Vaccines with Diluents in Poultry Flocks: How to Use Them?

Mohammad Abdoshah
Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, P.O. Box 14831975, Iran
m.abdoshah@rvsri.ac.ir

Due to flock sizes of commercial poultry operations, the most widely used vaccines are live attenuated virus vaccines which can be administered to birds by techniques which are practical within the limitations of the production environment. This is usually by means of the drinking water or by spray application, though some live vaccines require individual application by eye drop or administration by injection. Appropriate vaccine diluent, especially drinking water is essential for live vaccines to maintain vaccine efficacy and viability during storage and administration. Nevertheless, there are limited studies on how different diluents affect the live, attenuated vaccines effectiveness.

Live vaccines by their nature have a limited lifespan that must be taken into account during administration. As a rough guide 1.5 to 2.0 hours is a typical period of time over which live vaccines should be administered. The aim should be to present the vaccine in the water/diluent to all the birds in the colony over this period. Live vaccines are as susceptible to antiviral agents as field viruses, and it is important to ensure that the vaccine does not come into contact with disinfectant, chlorinated water or water sanitizing materials commonly used on farms. The volume of the water necessary for the vaccination period should be calculated and the influx of mains water into the tank interrupted until the completion of vaccination. Chlorinated water is hazardous to vaccines and can severely reduce the amount of vaccine virus presented to the birds. The addition of skimmed milk powder to all water, which will come into contact with vaccine, is a simple and effective way to overcome the detrimental effects of the chlorine. The milk powder must be mixed at the correct rate of 2-2.5 g/litre the powder should be mixed with the water at least 20 minutes prior to the vaccine being added.

Eye drop administration is probably the most effective (but labor intensive) means of administering live respiratory virus vaccines to birds. Eye drop diluent is available from vaccine manufacturers for use with certain vaccines and is presented in an easy to use bottle, which usually contains a dye in order to assess the efficiency of administration. Birds dosed effectively will show staining at the nares shortly after vaccination.

Finally, it can be recommended to make sure to reconstitute (mix) the poultry vaccines correctly before administering them. Only use the diluent provided by the manufacturer for that vaccine. Always check the expiration date on the diluent and vaccine. Never use expired diluent or vaccine and never freeze diluents

Evaluating the impact of feed additives on gut microbiota in broiler chickens using metagenomic analysis

Manoochehr Allymehr
Poultry Health & Diseases Department, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
m.allymehr@urmia.ac.ir

Introduction

It's expected that the world's population will reach over 9 billion by 2050, which poses food security challenges, especially for developing countries. Moreover, economic growth has increased the demand for livestock and poultry products, putting pressure on limited resources to produce more. Despite this, the livestock sector is one of the fastest-growing areas in agriculture, accounting for about 40% of the global value of agricultural production, supporting the livelihoods and food security of nearly 1.3 billion people (Bruinsma, 2003).

Despite the many benefits of increasing livestock and poultry production, it has created two major public health issues. First, the use of antibiotics as growth promoters in animal feed has raised widespread concerns, as their use has been banned in many countries, including the European Union (EU), due to the potential for developing antibiotic resistance in human-associated microbial populations and diseases in livestock and poultry. Second, certain zoonotic diseases such as salmonellosis, campylobacteriosis, and E. coli infections are serious public health concerns worldwide and can lead to significant economic losses.

Over the past three decades, the poultry industry has expanded rapidly, growing at an annual rate of over 5%, compared to the beef and pork industries, which have grown by 1.5% and 3%, respectively. Such rapid growth necessitates disease prevention and control strategies. The economic loss due to production losses associated with disease outbreaks constitutes 20% of the value of poultry production. Regulations regarding the use of antibiotics make it essential to find potential alternatives to replace antibiotic growth promoters and reduce the prevalence of bacterial diseases in livestock and poultry. Some of the alternatives that have been considered include probiotics, prebiotics, postbiotics, organic acids, and plant extracts (FAO, R. 2006).

Parker (1974) defined **probiotics** as "organisms and substances that help maintain the microbial balance in the gut," which includes both living organisms and non-living materials. Fuller (1989) criticized the inclusion of the word "substances" and redefined probiotics as "a live microbial feed supplement that beneficially affects the host animal by improving its microbial balance in the gut." The Food and Agriculture Organization of the United Nations (FAO) and a working group of the World Health Organization (WHO) defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (WHO/2001, FAO/). The main mechanisms of action for probiotics include: modifying the microbial population of the

digestive system and creating a favorable microflora in the gut, increasing the digestion and absorption of nutrients, producing antimicrobial substances, enhancing gut innate immunity, stimulating immune response, and resistance to colonizationAlthough probiotics are marketed as a replacement for AGPs, the mechanism of action of these different feed additives appears to be distinct (Fajardo et al., 2012).

Prebiotics are a relatively new concept that comes from the idea that indigestible food elements (like indigestible oligosaccharides) are selectively fermented by bacteria that are beneficial for gut function The increase of endogenous lactic acid bacteria and bifidobacteria in the gut is beneficial for host health, and prebiotics help promote the growth of bifidobacteria and lactobacilli in the gut, enhancing the host's microbial balance. (Schrezenmeir, J.; de Vrese, M., 2001).

Inulin, fructo-oligosaccharides (FOS), mannan-oligosaccharides (MOS), galacto-oligosaccharides (GOS), soy-oligosaccharides (SOS), xylo-oligosaccharides (XOS), pyrodextrins, and isomalto-oligosaccharides (IMO) are all types of prebiotics. They are the most commonly used prebiotics in poultry. (Everard, A., et al 2011,).

Organic acids are organic compounds that retain acidic properties. Most organic acids are formed from carboxylic acids (-COOH). Organic acids primarily consist of short-chain fatty acids (SCFA, ≤C6), which are commonly referred to as volatile short-chain fatty acids (VSCFA), such as fumaric, propionic, acetic, lactic, butyric, and others. Other organic acids include medium-chain fatty acids (MCFA; C7 to C10) and long-chain fatty acids (LCFA; ≥C11). (Papatisiros VG., et al. 2013). Organic acids were initially added to feed for cleaning and disinfecting purposes, aiming to reduce fungal and bacterial contamination in feed and to prevent salmonellosis in poultry. Organic acids are a reliable alternative to growth-promoting antibiotics, capable of reducing pathogenic bacteria and enhancing nutrient digestibility by affecting the pH in the digestive system of poultry.

Materials and Methods:

This study was conducted in a broiler farm with a capacity of 40,000 birds(Ross 308), which has 4 similar houses. Agrimos(prebiotic), CALSPORIN (probiotic) and Hexacid (organic acid) were added to the diets used at a rate of 1 kg/ton from one day of age until the end of the rearing period, and the control house received the recommended diets of Ross Company. At 14 and 28 days of age, 10 birds (5 male and 5 female) were randomly selected from each house and the contents of the ileum and cecum were sampled. DNA samples were extracted using the Stool DNA Isolation Kit and DNA extracted from ileum and cecum samples were sequenced using the shotgun metagenomics technique. The sequencing process was performed using the Illumina DNA PCR-Free Prep Kit and the Illumina NextSeq 1000 sequencing machine.

Results & Discussion

Pairwise comparison of the composition of the gastrointestinal tract microbiome showed that - there was a significant difference ($p \leq 0.05$) between the different groups in the composition of the

gastrointestinal tract microbiota due to the consumption of the studied additives, and this difference was greater at 14 days of age compared to 28 days of age, and was much greater and more extensive in the cecum compared to the ileum (Figures 1 and 2).

Table 1: summarizes the production performances of the tested groups.

performance of the broilers					
Group	**Live weight (Kg)**	**livability**	**FCR**	**Age (Day)**	**EEF**
Control	**1.605**	**91.45**	**1.73**	**35**	**242.39**
Organic Acid	**1.614**	**92.42**	**1.58**	**35**	**269.72**
Probiotic	**1.613**	**92.60**	**1.55**	**35**	**275.36**
Prebiotic	**1.609**	**90.25**	**1.60**	**35**	**259.30**

European Efficiency Factor (EEF): (Live weight in Kg X liveability) / (FCR X Age in days) X 100

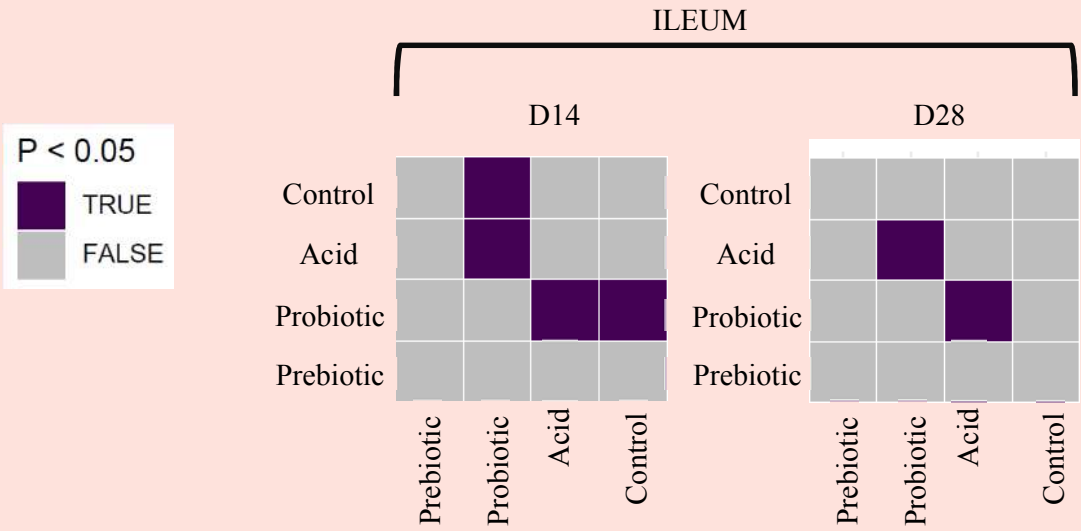


Figure 1: The differences in microbiome composition and structure between various sample groups in ileum of the broilers, Pairwise Comparison Heatmap based on Adonis p-values.

.

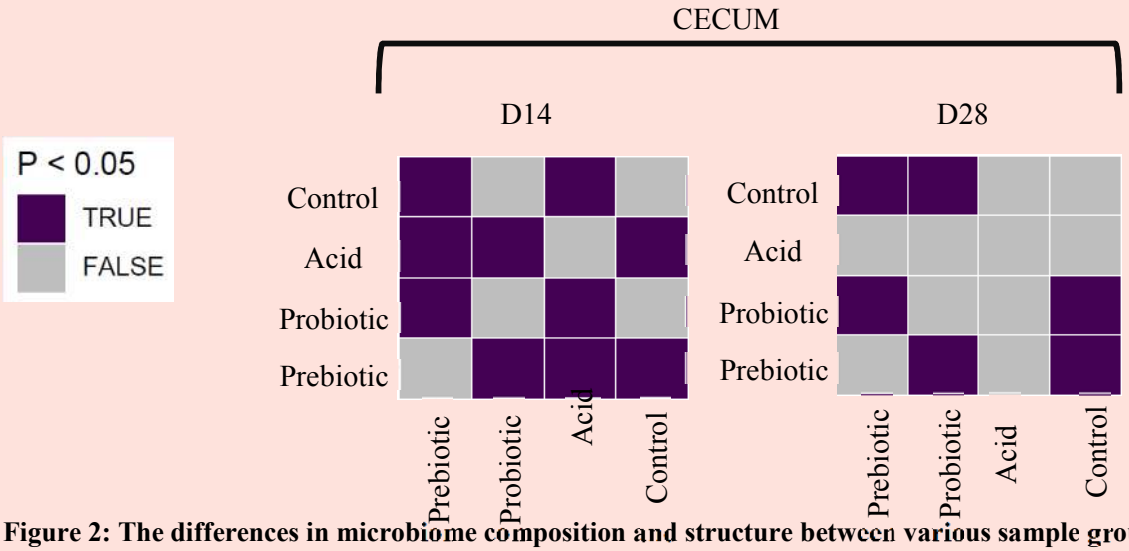


Figure 2: The differences in microbiome composition and structure between various sample groups in cecum of the broilers, Pairwise Comparison Heatmap based on Adonis p-values

Analysis of the results of this study showed that the additives used can affect the differential abundance of the microbiome in the digestive tract of broilers, and their effect is greater in the cecum than in the ileum, and their type of effect is mostly in the form of a reduction in the number

of bacteria in the digestive tract and less likely to increase the microbial population, and among the additives studied, prebiotics had the greatest effect (Figure 3).

