

Pathogens: undefined pathotypes of *E. coli* (***Angell and Duncan, 2020***)

IgY: Anti-*E. coli* O157:H7 IgY was tested in sheep and reduced fecal shedding of the pathogen (***Cook et al., 2005***).

Salmonellosis

Characteristics: Salmonellosis is an issue in immune-depressed, therefore, very young or old animals. Calves of two to six weeks of age, often when coming from the market, are affected by Salmonellosis. The calves are dull, do not want to suck, and have diarrhea. In the case of septicemia, the progress is rapid, and the calves die within 12 hours. Predisposing factors for Salmonellosis are a failure of passive transfer and bad hygienic conditions.

Pathogens: *Salmonella Dublin* and *Salmonella Typhimurium*, as well as *Salmonella Newport*, rod-shaped (bacillus) gram-negative bacteria belonging to the family of Enterobacteriaceae.

IgY: Specific IgY against *Salmonella Typhimurium* and *Salmonella Dublin* was tested in neonatal calves and significantly decreased the mortality rate from 100% to 0% (***Yokoyama et al., 1998***). Specific IgY against *Salmonella Newport* was tested in vitro (***Bustos et al., 2021***)

Clostridiosis (also Hemorrhagic Enteritis, Necrotic Enteritis, Lamb Dysentery)

Characteristics: Often, the only sign of the disease is the calf's death. Sometimes, the calves show severe dark, bloody diarrhea or other signs such as abomasal bloat, depression, kicking at the belly (painful abdomen), and anorexia (off-feed). Predisposing factors for the disease include the limited production of digestive enzymes and/or acid in the abomasum, insufficient passive immunity, and changes in the normal microflora. The responsible pathogens are typically found in the intestines. However, they can pose an issue when certain preconditions, such as feed change, weather, or poor digestibility of milk replacers, allow them to proliferate.

Pathogens: *Clostridium perfringens* types A, B, C; in lambs type D

IgY: IgY was successfully tested in mice (anti-*Clostridium difficile* spores – ***Pizarro-Guajardo et al., 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: Calf scours are not thought to be caused by Campylobacter, but Campylobacter might be found in fecal samples together with other bacteria or might be a secondary pathogen. Campylobacter, however, can be transmitted to people via meat and milk, so reducing the potential carriage in livestock is recommended.

Pathogens: *Campylobacter* spp.

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

Leptospirosis

Characteristics: Transmission via infected urine or contaminated water (ponds, lakes, slow-moving water sources), bite wounds, skin damage from other infected animals, or a transmission from the dam to the progeny.

Cows: Abortions and, therefore, new heat (estrus repetition) in the case of chronic disease and, in the case of acute infection, abortions at any time of the pregnancy and the birth of weak calves.

Goats and sheep rarely show symptoms and, therefore, are considered as resistant. Possible signs in goats and sheep can be abortions, stillbirth, weak progeny, and infertility.

Pathogens: *Leptospira spp (*e.g., *L. interrogans, L. weilii),* gram-negative bacteria belonging to the Leptospiraceae family. The pathogens enter the organism via skin and mucosa. Leptospira are sensitive to antibiotics (e.g., penicillin, streptomycin), most disinfectants, and drying.

IgY: Leptospira-specific IgY was tested in hamsters (***Lv et al., 2014***)

Meningoventriculitis (Streptococcus bovis)

Characteristics: Symptoms of the disease are depression, anorexia, pyrexia, and difficulty or inability to stand, followed by cloudiness of the ocular aqueous humor or cornea. An autopsy shows congestion, petechiae, and cloudy areas in the meninges (***Seimiya et al., 1992***).

Pathogens: *Streptococcus bovis*

IgY: Feeding polyclonal antibody preparations (PAP)-IgY against *Streptococcus bovis* and *Fusobacterium necrophorum* inhibited bacterial growth in beef steers and improved feed efficiency (***Medeiros da Silva, 2024***)

Parasites

Cryptosporidiosis

Characteristics: Cryptosporidiosis mainly occurs in beef calves at 14-21 days of age (dairy calves are more often held in single pens, reducing the risk of spreading). The animals suffer from diarrhea, and the feces are yellow/green, containing high amounts of mucus. Dehydration is mild; however, the calf does not want to suck, loses condition within 2-5 days, and looks dull. If the calves are not given fluids to overcome dehydration, they may die. Morbidity is high, but mortality in uncomplicated cases is low.

Cryptosporidiosis is a zoonotic disease transmittable to humans.

Pathogens: *Cryptosporidium parvum*, an obligate intracellular protozoon belonging to cryptosporidia. It is an oval parasite living in the small intestine.

IgY: Anti-cryptosporidium IgY was tested in calves and significantly decreased oocyst shedding compared to the control and a colostrum-fed group (***Nozaki et al., 2019***). 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***).

Coccidiosis

Characteristics: In general, infection with coccidia occurs more indoors, in warm and moist conditions, than outdoors and more in warm seasons than cold ones.

Clinical infection with apparent symptoms is seen between 1-2 months up to one year of age in calves, between 1-2 months in lambs and in kids. In adults, only some cases have been reported. Cows, ewes, and does show increased oocyst shedding toward delivery; the udders can become contaminated, altogether contributing to increased infection in the offspring. Infected lambs often do not show clinical signs. However, when the animals are infected at the age of 4-8 weeks, in a contaminated environment with all lambs of the same age, most of them may show clinical signs, including sudden onset of -possibly bloody- diarrhea, anorexia, abdominal pain, and dullness. The diarrhea probably results in dehydration, weight loss, and bad body condition.

Often, coccidiosis paves the way for other enteric diseases, such as giardiasis or necrotic enteritis.

Several factors contribute to the development of coccidiosis: Environment and management, including weather, housing, feeding practices, and how the animals are grouped.

Pathogens: Host-specific *Eimeria* spp. More than 20 species have been found in the feces of cattle worldwide. Location in the gut and cells of invasion depends on the Eimeria species, e.g., *E. bovis* and *E. zuernii* can damage the distal small intestine, cecum, and colon, and *E. ellipsoidalis* the small intestine.

For sheep, two Eimeria species are considered pathogenic, *E. crandallis* and *E. ovinoidalis*, both host-specific, and for goats, 17 species were identified, and most of them are considered pathogenic.

IgY: IgY was produced against different Eimeria species and tested in poultry:

Eimeria tenella (***Juárez-Estrada et al., 2021***; ***Lee et al., 2009b***), *Eimeria maxima* (***Lee et al., 2009b***), *Eimeria acervulina* (***Lee at al., 2009a***; ***Xu et al., 2013***). The production of IgY against cattle-, sheep-, and goat-specific Eimeria should be possible, but this has not yet been realized.

Giardiasis

Characteristics: Transmission happens via smear infection or the intake of infectious oocysts from the environment. In calves, prepatency is 4-7 days, and in lambs, 10-21 days. Oocyst shedding lasts for 6 to 16 weeks, sometimes also longer. Giardiasis is a multifactorial disease, meaning that other pathogens or changes in the gut environment can favor the proliferation of trophozoites and their attachment to the epithelial cells. A catarrhal infection of the small intestine is followed by villus atrophy and infectious infiltration of the lamina propria. This results in malabsorption and worse digestion activity.

Giardiasis in cattle, in most cases, does not provoke any symptoms. Calves and lambs, however, can suffer from slight or also obstinate, intermittent diarrhea at the age of 3 to 10 weeks.

Pathogen: *Giardia duodenalis* (other names: *G. intestinalis* or *G. lamblia*), a waterborne zoonotic protozoan

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. (2017)***