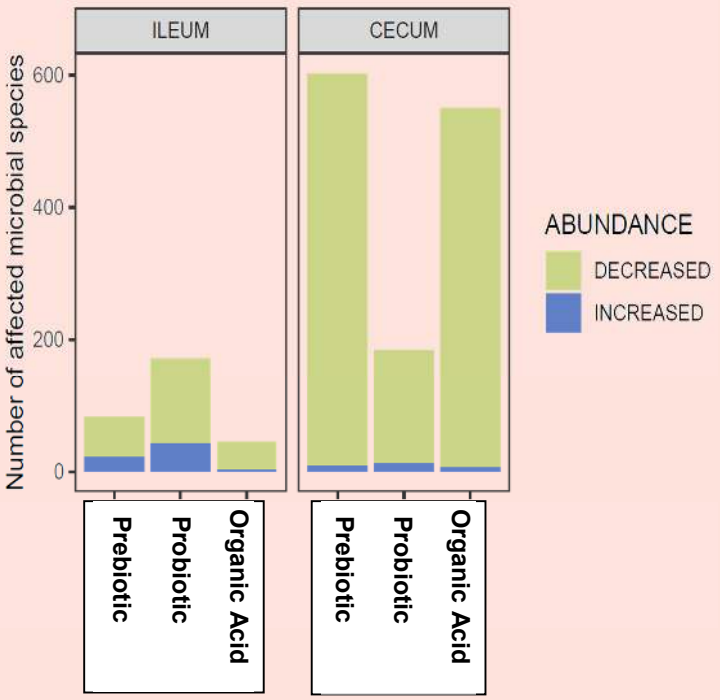


Figure 3: The impact of different treatments on the differential abundances of microbiota compared to the control group.

References

Bruinsma, J. 2003. Livestock Production. In: J. Bruinsma (ed.) *World agriculture: towards 2015/2030. An FAO perspective*. Earthscan Publications Ltd, London.

FAO, R. 2006,. Prospects for food, nutrition, agriculture and major commodity groups. World Agric. Towards 2030–2050

FAO/WHO. 2001. *Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria*. Food and Agriculture Organization of the United Nations.

Fuller, R. 1989. Probiotics in man and animals. *Journal of Applied Bacteriology,* 66(5): 365–378.

Fajardo, P., Pastrana, L., Mendez, J., Rodriguez, I., Fucinos, C. & Guerra, N. P. 2012. Effects of feeding of two potentially probiotic preparations from lactic acid bacteria on the performance and faecal microflora of broiler chickens. *Scientific World Journal, Art. No. 562635*.

Schrezenmeir, J.; de Vrese, M. Probiotics, Prebiotics, and Synbiotics-Approaching a Definition. Am J. Clin. Nutr. **2001**, 73, 361S–364S.5, 277–283.

Everard, A.; Lazarevic, V.; Derrien, M.; Girard, M.; Muccioli, G.G.; Muccioli, G.M.; Neyrinck, A.M.; Possemiers, S.; Van Holle, A.; François, P.l. Responses of Gut Microbiota and Glucose and Lipid Metabolism to Prebiotics in Genetic Obese and Diet-Induced Leptin-Resistant Mice. Diabetes **2011**, 60, 2775–2786.

Papatisiros VG, Katsoulos PD, Koutoulis KC, Karatzia M, Dedousi A, Christodoulopoulos G. Alternatives to antibiotics for farm animals. CAB Rev Ag Vet Sci Nutr Res. (2013) 8:1–15.

Cellular Immune Responses Against Colibacillosis in Chickens

Sina Bagheri
Clinical Centre for Population Medicine in Fish, Pig, and Poultry,
Clinical Department for Farm Animals and Food System Science,
University of Veterinary Medicine Vienna, Austria
Sina.Bagheri@vetmeduni.ac.at

Clinical Centre for Population Medicine in Fish, Pig, and Poultry, Clinical Department for Farm Animals and Food System Science, University of Veterinary Medicine Vienna, Austria

Colibacillosis, a bacterial disease caused by avian pathogenic *Escherichia coli* (APEC), represents a significant challenge in poultry production worldwide. The disease manifests primarily as respiratory and systemic infections, leading to considerable economic losses and welfare concerns. Despite its importance, the precise immunological mechanisms that confer protection against APEC infections remain insufficiently characterized. Enhancing our understanding of these mechanisms is critical for developing effective control measures, including vaccines and immunomodulatory therapies.

The immune system combats pathogens through humoral and cellular responses. In chickens, cellular immune responses are orchestrated by innate and adaptive immune components, including mononuclear cells, cytokines, and chemokines. These responses can be evaluated through the expression of immunoregulatory mediators such as interleukins (e.g., IL-1β, IL-6), interferons (e.g., IFN-γ), and surface markers on immune cell subsets like $CD4^+$, $CD8\alpha^+$, and TCR-$\gamma\delta^+$ T cells. However, detailed studies on avian cellular immunity have been constrained by a lack of sophisticated tools for probing these responses, unlike in mammalian systems.

Our recent investigations sought to unravel the cellular immune responses to APEC infection using innovative methodologies. To study the direct immune response to APEC, an 8-color flow cytometry panel was developed to assess cytokine production and phenotypic changes in chicken mononuclear cells following *ex vivo* stimulation. For immunophenotyping, panel matrices were designed firstly to target live cells and then populations of immune cells positive for CD45, CD8α, CD4, TCR-γδ and B cells as well as monocytes/macrophages of chicken. The cytometry panel was applied to investigate the effect of live and two different types of inactivated APEC (formalin-killed APEC and irradiated APEC) on the cellular immune response. For that, mononuclear cells from spleen, lung and blood of 10-week-old specific pathogen-free layer birds were isolated and stimulated with live, irradiated or killed APEC. Notably, stimulation with live and irradiated

APEC resulted in significantly increased cytokine production at both the protein and mRNA levels, particularly in CD8α$^+$, TCR-γδ$^+$, and CD4$^+$ T cells. However, formalin-inactivated APEC elicited a weaker cytokine response, indicating that gamma irradiation better preserves the immunostimulatory properties of APEC. These findings underscore the role of cellular immunity in controlling APEC infections. Naïve chicken mononuclear cells mounted a significant immune response upon *ex vivo* stimulation with live, gamma-irradiated, and formalin-killed APEC, as evidenced by the expression of IFN-γ and IL-17A in CD4$^+$, CD8α$^+$, and TCR-γδ$^+$ subsets. The preservation of immunogenicity in gamma-irradiated APEC, compared to formalin-inactivated preparations, highlights its potential as a vaccine candidate.

To extend these findings *in vivo*, we investigated the effects of vaccination with irradiated APEC and subsequent challenge with a homologous strain. Four experimental groups of commercial pullets (n = 16 per group) were studied: vaccinated only, vaccinated and challenged, challenged only, and sham-inoculated controls. Vaccination was performed via aerosol delivery of an irradiated APEC strain, while the challenge was administered intratracheally using the homologous strain. Lung and spleen were sampled for investigating gene expression of cytokines mediating inflammation by RT-qPCR and changes in the phenotype of subsets of mononuclear cells by flow cytometry. After re-stimulation of immune cells by co-cultivation with the pathogen, APEC-specific IFN-γ producing cells were determined. Challenged only birds showed more severe pathological and histopathological lesions, a higher probability of bacterial re-isolation and higher organ to body weight ratios compared to vaccinated and challenged birds. The *in vivo* results revealed significant changes in cytokine expression and immune cell phenotypes. Following vaccination and/or challenge, IL-1β and IL-6 expression was markedly elevated in the lungs, while IL-1β expression increased in the spleen. Phenotypic analysis of immune cells demonstrated dynamic changes: macrophages and TCR-γδ$^+$ T cells were altered in both the lung and spleen of challenged birds, while CD4$^+$ T cells were increased in the spleen. In vaccinated and challenged birds, an increase in Bu-1$^+$ cells was observed in the lungs. Additionally, IFN-γ-producing immune cells specific to APEC were significantly elevated in both vaccinated and challenged, as well as challenged-only groups, highlighting the vaccine's role in inducing a robust, antigen-specific cellular response. In the *in vivo* context, vaccination with gamma-irradiated APEC demonstrated promising immunostimulatory effects. Increased pro-inflammatory cytokine production (e.g., IL-1β and IL-6) and shifts in immune cell populations, including macrophages, TCR-γδ$^+$ T cells, and CD4$^+$ T cells, were observed following vaccination and/or challenge. Importantly, vaccinated and challenged birds exhibited heightened APEC-specific IFN-γ responses, further supporting the role of gamma-irradiated APEC in enhancing antigen-specific immunity. Our studies provide insights into the cellular mechanisms underlying immunity to colibacillosis in chickens. It underscores the role of APEC-specific IFN-γ-producing cells in mediating protective immunity and highlights the significance of pro-inflammatory interleukins, such as IL-1β

and IL-6, in orchestrating robust immune responses. The dynamic shifts observed in mononuclear cell populations during vaccination and challenge reveal the intricate interplay of immune cells in responding to APEC infection.

These findings establish a strong foundation for advancing vaccine development and exploring immunotherapy strategies to combat colibacillosis. Notably, the immunostimulatory properties of gamma-irradiated APEC position it as a promising vaccine candidate for improving poultry health and mitigating the impact of this economically significant disease.

The role of dietary melatonin supplementation in ameliorating ascites syndrome and related parameters in cold stressed broiler chickens

Shahab Bahadoran

Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord-Iran.

bahadoran4@yahoo.com

Pulmonary hypertension syndrome (PHS), also known as ascites syndrome, is a current metabolic disorder in broiler industry throughout the world. The syndrome is typified by a rise in pulmonary artery pressure that eventually leads to right ventricular hypertrophy, right heart failure and death.

This syndrome is usually influenced by different mechanisms, including hypoxia, endothelial dysfunction, vascular active material imbalance and oxidative stress.

The crucial role of reactive oxygen species (ROS) in the pathogenesis of PHS has been demonstrated in the several studies. The high production of reactive oxygen species (ROS) prone to PHS may cause this syndrome or exacerbate it. Proteins, DNA, and some molecules can be oxidized by ROS, resulting in a cascade of harmful reactions and initiation of tissue damage. Excessive production of ROS could cause damage to the pulmonary vascular endothelium and vascular remodeling. DNA damage particularly its ends (telomere) was confirmed in the chicken with PHS.

For birds, the first line of defense against ROS is endogenous antioxidants such as tocopherols, glutathione, and ascorbic acid. The levels of these antioxidants are reduced in liver and lung tissues and the mitochondria of ascitic birds in association with increased markers of ROS-mediated tissue injury, leading to oxidative stress during PHS. As with the chemical antioxidants, cells are protected against oxidative stress by an interacting network of antioxidant enzymes such as superoxide dismutases, catalases, and glutathione superoxides. In many studies, it has been attempted to modulate the onset of PHS by supporting the antioxidant status at the onset of the ascites-promoting condition.

Melatonin (N-acetyl-5-methoxytryptamine) is an indole amine derivative produced by the pineal gland and many extrapineal sources such as skin, immune system cells, brain, and gastrointestinal tract. Melatonin plays a critical role in different physiological functions, including the regulation of circadian, immunity, oxidative stress, apoptosis, and mitochondria. Most of these activities change during inflammation. Melatonin could easily enter the cellular organelles (e.g., nucleus and mitochondria) and act as a natural scavenger involving the neutralization and detoxification of several chemical agents such as H2O2, hydroxyl radicals, nitric oxide radical and peroxinitrite. It has been explained that this hormone contributes to oxidative stress protection via electron and hydrogen alterations.

Bahadoran et al. (2024) investigated the potential antioxidant effects of melatonin in birds exposed to conditions promoting pulmonary hypertension syndrome (PHS) under cold stress. Two groups of birds were offered different concentrations of melatonin (0.2% and 0.4%), while a control group received no melatonin treatment. Serum and cardiac tissue samples were collected to evaluate Glutathione Peroxidase (GPX) and Superoxide Dismutase 1 (SOD1) activities. Results showed a significant decrease in ascites mortality and the right ventricular to total ventricular weight ratio (RV: TV ratio) in the melatonin-0.4% supplemented group in comparison to the control. Melatonin supplementation at both 0.2% and 0.4% levels led to lower levels of malondialdehyde (MDA) in the serum and heart compared to the control group, indicating reduced lipid peroxidation. Both melatonin groups exhibited increased serum/cardiac (GPX) activities as compared to the control, however the serum SOD1 activity was only increased in the melatonin-0.4% group of birds compared to the control group.

It is concluded that melatonin as an antioxidant agent can reduce severity of PHS and ascites mortality in broiler flocks.

Reference

1. Bahadoran, S. Adinehvand, S. Hassanpour, H and Mohebbi, A (2024). Investigating the Role of Dietary Melatonin Supplementation in Ameliorating Pulmonary Hypertension Syndrome in Cold-stressed Broiler Chickens. *ImmunoRegulation*, 6, 127-136
2. Bahadoran, S. Teymouri, Y. Hassanpour, H. Mohebbi, A and Akbari, M (2023). Effect of sage (Salvia officinalis L.) extract in antioxidant status and intestinal morphology of pulmonary hypertensive chickens. *Veterinary Medicine and Science*, 9, 2176-2184.
3. Calislar S, Yeter B and Sahin, A. (2018). Importance of melatonin on poultry. *KSU Journal of Agriculture and Nature*, 21, 987-97.
4. Hassanpour, H., Farhadi, N., Bahadoran, S. and Akbari, M (2023). Cardiac telomere attrition following changes in the expression of shelterin genes in pulmonary hypertensive chickens. *British Poultry Science,* 64, 370-376.

Overview of infectious bursal disease, prevention and control

Mohammad Majid Ebrahimi
Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization, P.O.Box 31975-148, Karaj, Iran.
mm.ebrahimi@rvsri.ac.ir

Infectious bursal disease (IBD), also known as Gumboro disease, is a highly contagious disease of young chickens in which the immune system's tissues, especially the bursa of Fabricius, are targeted. This results in immunosuppression and susceptibility to secondary infections that can end in death or manifest as respiratory or gastrointestinal disease. Clinical signs in affected birds may include depression, ruffled feathers, poor appetite or anorexia, huddling, an unsteady gait, reluctance to rise, and diarrhea. The pathogenicity of IBDV can vary depending on the immune status, age of the infected birds, and the viral strain. Severe acute disease, typically found in birds aged 3 to 6 weeks, is linked to high mortality rates, while subclinical or acute infections are less common during the first 3 weeks of life.

The immunosuppressive disease caused by the IBD virus (IBDV), a double-stranded RNA virus, belongs to the genus *Avibirnavirus* in the family *Birnaviridae*, which is divided into two serotypes 1 and 2. The bi-segmented genome of the IBDV consists of two segments A and B. Segment A has two partially overlapping open reading frames (ORFs). The viral proteins VP2 (48 kDa), VP3 (33–35 kDa), and VP4 (24 kDa) are produced from the proteolytic processing of a polyprotein encoded by the larger ORF. The smaller ORF encodes the nonstructural protein VP5 (17 kDa). Segment B has a single open reading frame encoding VP1, an RNA-dependent RNA polymerase. VP2 is a crucial structural protein responsible for the production of neutralizing antibodies. The antigenic variation, especially in the hyper-variable region of VP2 is an important cause of high variation in IBDVs. IBDVs have been divided into four main serotype I classes ranging from mild to intermediate, classic, variants, and very virulent based on their pathogenicity and antigenicity.

Clinical IBD diagnosed by a combination of characteristic signs and postmortem lesions. Subclinical IBD can be confirmed by examining the presence of a humoral immune response in unvaccinated chickens or by identifying viral antigens or viral genomes in tissues. Viral antigens can be detected in the bursa of Fabricius before the extraction of anti-IBDV antibodies in order to early diagnosis. The agar gel immunodiffusion (AGID), immunofluorescence, antigen-capture enzyme-linked immunosorbent assay (AC-ELISA), and molecular techniques should be applied. In the AGID test, a bursa homogenate is used as the antigen against a known positive antiserum. AC-ELISA using plates coated with IBDV-specific antibodies can also detect IBDV antigens in bursal homogenates. IBDV antigens may be identified by immunostaining of infected tissues using IBDV-specific chicken antiserum. Only a small percentage of the flock should be tested for antibodies. If positive reactions are observed in unvaccinated birds, the entire flock should be considered infected. Both serotypes of IBDVs share common group antigens that can be detected by the fluorescent antibody test and ELISA. Therefore, it is not possible to distinguish between serotypes or their antibodies. The common group antigens for both serotypes are found on VP2 (40 kDa) and VP3 (32 kDa). VP2 also contains serotype-specific group antigens that trigger virus neutralization (VN) antibodies, while

antibodies against VP3 do not provide any protective effect. Molecular techniques have become popular due to their sensitivity, time-saving nature, ability to be used on crude or inactivated samples, and the fact that they do not require virus replication. The most frequently used molecular method to detect the genome of viruses is the reverse-transcription polymerase chain reaction (RT-PCR). In the absence of such tests, histological examination of bursa is useful.

The principal target for IBDV strains is surface IgM-bearing B cells in the bursa of Fabricius. For this reason, specific pathogen free (SPF) chickens, 9-11-day-old embryonated eggs, and chicken embryo fibroblast cells are the best options for virus isolation *in vitro*. Due to finite lifespan and laborious preparation of these substrates, some chicken embryo related cells and mammalian cell lines have been studied as alternatives for IBDV field samples isolation. The identity of the isolated virus should be followed by VN assay. IBDV strains can be further identified by testing their pathogenicity in specific antibody negative (SAN) chickens, by investigating their antigenic reactivity in cross VN tests or using maternal antibodies, by determining the number and size of the restriction fragments obtained in the restriction enzyme fragment length polymorphisms based on RT-PCR (RT-PCR/RFLP) of the genomic region encoding the VP2 variable domain. Both serotypes of IBDVs share common group antigens that can be detected by the fluorescent antibody test and ELISA. Therefore, it is not possible to distinguish between serotypes or their antibodies. The common group antigens for both serotypes are found on VP2 and VP3. VP2 also contains serotype-specific group antigens that trigger VN antibodies, while antibodies against VP3 do not provide any protective effect. This approach has also been implemented for the molecular grouping of IBDV strains based on their segment B restriction profile. Moreover, genome sequencing and phylogenetic evaluation of the isolates with reference IBDVs are suggested for an optimal assessment of genetic relatedness.

IBDV excreted in the feces of infected birds, it is very stable in the environment and may remain for several weeks. Difficult to eradicate from premises. The virus can be spread via direct contact between infected and susceptible birds and indirect transmissions via contaminated feed, water, equipment, or employees, and through rodents, wild birds, and insects as carriers. With decreased productivity, higher mortality rates, decreased egg production, and trade restrictions, IBDV can significantly affect the economics of poultry production. Vaccination of chickens at the right time is an effective way for providing protection against IBDV. Four major types of vaccines are available for the control of IBD including live attenuated vaccines, immune-complex vaccines, live recombinant vectored vaccines expressing IBDV VP2 antigen, and inactivated oil-emulsion adjuvanted vaccines. To date, all commercial vaccines are developed from serotype 1 of the virus. The immunization of breeder flocks to confer parental immunity to their progeny is crucial. Maternal antibodies protect chicks from early immunosuppressive infections. Normally, maternal antibodies protect chicks for 1-3 weeks, but by boosting immunity in breeder flocks with oil-adjuvanted vaccines, passive immunity can be extended to four or five weeks.

Field exposure or vaccination triggers active immunity against IBDV. The role of humoral immunity in protection against IBDV has been well documented, as evidenced by the protection provided by passive transfer of antibodies. Specific antibody levels are typically high and VN titers greater than 1:1000 being common. Antibodies passed from the hen to the chick via the yolk of the egg can protect chicks from early infections with IBDV, providing protection against the virus's immunosuppressive effects. The half-life of maternal antibodies to IBDV is between three and five days. Therefore, knowing the titer of neutralizing antibodies in the offspring can predict when chicks will become susceptible. Once antibody titers drop below 1:100, chicks become 100% susceptible to infection, while titers between 1:100 and 1:600 offer around 40%

protection against challenge. The immunization of breeder flocks to confer parental immunity to their progeny is crucial. Maternal antibodies protect chicks from early immunosuppressive infections. The main challenge with actively immunizing young maternally immune chicks with attenuated IBDV vaccines is determining the correct time for vaccination. This timing varies depending on levels of maternal antibodies, vaccination route, level of exposure, and virulence of the vaccine virus. Environmental stresses and management practices should also be considered when developing an effective vaccination program. Monitoring antibody levels in a breeder flock or its progeny (flock profiling) can help determine the best time to vaccinate. It is important to note that while ELISA and VN tests produce correlated antibody titers, they may suggest different vaccination dates for progeny chicks. Therefore, the formula used to calculate vaccination dates should be thoroughly evaluated.

The use of killed vaccines in oil emulsions to stimulate high levels of maternal immunity is common in practice. Oil emulsion IBD vaccines can generate sufficient maternal immunity to protect chicks for 4-5 weeks, whereas progeny from breeders vaccinated with live vaccines are protected for only 1-3 weeks. Killed vaccines are usually not practical or desirable for inducing a primary response in young chickens and most effective in chickens that have been primed with live virus either through vaccination or field exposure to the virus. A universal vaccination program cannot be implemented due to variations in maternal immunity, management practices, and operational conditions. If high levels of maternal antibodies are achieved and field challenges are reduced, vaccination of broilers may not be necessary. The timing of vaccination with attenuated and intermediate vaccines can range from as early as seven days to two or three weeks. If broilers are vaccinated at 1-day-old, the IBDV vaccine can be administered by injection along with the Marek's disease vaccine. Priming of breeder replacement chickens may be necessary, and many producers vaccinate with a live vaccine at 10-14 weeks of age. Killed oil-adjuvant vaccines are typically given at 16-18 weeks. Revaccination of breeders may be needed if antibody profiling shows a significant drop in flock titers.

Like many diseases, passively acquired immunity to IBDV can interfere with the stimulation of an active immune response. It is advised to conduct antibody profiling of breeder flocks to assess the effectiveness of vaccination and the persistence of antibodies. Adult birds are resistant to oral exposure to the virus but produce antibodies after intramuscular or subcutaneous inoculation of IBDV. Partial protection against IBD has been achieved in chickens even without detectable neutralizing antibodies, through experimental immunization with a recombinant virus expressing the VP2 protein, suggesting a role for cell-mediated immunity (CMI) in protection against IBDV. There is growing evidence of the additional effect of CMI in protecting against the disease. Additionally, live recombinant virus vectors expressing IBDV immunogens including fowlpox virus, herpes virus of turkey, Marek's disease virus, chicken adenovirus, and Newcastle disease virus induce an active anti-IBDV antibody response even in the presence of high levels of neutralizing maternally-derived antibodies.

In ovo vaccination of chickens for IBD and other agents at 18 days of incubation is a labor-saving technique that may help vaccines bypass the effects of maternal antibodies and initiate a primary immune response. *In ovo* injection of an intermediate IBD vaccine alone has experimentally resulted in faster recovery of bursal lesions compared to post-hatch vaccination, and it provides similar protection against challenge.

Increasing concern of mycoplasma gallisepticum and economic design of treatment program

Adel Feizi
Department of clinical sciences, faculty of veterinary medicine, Islamic Azad University, Tabriz, Iran.
adel_feizi@yahoo.com

Infections caused by Mycoplasma gallisepticum, commonly known as chronic respiratory disease, have a global distribution and are a very important disease in broiler, layer, and breeder flocks. In this study, 9 MG-positive broiler farms, each consisting of two similar house, were selected. A medical regimen was applied in two phases to control mortality and complications caused by MG: in the first week for 4 days, and in the third week for 5 days across three groups. In all groups, the doxycycline-Tylvalosin treatment regimen was used in one house. Among the 4 MG-positive broiler farms, three farms used Tilmicosin-doxycycline, three farms used Tiamulin-doxycycline, and three farms used Enrofloxacin. In each group, 150 chickens were kept separately as controls and did not receive any medication. The examination of performance indices in the tested groups showed that the combined drug Tylvalosin-doxycycline had the best effect compared to the other groups, but this difference was significant with the Enrofloxacin drug group and the control group ($p \leq 0.05$). In this study, the combination therapy program of Tylvalosin-Doxycycline was more effective than other treatment programs in controlling disease symptoms and improving the performance of infected broiler flocks. The final recommendation is that in MG-positive broiler flocks, early treatment programs at young ages significantly reduce treatment costs compared to treatment at older ages.

Introduction:

The species designation Mycoplasma gallisepticum was made in 190 by Edward and Kanarek. Infections caused by Mycoplasma gallisepticum, commonly known as chronic respiratory disease, have a global distribution and are a very important disease in broiler, layer, and breeder flocks. Mycoplasma gallisepticum infection are commonly known as chronic respiratory disease of chickens and infection sinusitis of turkeys. MG is the most pathogenic and economically significant Mycoplasma pathogen of poultry MG causes mortality, condemnation, reduced egg production, feed efficiency and drop in hatchability. Due to the vertical transmission of MG, infected breeder hens should be eliminated as a source of infection. Effective biosecurity measures are valuable tools in controlling Mycoplasma infections. In addition to vertical transmission, horizontal transmission also occurs in poultry farms, and some predisposing factors such as stress, cold, overcrowding, immune-suppressive diseases, and viral respiratory diseases (e.g., ILT, IB, ND) make poultry farms susceptible to MG. Therefore, in case of MG infection, especially in broiler farms, an effective and

economical treatment plan is necessary. Given that most infections caused by MG, which is referred to as chronic respiratory disease (CRD), are complicated by E. coli bacteria, turning it into a CRD complex. Therefore, the use of combination drug programs in this infection has become more widespread. Due to the high treatment costs, timely use of medication at a young age will reduce these costs. Thus, prompt and rapid treatment is recommended for both MG-positive broiler flocks and cases of airsacculitis.

Material and method:

In this study, 9 MG-positive broiler farms, each consisting of two similar house, were selected. To confirm the MG-positive status, serological tests RSA and ELISA were used. A medical regimen was applied in two phases to control mortality and complications caused by MG: in the first week for 4 days, and in the third week for 5 days across three groups. In all groups, the doxycycline-Tylvalosin treatment regimen was used in one house. Among the 4 MG-positive broiler farms, three farms used Tilmicosin-doxycycline, three farms used Tiamulin-doxycycline, and three farms used Enrofloxacin. In each group, 100 chickens were kept separately as controls and did not receive any medication. All nutritional, breeding, and vaccination programs were applied in both house and the control group. The drug doses of doxycycline-Tylvalosin-Tilmicosin-Tiamulin were 40mg/kg in the first week and 20mg/kg in the third week, while the Enrofloxacin dose was 20mg/kg in the first week and 10mg/kg in the third week. In all groups, clinical symptoms including respiratory distress, aggregation, feed consumption, postmortem signs such as air sac lesions, mortality rate, feed conversion ratio, and final weight were compared."

Results:

The examination of performance indices in the tested groups showed that the combined drug Tylvalosin-doxycycline had the best effect compared to the other groups, but this difference was significant with the Enrofloxacin drug group and the control group. The reduction in respiratory reactions, improvement in feed consumption, better distribution of chickens in the house, significant reduction in air sac lesions, reduced mortality, condemnation, and improved feed conversion ratio were observed in all experimental groups except the Enrofloxacin and control groups.

The effect of treatment programs on the performance

FACTOR / GROUP	WG(gr)	F.C.R	CONDEMINATION(%)	MORTALITY(%)
TYLVALOSIN + DOXYCYCLIN	2920±73.10 [a]	1.85 [a]	1.2 [a]	8.3 [a]
TILMICOSIN + DOXYCYCLIN	2900±52.24 [a]	1.91 [b]	1.7 [b]	9.5 [b]
TIAMULIN + DOXYCYCLIN	2930±72.11 [a]	1.93 [b]	1.7 [b]	9.8 [b]
ENROFLOXACIN	2800±52.13 [b]	2.1 [c]	2.5 [c]	13.2 [c]
CONTROL GROUP	2650±77.15 [c]	2.4 [d]	3.2 [d]	15 [d]

Improve performance in qualitative factors after medication

FACTOR / GROUP	Air Sacculitis	Feed intake	Tracheal vales	huddling
TYLVALOSIN + DOXYCYCLIN	++++	+++	+++	+++
TILMICOSIN + DOXYCYCLIN	+++	+++	+++	+++
TIAMULIN + DOXYCYCLIN	+++	+++	+++	+++
ENROFLOXACIN	+	+	++	+
CONTROL GROUP	-	-	-	-

++++: Excellent, +++: Good, ++: Average, +: weak, -: Negative

Conclusion:

In this study, the combination therapy program of Tylvalosin-Doxycycline was more effective than other treatment programs in controlling disease symptoms and improving the performance of infected broiler flocks. Although the treatment programs of Tilmicosin-Doxycycline and Tiamulin-Doxycycline also had significant effects on reducing symptoms and complications related to MG and improving the performance indices of MG-positive flocks, the use of Enrofloxacin did not have a significant effect on reducing symptoms and improving indices. The final recommendation is that in MG-positive broiler flocks, early treatment programs at young ages significantly reduce treatment costs compared to treatment at older ages. Therefore, treatment is recommended in the first and third weeks, starting with the appearance of clinical signs and necropsy associated with MG, and the treatment program should continue for up to 5 days.

Gut Microbiota, Immunity, and Health in Poultry

Mahdi Ghanbari
dsm-firmenich, Animal Nutrition and Health R&D Center, Tulln, Austria
mahdi.ghanbari@dsm-firmenich.com

Abstract: The gut microbiota plays a vital role in maintaining the health and immunity of poultry. Research has shown that a balanced microbiome enhances immune responses, supports digestion, and helps prevent pathogen colonization. However, when disrupted, the microbiota can contribute to various health issues, including inflammation, infections, and poor growth performance. In poultry farming, where antibiotics have been traditionally used to manage health challenges, the need for alternatives is increasingly critical, especially in light of the rising concerns about antimicrobial resistance (AMR). This paper explores the importance of gut microbiota in poultry health and immunity, with a particular focus on how feed additives support microbial balance, enhance immunity, and offer sustainable solutions to reduce antibiotic reliance, thus helping to mitigate the risks associated with AMR.
Kew words: Gut microbiota, poultry health, immune modulation, antimicrobial resistance (AMR)

Understanding the Intestinal Tract and Microbiota

The intestinal tract is essential for nutrient absorption, energy production, and waste elimination, and plays a critical role in maintaining the health of the animal. As the largest mucosal surface in the body, it is continuously exposed to external agents such as pathogens, making it a key site for immune activity. The intestinal mucosal barrier and mucus layers act as physical defenses, preventing pathogens from translocating into the body. Furthermore, the gut houses a significant portion of the animal's immune cells, highlighting its central role in immune regulation. The gut microbiota, composed of bacteria, archaea, fungi, and protozoa, is crucial in supporting intestinal health in poultry. These microorganisms, often termed "commensals," coexist with the host and provide a range of benefits. Their role in digesting complex carbohydrates, synthesizing essential vitamins, and modulating immune responses is well established. The gut microbiota interacts with the immune system, shaping defense mechanisms and protecting the host from pathogenic invasion. The composition of the gut microbiota can vary significantly across species and even between individuals. In poultry, microbial communities in different sections of the gut (e.g., small intestine, cecum, and large intestine) differ in structure and function. Understanding these variations is essential for optimizing gut health and improving poultry production outcomes.

Gut-Immune System Interactions and Health

The gut is not only responsible for nutrient absorption but also serves as one of the most active immune organs in the body. A dense network of immune cells resides within the gut, constantly interacting with the microbiota. These interactions are crucial for the development of immune tolerance and defense against pathogens. The gut microbiota supports immune regulation by stimulating the production of immune mediators, enhancing gut mucosal barriers, and preventing the overgrowth of harmful pathogens. The balance between beneficial and harmful microbes in the gut is essential for maintaining immune health, preventing unnecessary inflammation, and reducing the risk of disease. Additionally, the gut-brain axis, a network of biochemical signaling between the gastrointestinal tract and the brain, plays a pivotal role in influencing behavior, stress resilience, and overall well-being. Gut microbiota modulate the production of neurotransmitters, affecting mood and behavior. This has direct implications for animal welfare and productivity in poultry farming, making it an area of significant interest. In poultry, the microbiota not only supports digestion but also enhances energy production. Microbial fermentation of complex carbohydrates in the hindgut produces short-chain fatty acids (SCFAs), which serve as an energy source for intestinal cells. SCFAs are essential for efficient digestion, growth, and feed conversion. Furthermore, the microbiota contributes to immunity by serving as a first line of defense against pathogens. Stable microbiota populations prevent pathogenic bacteria from colonizing the gut, thereby reducing the risk of inflammation and infection. By producing antimicrobial peptides, the microbiota helps maintain gut integrity, which is essential for overall health.

Modern Methods of Analyzing the Gut Microbiome

Traditional methods of analyzing the gut microbiome, such as culturing specific microorganisms, provided limited insights into microbial communities. These approaches were biased toward culturable bacteria, often overlooking less abundant or non-culturable species that might play essential roles in health. Recent advancements, however, have revolutionized our ability to study the microbiome in more detail.

16S ribosomal RNA (rRNA) sequencing has become a standard technique for profiling bacterial communities. By targeting a conserved region of the 16S gene, this method allows for the identification of microbial taxa across a variety of environments, including the gut. It provides an in-depth view of the diversity and abundance of microbial populations, enabling researchers to correlate microbial profiles with health outcomes. However, while 16S sequencing focuses primarily on bacterial composition, it does not fully capture the functional roles of microbes. To address this, metagenomic sequencing offers a more comprehensive approach. Metagenomics involves sequencing the entire genomic content of the microbiome, providing insights not only into the taxonomy of microbes but also into their functional potential. This approach allows researchers to identify microbial genes and metabolic pathways, offering a better understanding of how microbes contribute to immunity, nutrient metabolism, and pathogen defense.

These modern microbiome analysis techniques have significantly advanced our understanding of the gut microbiota's role in health and disease. By providing a clearer picture of microbial diversity and function, these methods are crucial for developing strategies to optimize gut health in poultry and other livestock species.

Antimicrobial Resistance (AMR) and the Gut Microbiome

The use of antibiotics in animal production, while essential for disease management, has led to the emergence of antimicrobial resistance (AMR). The gut microbiota is a major reservoir for AMR genes, and its disruption through antibiotic treatments can lead to the selection of resistant bacteria, which may have significant public health implications. In poultry, antibiotic use in feed has been linked to changes in the gut microbiota composition, potentially promoting the overgrowth of resistant strains. However, studies have shown that the gut microbiota can also play a role in mitigating the development of resistance by maintaining a diverse microbial community that limits the spread of pathogenic bacteria. Research into alternative methods of managing gut health, such as the use of prebiotics, probiotics, and phytochemicals, is gaining momentum as a way to reduce reliance on antibiotics and minimize the risk of AMR. By modulating the gut microbiota, these interventions may not only improve poultry health and performance but also contribute to a more sustainable and antibiotic-free poultry industry.

Conclusion

Understanding the complex interactions between gut microbiota, immunity, and overall health is key to improving poultry health and productivity. Advances in microbiome research have revealed that microbial communities are not merely passive inhabitants of the gut but active participants in nutrient metabolism, immune defense, and disease prevention. As the poultry industry faces increasing pressure to reduce antibiotic use and improve animal welfare, strategies aimed at optimizing gut microbiota offer promising solutions. Further research into the mechanistic role of microbiota in immune modulation and AMR resistance is critical for advancing our ability to manage gut health effectively in poultry and other livestock species.

References:

1. Bortoluzzi, C., M. Ghanbari, J. C. Gonzáles, J. O. Bohórquez, R. Paredes, Y. Mauri, and C. A. Lozano-Poveda. 2024. Precision biotic as an effective replacement of hydrolyzed yeast and butyrate in antibiotic free diets of broiler chickens raised under field conditions. Poultry Science:104664. doi https://doi.org/10.1016/j.psj.2024.104664
2. Ghanbari, M., V. Klose, F. Crispie, and P. D. Cotter. 2019. The dynamics of the antibiotic resistome in the feces of freshly weaned pigs following therapeutic administration of oxytetracycline. Scientific Reports 9:4062. doi 10.1038/s41598-019-40496-8
3. Koorakula, R., M. Schiavinato, M. Ghanbari, G. Wegl, N. Grabner, A. Koestelbauer, V. Klose, J. C. Dohm, and K. J. Domig. 2022. Metatranscriptomic Analysis of the Chicken Gut Resistome

Response to In-Feed Antibiotics and Natural Feed Additives. Frontiers in microbiology 13. doi 10.3389/fmicb.2022.833790

4. Peng, C., M. Ghanbari, A. May, and T. Abeel. 2024. Effects of antibiotic growth promoter and its natural alternative on poultry cecum ecosystem: an integrated analysis of gut microbiota and host expression. Frontiers in microbiology 15:1492270. doi 10.3389/fmicb.2024.1492270
5. Segura-Wang, M., N. Grabner, A. Koestelbauer, V. Klose, and M. Ghanbari. 2021. Genome-Resolved Metagenomics of the Chicken Gut Microbiome. Frontiers in microbiology 12. doi 10.3389/fmicb.2021.726923
6. Temmerman, R., M. Ghanbari, G. Antonissen, G. Schatzmayr, L. Duchateau, F. Haesebrouck, A. Garmyn, and M. Devreese. 2022. Dose-dependent impact of enrofloxacin on broiler chicken gut resistome is mitigated by synbiotic application. Frontiers in microbiology 13:869538. doi 10.3389/fmicb.2022.869538
7. Wegl, G., N. Grabner, A. Kostelbauer, V. Klose, and M. Ghanbari. 2021. Toward Best Practice in Livestock Microbiota Research: A Comprehensive Comparison of Sample Storage and DNA Extraction Strategies. Frontiers in microbiology 12:627539. doi 10.3389/fmicb.2021.627539

Newcastle virus genotype 7, a global threat to the poultry industry

Farhid Hemmatzadeh
Associate Professor/Reader
School of Animal and Veterinary Science
Faculty of Sciences, Engineering and Technology, University of Adelaide
farhid.hemmatzadeh@adelaide.edu.au

After the outbreak of genotyoe VII of Newcastle disease virus (NDV-GVII), it became evident that the pathogenedis and tropism of this virus differ significantly from other NDV genotypes. Common NDV genotypes tend to cause more pronounced pathological lesions in the respiratory and digestive tracts of poultry, leading to the characterization of NDV as primarily a respiratory/gastrointestinal pathogen. Importantly, neurotropic strains of the virus, while exhibiting a strong affinity for the nervous system, also induce severe gastrointestinal and respiratory lesions.

In contrast to other genotypes, the NDV-GVII exhibits a pronounced lymphotropism, leading to the destruction of lymphatic tissues and causing necrosis and necroptosis prior to significant respiratory and digestive lesions. The destruction of lymphatic tissue caused by the genotype 7 virus often results in shock and rapid death in affected birds. In less immediate cases, it can lead to severe immunosuppression in poultry.

Numerous studies have demonstrated that conventional vaccines, which belong to genotypes I or II, do not provide adequate immunity against genotype VII. Research conducted at the University of Adelaide, Australia, has shown that GVII homologous virus vaccines can induce stronger immunity, reduce mortality rates following exposure to the virus, and decrease virus shedding in vaccinated birds after the challenge with the wild type strains.

Therefore, considering the dominance of NDV-GVII in the country's poultry industry, it is recommended that the NDV-GVII vaccine be utilized to reduces the viral load in birds while providing homologous immunity to circulating strains.

Analytical Review of Newcastle Disease in Iran from 1950 to 2024: Challenges, Changes, and the Road Ahead

Alireza Homayouni Mehr
Symoorgh Avian Special clinic
homayounimehr@gmail.com

Abstract:
Newcastle disease (ND) is one of the most economically significant viral diseases affecting the poultry industry worldwide, including Iran. Since the first recorded outbreak in 1950, ND has remained a persistent challenge for poultry farmers, veterinarians, and policymakers. Over the past decades, Iran has witnessed substantial changes in the epidemiology of the disease, vaccination strategies, and biosecurity measures. This paper provides a comprehensive analytical review of the evolution of Newcastle disease in Iran, highlighting key challenges, advancements, and future directions for controlling this disease.

Introduction:
Newcastle disease is caused by Avian Paramyxovirus type 1 (APMV-1), which can lead to high mortality in poultry populations. Since its first outbreak in Iran, ND has become endemic and continues to pose a significant threat to the country's poultry industry. This paper examines the historical progression of ND in Iran and analyzes its epidemiology, control measures, vaccination strategies, and emerging challenges.

Epidemiology and Historical Perspective:
The first official case of Newcastle disease in Iran was reported in 1950. In the following decades, multiple outbreaks occurred, primarily affecting backyard and commercial poultry farms. The epidemiology of ND in Iran has evolved due to changes in poultry farming practices, increased bird density, and expanding global trade. Despite extensive vaccination efforts, recurrent outbreaks indicate the persistence of virulent viral strains and potential vaccine inefficacy.

Evolution of Newcastle Disease Virus Strains: Molecular studies have demonstrated genetic evolution in the dominant strains of Newcastle disease virus (NDV) circulating in Iran. Early outbreaks were primarily associated with class II genotype II viruses, whereas recent outbreaks have been linked to genotype VII and other emerging genotypes. These genetic changes impact vaccine efficacy and disease control strategies.

Vaccination Strategies and Their Effectiveness: Vaccination remains the primary tool for ND control in Iran. Over the years, both live attenuated and inactivated vaccines have been widely used.

However, field studies indicate that despite high vaccination coverage, sporadic outbreaks continue to occur. Factors contributing to these failures include:

- Improper use and storage of vaccines.
- Emergence of antigenic variants not fully covered by existing vaccines.
- Weak biosecurity measures allowing viral circulation within poultry farms.

Challenges in Newcastle Disease Control: Despite significant advancements, controlling ND in Iran faces multiple challenges:

1. **Vaccine Effectiveness:** Mismatch between circulating viral strains and vaccine strains may result in inadequate protection.
2. **Biosecurity Deficiencies:** Many small-scale and backyard farms lack proper biosecurity measures, facilitating virus spread.
3. **Illegal Poultry Trade and Movement:** Informal trade and unauthorized transportation of live birds contribute to disease transmission.
4. **Diagnostic Limitations:** Rapid and accurate NDV detection remains challenging in some regions, leading to delays in outbreak response.

Government Policies and Control Programs: Iran has implemented several national programs for ND control, including mandatory vaccination, surveillance systems, and biosecurity regulations. However, enforcement challenges and non-compliance among some producers reduce the effectiveness of these programs.

Future Directions: Strategies for Newcastle Disease Control: To effectively manage ND in Iran, the following measures are recommended:

1. **Enhanced Surveillance:** Developing molecular surveillance to monitor NDV evolution and detect emerging strains.
2. **Improved Vaccine Development:** Investing in next-generation vaccines tailored to Iranian NDV strains.
3. **Strengthening Biosecurity Measures:** Implementing stricter regulations and educating poultry farmers on best disease control practices.
4. **Public-Private Sector Collaboration:** Encouraging private sector participation in vaccine production, distribution, and control programs.

5. **International Cooperation:** Establishing collaborations with global veterinary institutions for research and disease monitoring.

Conclusion:

Newcastle disease remains a major threat to Iran's poultry industry, necessitating a multifaceted control approach. Despite advancements in vaccination and surveillance, challenges such as vaccine failures, emerging strains, and weak biosecurity measures hinder complete eradication. A combination of extensive surveillance, improved vaccines, and stricter regulations will play a crucial role in mitigating the impact of ND in Iran. By adopting a scientific and preventive approach, Iran can better protect its poultry sector against future Newcastle disease outbreaks.

Broiler multi-causal respiratory diseases: Points of Consideration

Hossein Hosseini
PCR Veterinary Diagnostic Laboratory, Tehran, Iran
hosseini.ho@gmail.com

A lot of information is available about pathogens responsible for poultry respiratory disease. However, the occurrence of diseases with single pathogen under farm conditions is rare and respiratory disease is usually the result of the involvement of two or more different agents. So, respiratory disease in broiler is known as multicausal respiratory disease. Respiratory disease in broiler commonly is result of multiple pathogens and unfavorable environmental conditions. Infectious bronchitis (IB), Newcastle disease (ND), Avian Influenza (H9N2), *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), *Escherichia coli* (E. Coli) and *Ornithobacterium rhinotracheale* (ORT) are most important agents known to be involved in respiratory disease. In addition, immunosuppressive agents including Marek's disease (MD), Infectious bursal disease (IBD) and Chicken infectious anemia (CIA) may adversely affect susceptibility to respiratory infections. Combination between two or more agents resulted in more sever respiratory diseases. This combination is known to be responsible for great economic loss in broiler flocks in Iran. Because of multiple agents, identifying the role and importance of each of them is of great importance in the control and prevention of complex respiratory disease. Based on clinical sample results infectious bronchitis and H9N2 are two most important and prevalent agents responsible for respiratory disease and economical loss in broiler flocks in Iran. In this presentation more details will be provided about them and the role of Newcastle disease virus in respiratory disease.

Immunogenicity of Inactivated H5 Avian Influenza Vaccine Used in Commercial Laying Pullet in Tehran Province, Iran

Karami, S
Iran Veterinary Organization, Tehran, Iran
karamivet2005@gmail.com

Karami, S1, Karimi, V1*, Barin, A2, Rouigari, MR3, Bozorgmehri Fard, MH1

1. Department of Poultry Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2. *Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
3. *Department of Poultry Diseases, Iran Veterinary Organization, Tehran, Iran*

Highly pathogenic avian influenza (HPAI) is a viral disease caused by some H5 and H7 subtypes of influenza virus type A in most species of birds, especially poultry. HPAI viruses are among the most challenging viruses that threaten both human and animal health. Consequently, various strategies, such as the use of vaccines have been proposed to control the disease. After a catastrophic pandemic and the failure of conventional methods (elimination and extermination) in Iran, multiple vaccines have been used to control the disease. This study investigates the immunogenicity of two recombinant inactivated commercial vaccines of H5N1 and H5N3 subtypes in laying pullet flocks in Tehran Province, Iran. From 32 halls in six breeding units of laying pullets, 3,200 sera, and 800 tracheal and cloacal swabs were collected. After collecting the samples, Serum neutralisation (SN) and hemagglutination inhibition (HI) tests were conducted on sera to determine the serum titers of H5 specific antibody obtained from vaccine inoculation in three steps: before, after the first vaccination, and after the second vaccination (booster). The SN and HI tests were carried out by the alpha and beta methods on the pooled samples by the vaccine type (as antigen for HI and SN), and the results were compared. The PCR was performed on the tracheal and cloacal swab samples to possibly detect the HA (H5)virus in the studied flocks. The HI test results showed that both vaccines had a Serum antibody titre above 5 (log2) after two vaccination rounds, indicating a desirable immunogenic response. The SN test results also showed a neutralisation index above 104.5 for both vaccines, indicating more than 50% reduction in antigenicity of the virus. The PCR results were negative. This study was the first investigation of immunogenicity following two-time vaccination against H5 subtype vaccines in Iranian poultry flocks, indicating suitable antibody titer against the influenza virus in vaccinated flocks.

Keywords: Heamagglutination inhibition, Highly pathogenic avian influenza, PCR, Serum neutralisation, Vaccine

The principles of drinking water management in poultry farms

Seyed Ahmad Madani
Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
madani@ut.ac.ir

Introduction: Water is the most essential nutrient; however, its accessibility and quality for avian species are frequently overlooked. Poultry drink almost twice the amount of water compared to their feed intake. Consequently, the quality of drinking water is a critical factor influencing their health and productivity. It is essential that the chemical and microbiological characteristics of poultry drinking water meet acceptable standards.

Current status: In a study aimed to assess the chemical and microbial quality of drinking water from poultry farms across various provinces in Iran, 311 water samples were collected from 18 provinces. Significant variations in chemical parameters were observed across the provinces and sampling years, while seasonal changes did not notably affect the chemical and microbial parameters. Well water was the most prevalent (96%) water source in poultry farms. Acceptable hardness levels were found in 88.9% of spring water samples and 43.9% of well water samples. Salinity levels were favorable in 100% of spring water and 66% of well water samples. The detection of *E. coli* did not reveal significant seasonal differences. The results indicated that both chemical and microbial parameters exceeded standard thresholds in numerous poultry farms across different provinces, potentially compromising health and production outcomes. Therefore, it is imperative that many poultry farms implement water purification and sanitation systems, and that regular monitoring of water quality is an obligation in all facilities.

Physical evaluation: Drinking water temperature should be at most equivalent with room temperature. 5-10℃ lower temperature is usually favorable. Drinking water should be completely clear, colorless, tasteless and odorless. Any color may be considered a sign of contaminants. Turbid water is less palatable and can cause clogging of the water system. A pH of 6.5 to 8.0 is acceptable for poultry.

Chemical evaluation: While numerous chemical parameters can be evaluated, salinity and hardness are particularly significant factors. Total hardness or carbonate hardness are both applicable in poultry farms. Total dissolved solids (TDS) is a valuable parameter for on-farm evaluation of the drinking water as it could be assessed using a pocket size digital TDS meter. Iron, nitrate, nitrite, heavy metals, sulfate and other salts or minerals can be evaluated in the water, but they are much less important than hardness and salinity regarding the poultry health and production.

Microbiological evaluation: Drinking water can serve as a vector for various pathogens, especially in cases of sewage contamination, necessitating the assessment of microbial indicators. Total viable

count (enumeration of mesophilic bacteria), coliform count and *Escherichia coli* detection in the water are three precious microbiological tests for the evaluation of drinking water in poultry farms. TDS or electric conductivity is also an indicator for microbial contamination.

Water sources: Well, qanat, spring, surface waters and tap water are the main sources for poultry farms. More than 90% of poultry farms provide their water from ground wells. The wells can be categorized based on their construction and depth. They can be dug, driven and drilled, or shallow (<50 m), intermediate (50-100 m) and deep (>100 m). Microbiological contamination is usually less frequent in deeper wells. The chemical characteristics of well water mostly depend on the geological situation of water table. Chlorine retention in tap water is harmful of vaccination via drinking system.

Conceptual planning: Water availability should be definitely assessed while running a feasibility study for construction of a poultry farm. The availability in neighboring lands and/or geographic location of the planned farm in a region with huge amount of precipitation cannot guarantee the water accessibility in the farm. There are many farms in Northern Iran with great challenges in water resources and availability throughout the year.

Structure of watering system: Water supply for at least three days of routine consumption should be preplanned on each poultry farm. Sanitation of watering system including vessels, tanks, and tubes are as important as disinfection of drinking water. Cleaning and disinfection of water distribution system is a vital operation between each flocks. Descaling and biofilm removal should be performed intermittently or even continuously. Different type of drinkers are available on the market. Advantages and disadvantages of each system should be considered.

Water purification and disinfection: Different physical and chemical methods are used for water purification and sanitation. Sedimentation tanks/vessels are applied for reducing the turgidity and sediments. Turbid water should be filtered prior to use. Sand and active charcoal are commonly used for filtration at first place. Paper filters with different pore size (1-10 µm) are used for filtration. Reverse osmosis is the most efficient method for water purification. However, in addition to required capital investment for this system, the maintenance is costly. Chlorine is still the most effective water disinfectant. Water chlorination can be accomplished by different products like sodium hypochlorite, calcium hypochlorite, chlorine dioxide, and sodium dichloroisocyanurate. Ozone, hydrogen peroxide, peroxyacetic acid, and potassium peroxymonosulfate are alternative oxidizing agents that are applied for water sanitation. Ozone and chlorine gases required dedicated facility and equipment for application. A cost effective dosing pump system is essential for most combined chlorine substances. Peroxide based disinfectants are mostly used manually in the supply tanks. Free chlorine can be easily evaluated with colorimetric orthotolidine test (OT) on farm. At least 3 ppm of chlorine is needed for efficient water disinfection.

Conclusion: Drinking water in poultry farms should be tested for salinity (<100 ppm), total or carbonate hardness (< 300 ppm) and microbiological contamination (TVC < 500/ml and free of fecal coliforms) twice each year. Cleaning and disinfection of watering system is an important part of C&D program in each farm and should be thoroughly managed and monitored by poultry veterinarians.

Zoonoses in pet birds: A Review, Perspectives and Control Strategies

M Sadegh Madadi
Associate Professor,
Department of Clinical Sciences, Faculty of Veterinary Medicine,
University of Tabriz, Tabriz, Iran
madadims@gmail.com

The term "pet bird" refers to birds kept and bred primarily for ornamental purposes. Pet birds can carry organisms that may pose an infectious risk to humans. Among the most popular groups of pet birds are psittacine, passerine, and columbiform species. Zoonoses can manifest as parasitic, fungal, bacterial, or viral diseases. Some of these conditions, such as Chlamydophilosis, Salmonellosis, and highly pathogenic Avian Influenza A, can significantly impact human health. This paper aims to explore the risks faced by individuals who handle birds. It will evaluate the potential health and economic impacts on the broader human population and provide recommendations for reducing the transmission of diseases from birds to humans.

The primary mode of transmission and dissemination occurs through direct contact, while in limited circumstances, it can also occur via vectors. Pathogens are released from the feathers, feces, and various secretions of infected birds, including saliva, ocular fluids, tracheal secretions, and other bodily fluids. These infectious agents can contaminate food dishes, water bowls, and the surrounding environment, including the birds' cages and the soil. Veterinarians, pet bird owners, ornamental bird breeders, zoo personnel, and workers in pet industry facilities represent the occupational categories most at risk of encountering birds and their associated zoonotic diseases. The probability of an individual acquiring a disease from a bird is quite low. Individuals with underdeveloped or weakened immune systems are particularly vulnerable to various diseases.

Major Zoonotic diseases

Psittacosis: Chlamydiosis, also known as psittacosis, is an infectious disease caused by the bacterium Chlamydia psittaci. The transmission of this organism occurs through exposure to feather dust and fecal matter.

Salmonellosis: is an infection caused by various species of Salmonella bacteria. The transmission of these bacteria occurs via feather and fecal dust, in addition to being spread through contaminated eggs.

Campylobacteriosis: is an infection caused by the bacterium Campylobacter jejuni, which can inhabit the small intestines of both symptomatic and asymptomatic avian species.

Collibacillosis: is an infection caused by Escherichia coli bacteria, which are typically present in the intestinal tracts of all animal species.

Mycobacteriosis:, also known as avian tuberculosis, is caused by the Mycobacterium avium, which is transmitted through food and water contaminated with feces.

Newcastle disease: is an infectious condition caused by the Paramyxovirus, which is excreted through oral and respiratory secretions, as well as fecal matter.

Avian influenza A virus H5N1, when administered intranasally, has been shown to induce clinical symptoms that can result in mortality in pet bird species, such as zebra finches and common budgerigars. While the overall risk posed by avian influenza to the general population remains low, as these viruses typically do not infect humans, H5N1 stands out as one of the rare avian influenza viruses that have successfully crossed the species barrier to infect human hosts.

Allergic alveolitis: also known as bird breeder's lung, is a condition characterized by a hypersensitivity reaction to feather dander, dust, and fecal particles.

Cryptosporidiosis: is an illness induced by a protozoan belonging to the genus Cryptosporidium. In avian species, this disease can lead to respiratory complications, as well as gastrointestinal disturbances including gastroenteritis and diarrhea. In humans, cryptosporidiosis is characterized by symptoms such as abdominal discomfort, nausea, and profuse watery diarrhea, which generally lasts for a duration of three to four days.

Erysipelothrix rhusiopathiae: a Gram-positive, catalase-negative, rod-shaped bacterium, is primarily associated with eye disease in turkeys, ducks, and budgerigars, although its presence is not confined to these species. In humans, this bacterium causes a systemic illness that is characterized by skin lesions and joint disorders.

Aspergillosis: caused by Aspergillus fumigates. Observations may reveal yellow to gray nodules or plaques located in the lungs, air sacs, and trachea, as well as plaques present within the peritoneal cavity of birds and may lead to recurrent hemoptysis and, in some instances, can result in significant, potentially lethal hemorrhaging in both humans and avian species

Other diseases: include Yersiniosis, Lyme disease, Pasteurellosis, Erysipeloid, Listeriosis, Spirochetosis, Staphylococcal infections, necrotic enteritis, botulism, nontuberculous mycobacteriosis, giardiasis, sarcocystosis, toxoplasmosis, avian mite dermatitis, histoplasmosis, Q fever, and cryptococcosis.

Avoiding Trouble: The transfer of zoonotic pathogens from animals to humans can be significantly reduced through the implementation of basic hygiene practices. It is essential to ensure that clothing and footwear are thoroughly cleaned following any interaction with other birds, such as during bird

club meetings, bird fairs, or when handling pet birds, thoroughly wash your hands both prior to and following any interaction with birds, which includes the cleaning of their cages. It is recommended to clean the cages on a weekly basis.

For exotic pet birds that have been imported, it is necessary to provide a veterinary certificate, a passport, and an import authorization along with the birds.

Veterinarians ought to inform pet owners by supplying comprehensive documentation that outlines the warning signs associated with infectious diseases in pet birds. Biosecurity measures and hygiene protocols should be implemented in facilities dedicated to breeding and selling birds. It is essential to enforce a quarantine period for newly arrived birds as a critical preventive strategy.

Phenotypic investigation of circulating avian influenza viruses

Masoud Moghaddampour
Razi Vaccine & Serum Research Institute, Karaj
M.MOGHADDAM@rvsri.ac.ir

Iranian researchers perform genotypic analysis of many isolates of H9N2 influenza virus every year. By discovering the genotypic changes, they announce the possibility of the emergence of a new virus, and it is generally indirectly induced that the vaccinal seed virus of the commercial vaccine is not effective and must be replaced. To prove the newness of the isolated virus, as CDC researchers have said, it is necessary to check the performance of the virus phenotype against the standard antiserum with a specific HI test. That if the standard antiserum cannot completely neutralize the isolated virus, the genotypic changes have reached such a level that it has changed the phenotype of the virus. According to phenotypic investigation in the first phase, three H9N2 isolates, which are A, B and C, and the vaccine strain were propagated in SPF eggs. Then polyvalan serum against H9N2 avian influenza vaccinal virus strain of primary isolate (1377) was prepared. Hemagglutination inhibition test was performed with the harvested antigens and standard antiserum. The comparison of hemagglutination inhibition between the three isolated viruses and the vaccine virus was evaluated. The results showed that, among the three selected virus isolates, only sample C showed a difference of five wells compared to the vaccine virus. This means that the standard antiserum could not completely inhibit the hemagglutination of C virus. The results of the isolates A & B were almost identical to the vaccinal influenza virus. To ensure the accuracy of the test results, the test was repeated three times and the results of each 3 repetitions were almost the same for each virus.

Key words: Avian influenza, HI test, phenotypic investigation, vaccine seed, circulating virus

New methods for design and development of poultry vaccines

Zohreh Mojahedi
Razi Vaccine & Serum Research Institute, Karaj
z.mojahedi@rvsri.ac.ir

Zohreh Mojahedi, Ali Rezaei Mokaram, Naser Ghodsian, Seyed Reza Ebrahimi, Nadieh Shiri, Mohammad Abdoshah

With the rapidly increasing demand for poultry products and the current challenges facing the poultry industry, the application of biotechnology to enhance poultry production has gained growing significance. Biotechnology encompasses all forms of technology that can be harnessed to improve poultry health and production efficiency. Notably, biotechnology-based approaches have fueled rapid advances in biological research, including production of biological products such as vaccines and various types of immunostimulants to increase the defensive activity of the immune system against pathogenic infection However, recent strides in vaccine technology are demonstrating significant promise for disease prevention and control.

Until recently, vaccine technologies encountered many technical challenges associated with vaccine formulation, delivery, and the durability of protection.

The conventional vaccine delivery system in poultry involves routes such as oral, nasal spray, wing web injection, and intramuscular and subcutaneous injections. The oral route for vaccination is the most common and non-invasive method. This review focuses on the evolving some applications of biotechnology such as Gel-based & EFF tablet vaccines aimed at enhancing vaccine immunogenicity, efficacy, stability, and delivery

Live attenuated vaccines and Protection against avian infectious bronchitis variants

Najmeh Motamed
Poultry Vaccine Production Department, Razi Vaccine and Serum research Institute, Agricultural Research, Education and Extension Organization, Karaj, Iran
drmotamed95@gmail.com

One of the most contagious viral diseases in chicken flocks, have been frequently reporting around the world since 1930s, Avian Infectious Bronchitis (IB) disease, The disease primarily impacts the respiratory, urinary, and reproductive systems, leading to significant clinical issues and substantial economic losses for the poultry industry. The IBV genome is a single-stranded, positive-sense RNA, 27.6 kb, making it the largest among RNA viruses. Its evolution is driven by a low proofreading capability, coupled with natural mutations and recombination events, resulting in various genotype groups and lineages. Currently, IBVs are classified into seven genotype groups (Group I to Group VII), which include 35 distinct lineages and several unassigned inter-lineage recombinants. The Massachusetts (Mass) serotype was the first to be developed vaccine, and other strains belonging to Mass including H120 and Ma5 are used all over the world. 793B is the other serotype widely distributed in Europe, Asia, and other parts of the world. In the 1990s Strains identified as the 793B serotype, including CR88 and 1/96, 793B emerged. Moreover, the distribution and multiplicity of IBV genotypes vary with different geographical areas. The major IBV serotypes are characterized in Massachusetts in the USA, 4/91 (793B or CR88) in the UK, D274 in Europe and QX-like reported in China. Different types of serotype or genotype of IBV failed to cross-protection against multiple genotypes due to continuous evolution of IBV. Core controlling strategy is vaccination in combination with biosecurity measures in poultry farms. Factors influencing Chicken immune responses to IBV vaccination depends on route of administration, type of vaccine, vaccine strain and bird age. Today live attenuated and inactivated vaccine are used either individually or in a combined regime. Quality IB vaccines in both types are available, but what complicates the situation is the number of the virus variants continues to increase and it is impossible to develop vaccines against each new variant. Consequently, it is desirable to implement studies under organized conditions to define which IBV vaccine(s) deliver the best protection against different virus challenges and minimizing losses due to virulent infections. In spite of being laborious, not economical and practical in large farms the gold standard is oculonasal route however usually in commercial poultry systems, live attenuated vaccines are administered by drinking water, spray or aerosol methods at first day or week of age. Farmers normally follow rotational vaccination programs for controlling IBV; However, the incidence of disease has become a repeated occurrence with local variants indicates the failure of the vaccination

which is a consequence of the inability of birds to produce a proper immune response after vaccine administration. Different factors are associated with successful IBV vaccinations, such as the overlook of long-term immunity, the selection of the proper serotypes, and the timing of revaccination. Farmer's usually guilt the vaccine's lack of efficiency for miscarriage to immunize chicken flocks. According to recent studies more than 50% of vaccination failures are recognized as improper application of the vaccine not the different genotype or variant occurrences. Furthermore, incorrect or impaired cold-chain maintenance and storage are other important issues for vaccine failure leading to outbreaks of IBV in vaccinated farms. Stress is "the nonspecific response to environmental stimulation and is interpreted as "the result of extreme demands happens after the physiological and behavioral experiences of birds to adaptation". All kinds of stress can decline a bird's immune response, such as temperature instability, high humidity, poor quality feed, parasitic infestation, and immunosuppressive diseases. Vaccinations should be used in healthy and in good conditions birds to avoid stress. Glucocorticosteroid hormone are secreted following stress which causes adverse effects on the immune system. Research indicates that stress can hinder antibody responses to vaccines, particularly affecting thymus-related vaccines, with the most noticeable effects observed a significant time after vaccination.Another question is, Is there any differences between different route of vaccination when it comes to challenge or even immune response evaluations? In general occulo-nasal is the most reliable but less applicable route which induces mucosal immunity in upper respiratory system. In addition immune gene mRNA transcription profiles of IFNα, IFNβ and TLR3 in target tissues are significant indices. There is no doubt about that the innate, mucosal, cellular and humoral immune responses are vital for protection against the virus. Considering those determining factors vaccination protocol becomes a significant challengeable decision. The point is, What, How and when different vaccines must be used? "Protectotype concept" is the Immunization strategy with multiple serotype vaccines resulting in up to 90% protection against different types of IBV infection instead of homologous vaccines. So a well-designed vaccination regimen is essential, even for homologous IBV vaccines. A prime-boost vaccination regime with classical and variant IB virus's combinations provides broad spectrum protection. It is worth to mention that "IBV protectotype vaccine theory" presents a paradox: it can effectively counter newly emerged strains of IBV that may cross-react with widely used vaccines, yet it may also increase the mutation rate and biodiversity of these new genotypes. For example, heterologous IBV challenge viruses (QX) rapidly evolved under experimental conditions when mixed with the IBV vaccine (H120), raising concerns about the efficacy of heterologous vaccination in the field and it's potential to accelerate IBV evolution and the potential for damaging immune system of the bird. Initial works showed that broader protection against heterologous IBV types could be realized by giving two different IBV vaccine types either simultaneously or as a prime and boost. Since then, there have been a number of experiments examining protection discussing administration of different IBV vaccine types and three or four vaccine serotypes.

In conclusion, vaccination is essential as an aid in protecting birds against IBV infection. The better immune response is observed after homologous vaccination but Heterologous IBV protection is confirmed in birds and today it is done commonly and safely by using at least 3 different genotypes. Live priming is a beneficial part of the IBV vaccination program in breeders and IBV inactivated vaccine improves IBV protection after prime boosting with live vaccines. Next generation vaccines must not only be ignored but stay in parallel with protectotype research. Using recombinant viruses with different Spike glycoproteins with the ability to be grown on cell cultures or different subunit vaccines are promising the future of Infectious Bronchitis disease vaccination.

Isolation and Characterization of *Mycobacterium* Species from Poultry submitted to Reference Laboratory for animal Tuberculosis, Razi Vaccine and Serum Research Institute

Nader Mosavvari
Reference Laboratory for Bovine Tuberculosis, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran
nmosavari@gmail.com & n.mosavvari@rvsri.ac.ir

Nader Mosavvari[1]*, Keyvan Tadayyon[2], Shojaat Dashtopoor[1], Alireza Paradise[2]

Affiliations:

1. Reference Laboratory for Bovine Tuberculosis, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran
2. Department of Veterinary Aerobic Bacterial Research & Vaccine Production, Razi Vaccine and Serum Research Institute

Objectives: To isolate and identify *Mycobacterium* species from poultry samples and evaluate their molecular identity, antibiotic susceptibility, and strain diversity.

Materials & Methods:Since 2009, the reference laboratory for animal pathogenic mycobacteria at the Razi Institute has received samples from 18 poultry species. These samples, totaling 345, were processed using culturing, PCR amplification, molecular identification, antibiotic susceptibility testing, and genomic DNA fingerprinting with the RFLP-IS901 method using the PvuII enzyme. Samples included pigeons, peacocks, parrots, lovebirds, Israeli duck, pheasants, mallard ducks, phosphorescent geese, tawny geese, terns, geese, black swans, flamingos, white-tailed eagles, ostriches, vultures, owls, and turkeys.

Results & Conclusion: From the samples, 173 *Mycobacterium* isolates were obtained. Among these, 135 isolates belonged to the *Mycobacterium avium* complex, with 107 identified as *Mycobacterium avium subsp. avium* and 28 as *Mycobacterium avium* subsp. *silvaticum*. Additionally, 11 isolates of *Mycobacterium intracellulare*, 15 isolates of *Mycobacterium fortuitum*, and 12 isolates of other *Mycobacterium* species were identified.

Clinical signs included swollen joints in pigeon legs and wings and atrophy of breast muscle. The liver was the most affected organ in necropsy findings. No significant difference in susceptibility between male and female was found. Disease manifestations were categorized into four forms: classic tuberculosis, intestinal form, liver form, and non-TB form. The study confirmed infections by *Mycobacterium avium subsp. avium* through clinical signs, necropsy findings, histopathology, ZN staining, and molecular identification. PvuII–IS901 RFLP typing produced 15 patterns. Drug susceptibility testing showed resistance to Streptomycin, Kanamycin, Ethionamide, and Thiophene carboxylic acid hydrazide, but susceptibility to Isoniazid, Rifampin, and Ethambutol.

Keywords: *Mycobacterium*, *Mycobacterium avium* complex, Poultry, PCR, Antibiotic susceptibility

Exogenous Enzymes: Modulators of Poultry Gut Health, Efficiency, and Sustainability

Seyed Naser Mousavi
Department of Clinical Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran
snmousavi@hotmail.com

Exogenous enzymes play a pivotal role in optimizing gut health, improving feed efficiency, and promoting sustainability in the poultry industry. Recent studies reveal that the improvement in growth performance goes beyond the release of indigestible nutrients. The positive impacts extend to the intestinal microbiota, immune system, and antioxidant status. Enzymes mitigates the negative effects of anti-nutritional factors commonly found in feed ingredients, such as non-starch polysaccharides and phytates. They help improve gut health in poultry by shifting digestion to the anterior gut, producing fermentable oligosaccharides that benefit intestinal pH, and supporting mucin integrity. Proteases can enhance gut stability by reduce undigested protein flow to the gut and minimizing substrates for harmful bacteria and promoting beneficial microbes. The use of mannanases has been shown to reduce the inflammatory effects of Galactomannans, with studies indicating the greatest benefits in older birds, suggesting that the effects may also involve prebiotic or stimbiotic mechanisms alongside the direct removal of inflammatory agents. High levels of phytase, dephosphorylate phytate to inositol monophosphate (IP1), which is further dephosphorylated by intestinal alkaline phosphatase (iALP) in the small intestine. iALP plays a crucial role in preserving intestinal integrity by dephosphorylating inflammatory components like lipopolysaccharides (LPS) and other pathogen-associated molecular patterns (PAMPs), thus enhancing gut health and stimulating the immune system. These effects support gut resilience and protect against unwanted bacterial growth, making exogenous enzymes a promising alternative to antibiotics. This is especially important in modern poultry production systems, where digestive disorders are a significant challenge and can have profound effects on performance and welfare. From an environmental sustainability perspective, the incorporation of exogenous enzymes reduces the excretion of nitrogen and phosphorus. By improving nutrient digestibility and reducing the volume of undigested feed excreted, enzymes help minimize the environmental footprint of poultry production. This is of increasing importance in the context of global concerns regarding waste management, resource utilization, and the environmental impacts of intensive livestock farming.

Key Words: Exogenous enzymes, poultry, gut health, sustainability

Application of artificial intelligence in laboratory diagnosis of poultry disease

Gholamreza Nikbakht Brujeni
Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Nikbakht@ut.ac.ir

Poultry farming faces numerous challenges from infectious and non-infectious diseases that impose high costs on the industry. Since most clinical measures to combat and control diseases are based on valid laboratory diagnoses, the preliminary and crucial step is to obtain reliable and evidence-based data that can be analyzed. These data, which is obtained through various laboratory diagnostic methods, can sometimes be multitude, voluminous and requires storage, curation, classification, comparison, and complex analyses. Typically, biological samples such as blood, feces, or respiratory secretions are widely used for laboratory diagnosis of diseases. However, current advancements in computer science have made it possible to use image, audio, and digital samples for diagnosing various conditions. One of the advanced methods for analyzing and diagnosing diseases is the use of artificial intelligence and machine learning, which are applicable to various biological and informatics samples. Artificial intelligence has several effective applications in laboratory diagnoses of poultry diseases that can help improve the accuracy and speed of diagnosis. Pattern recognition in microscopic images, predictive modeling and determining references range and cut-off points for analyzing quantitative serum and biochemical tests, genetic data analysis, diagnosis through sound analysis, and diagnostic imaging are among the areas that have been researched so far. In this discussion, we will elaborate on the fundamentals of artificial intelligence and machine learning and highlight some of their applications for laboratory diagnoses.

Molecular characterization of IBD virus in Iran

Hassan Norouzian
Department of Clinical Sciences, Faculty of Veterinary Medicine,
Lorestan University, Khoramabad, Iran.
Noroozianh@yahoo.com

Infectious Bursal Disease (IBD) virus is responsible for a highly contagious and immunosuppressive disease in chickens. Typical macroscopic and histopathological lesions seen in the bursa of Fabricius are common findings in infected flocks, making them more vulnerable to opportunistic pathogens and leading to failures in vaccination.

Traditionally, IBD virus strains have been classified into four groups based on their phenotypes: classic, very virulent (vvIBD), variant, and attenuated. However, due to increasing difficulties in classifying new isolates using this traditional system, researchers have recently shifted to a classification based on "gengroups."

To explore the molecular characteristics of the IBD virus, researchers often sequence and analyze the hypervariable region of the VP2 gene (hvVP2). Recent studies of Iranian isolates have shown that these strains possess amino acid residues typical of very virulent IBD (vvIBD) viruses. Notably, some Iranian vvIBD isolates have demonstrated a unique G to S mutation at position 254, a mutation not found in prototype vvIBD isolates. Additionally, some attenuated isolates showed the mutation at position 253Q, which is absent in the D78 vaccinal strain.

The high degree of similarity among the hvVP2 sequences of Iranian vvIBD isolates suggests that they share a common ancestor. Phylogenetic analysis indicates that Iranian vvIBD isolates cluster with other isolates from the Middle East. Furthermore, recent studies in Iran have confirmed the ongoing circulation of vvIBD strains, highlighting the need to reinforce IBD control strategies. Therefore, additional research on the genetic characteristics and pathogenicity of new isolates is crucial for accurately assessing the current status of IBD in Iran.

Keywords: Infectious Bursal Disease virus, vvIBD, molecular characterization, phylogenetic analysis.

A review on Infectious Coryza in Iran

Abbas Nouri

Department of Avian Disease Research and Diagnostic, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

nouri@rvsri.ac.ir

Infectious Coryza (IC) is an acute respiratory disease in poultry caused by the *Avibacterium paragallinarum*. IC has a global distribution. In recent years, there has been reports of causative agent identification in laying hens and local poultry flocks In Iran. Infectious Coryza is one of the diseases that veterinarians can easily diagnose clinically, especially in local birds, because vaccination is not carried out in these flocks, and the characteristic symptoms of the disease are in typical forms. In commercial farms, particularly in parent flocks and commercial laying hens, the use of the infectious Coryza vaccine alongside other vaccines is included in the vaccination program before the egg production period begins. The occurrence of the disease in these types of flocks, due to initial immunization, may manifest clinically if there is not a complete match between the prevalent strains and the vaccine strains. In this case, due to the mildness of the disease symptoms, it may always be misdiagnosed with other respiratory diseases or coexisting illnesses and remain unnoticed. On the other hand, due to the lack of suitable serological tests, there is no way to estimate the spread of the disease except through molecular diagnostic tests and isolating the bacterial agent from clinical samples. These two issues are among the challenges in mapping and assessing the status of this disease in country. The economic losses from this disease are relatively significant, manifested in the costs of vaccine imports, antibiotic use along with the risks of drug resistance, and reduced production efficiency. Studies conducted so far in Iran have been limited to isolations and determining the antibiotic susceptibility of the causative agent. Therefore, information regarding the epidemiology of the prevalent strain of this *Av. paragallinarum* in the country and the level of immunity coverage of imported vaccines against local strains is not available. Here, a report on the findings of research conducted at the Razi Vaccine and Serum Research Institute, which has been more extensive in terms of geographical and isolation rate in recent years, will be briefly reviewed. This study involves the isolation of the *Av. paragallinarum* from several provinces in the country, which underwent antimicrobial sensitivity testing and genotyping .

HVT vector-based ND vaccine (rHVT-ND) can strongly suppress challenge virus shedding and prevent virus transmission in vaccinated population.

Vilmos;Palya;

Scientific Support and Investigation Unit, Ceva-Phylaxia Vet. Biol. Co., Budapest, Hungary

palya@ceva.com

Objectives: Vaccination against Newcastle disease (ND) is applied in many parts of the world. The goal of vaccination is usually aimed at preventing clinical disease, mortality, and production losses. However, beside these aspects, it is also an important requirement how effective is the vaccine in preventing viral spread.

Herpesvirus of turkeys (HVT) has become a widely used vector for poultry vaccines since it is proven to be a very safe and stable virus. One of the commercially available vector vaccines expressing the F gene of a genotype I NDV (Vectormune® ND) has already been shown to provide high level of protection against challenges with different genotypes of velogenic ND virus (vNDV) isolates in commercial broilers and layers. However, when assessing the efficacy of a vaccine, apart from measuring the level of clinical protection and the suppression of challenge virus shedding, it is also a critical parameter how efficiently it can prevent virus transmission in vaccinated population. In the study, which results are going to be presented, the effect of a single rHVT-ND vaccine administration at day-old was examined on the transmission rate of genotype VII vNDV within a group of commercial broilers with maternally derived antibodies to NDV.

Materials & Methods: At hatch, day-old broiler chicks were allocated to two groups. One of them was vaccinated with one dose of rHVT-ND vaccine (Vectormune® ND), while the other group was left without vaccination. Blood samples were taken on the day of hatch to determine the maternally derived antibody level against NDV.

Six weeks post-vaccination, broilers of both groups were assigned into two subgroups (i.e., seeder and contact). Seeder subgroups were challenged intranasally with a viscerotropic velogenic NDV (representative of genotype VII.2 (VIIi), and eight hours post-challenge contact subgroups were introduced to the corresponding seeder subgroup. During the post-challenge observation period of 14 days, clinical signs and mortality were recorded; and oro-nasal and cloacal swabs were collected daily. Blood samples were collected as well at the termination of the study. Measurement of challenge virus shedding was done by One-Step RT-Real-Time PCR. Transmission was quantified

by the reproduction ratio (R), which is the average number of new infectious animals produced by one infectious animal during its entire infectious period.

Results & Conclusion: No clinical signs or mortality attributable to the vNDV infection was seen in the vaccinated subgroups during the 14-day post-challenge observation period, and virus excretion was strongly reduced, compared to the unvaccinated group where all challenged chickens died and showed strong shedding of challenge virus.
Unvaccinated contact birds had a mean infectious period of 4.8 days, which was significantly longer than the infectious period of 3.4 days for the vaccinated contact birds. The reproduction ratio (R) for the unvaccinated animals was estimated at 3.20, which was significantly above the threshold for epidemic spread, while in the vaccinated animals the reproduction ratio was 0.82.
This experimental study showed that rHVT-ND vaccine was able to markedly reduce the transmission of vNDV infection within the vaccinated group (R was below 1), indicating that the infection would fade out after introduction in a vaccinated population. This study showed also the potential of rHVT-ND to prevent and control ND outbreaks even when they are caused by a virus belonging to a heterologous genotype than the vaccine insert. As such, this vaccine is suitable for use in vaccination programs to eliminate vNDV from chicken populations and prevent outbreaks upon introduction of the virus. It should also be noted that rHVT-ND is not normally applied as a single ND vaccine in areas with high ND virus pressure. The combination of rHVT-ND with live vaccines that is the usual prevention strategy in endemic areas, will probably more efficiently reduce the transmission rate.

Keywords: Newcastle disease, Vaccination, Recombinant HVT-ND vaccine, viru shedding and transmission.

Effective vaccination in coccidiosis control

Kaveh Parvandar Asadollahi
Department of Clinical Sciences, Faculty of Veterinary Medicine,
Science and Research Branch, Islamic Azad University, Tehran, Iran
kaveh_parvandar@yahoo.com

Coccidiosis is a major concern in poultry production due to its negative impact on nutrient absorption and growth. The resilience of Eimeria parasites to environmental changes and their prolonged infectivity make coccidiosis one of the most persistent and challenging diseases in the poultry industry. As the leading enteric disease in poultry, coccidiosis causes global annual losses exceeding USD 14.5 billion, including mortality, treatment and prevention costs. It accounts for approximately 30% of total pharmaceutical expenditures for poultry diseases control.

In poultry houses, each successive coccidial infection cycle exponentially increases the number of oocysts in the environment. Without anticoccidial intervention or the development of immunity, naïve birds face overwhelming exposure to infective sporulated oocysts, leading to severe disease outbreaks. Anticoccidial drugs, introduced in the 1950s alongside the modern poultry industry, have been widely used for coccidiosis control. However, repeated exposure has led to drug resistance, a genetic trait that, once established in Eimeria strains, can persist for many years.

To address this challenge, vaccination has emerged as the most practical and sustainable approach to coccidiosis control. The first live coccidiosis vaccine was developed over seven decades ago, and in 1992, vaccination programs for laying pullets, commercial broilers, and replacement breeders began in the EU. Unlike anticoccidials, vaccines stimulate a protective immune response without the risk of resistance development. However, because Eimeria species confer species-specific immunity, a vaccine will only protect against the specific strains included in its formulation. T-cell-mediated immunity plays a key role in this defense mechanism.

Various vaccine types and administration methods including drinking water, spray, eye-drop, and in-ovo injection—are used in both field and hatchery settings. The success of coccidiosis vaccination depends on three critical factors: vaccine efficacy, uniform vaccine uptake, and successful recycling of vaccine-derived oocysts in poultry houses.

Proper vaccine selection is essential, as we have various vaccine types and different vaccines contain varying Eimeria species.

Uniform vaccine uptake ensures all birds receive an adequate dose, which can be achieved through precise application techniques such as spray or in-ovo administration.

Successful oocyst recycling in the litter is vital for sustained immunity development. This requires optimal litter moisture control, proper stocking density management, and the avoidance of anticoccidial drug use post-vaccination to allow natural immunity to develop fully.

When these factors are well-managed, coccidiosis vaccination provides long-lasting protection, reduces disease outbreaks, enhances poultry performance, and decreases reliance on anticoccidial drugs promoting sustainable poultry production.

Comparison and interpretation of serological test results in the detection of *Mycoplasma synoviae* infection in Iranian breeder broiler farms

Seyed Ali Pourbakhsh

Mycoplasma Reference Laboratory, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization, Karaj, Iran

poursaba@yahoo.com

Pourbakhsh SA[1]*, Pourbakhsh SH[1], Ahangaran S[1], Salahi Z[1]

Objectives: *Mycoplasma synoviae* (MS) infection causes primary damage to the respiratory tract of poultry, leading to respiratory diseases in poultry and causing mortality, loss of production, and slaughterhouse elimination in industrial poultry. The most important way to control and prevent MS disease and infections is to identify infected breeder broiler and grandparent flocks and eliminate positive cases. Serological tests play a significant role in the control and prevention programs of this disease. Rapid serum agglutination (RSA), Enzyme-linked immunosorbent assay (ELISA), and hemagglutination inhibition (HI) tests are the most common serological methods used in the identification of infected flocks in diagnostic laboratories. The aim of this study was to compare and interpret the results of three serological tests (RSA, HI, and ELISA) in the detection of *Mycoplasma synoviae* infection in breeder broiler farms in Iran.

Materials & Methods: Total of 2463 sera belonging to 153 flocks from breeder broiler farms suspected of MS infection and disease from different provinces of Iran, which were sent to the Mycoplasma Reference Laboratory of Razi Institute, were simultaneously and separately tested for MS antibodies, in accordance with existing standards, by RSA, ELISA, and HI. The results obtained were compared with each other and each of the tests was evaluated in detecting MS contamination of farms.

Results & Conclusion: Total 2463 sera received, 1131, 1003 and 583 were positive in RSA, ELISA and HI tests respectively, and 1332, 1460 and 1880 were negative. 1131 positive sera in RSA, 982 (86.8%) were positive in ELISA and 575 (50.8%) were positive in HI. There was a high correlation coefficient (93.1%) between the results obtained from RSA and ELISA, while the correlation coefficient was 77.1% between RSA and HI. 1003 positive sera in ELISA, 573 (57.1%) were positive in HI, and there was a good correlation coefficient (82.1%) between the results obtained from ELISA and HI. Given the high sensitivity and low specificity of the RSA test, the high specificity and sensitivity of the ELISA test, and the high specificity and low sensitivity of the HI test, the use of the RSA test in initial screening for the identification and diagnosis of MS

infection in breeder broiler farms is recommended, and to eliminate false positives from the RSA test, the use of a supplementary ELISA test is necessary.

Keywords: *Mycoplasma synoviae*, Serological tests (RSA, ELISA, HI), Breeder broiler farms, Iran

Application of Artificial Intelligence in the Livestock and Poultry Industries

Shaban Rahimi
Professor Department of Poultry Science, Faculty of Agriculture,
Tarbiat Modares University, Tehran, Iran
rahimi_s@modares.ac.ir

Shaban Rahimi[1]*, Tahereh Ebrahimi[2], Haleh Ehtesham[2]
[1]Professor Department of Poultry Science, Faculty of Agriculture,
Tarbiat Modares University, Tehran, Iran
[2]Ph.D Candidate, Department of Poultry Science, Faculty of Agriculture,
Tarbiat Modares University, Tehran, Iran

Considering the rapidly increasing global population, the poultry and livestock sectors play a critical role in meeting the growing demand for meat, eggs, and dairy products. These industries serve as essential sources of food for many communities around the world. However, ensuring the health and welfare of broiler birds and livestock is paramount to achieving both optimal productivity and maintaining ethical animal treatment. In recent years, the livestock and poultry industries have embraced automation, leveraging technological advancements such as a wide array of monitoring and sensing tools, the Internet of Things (IoT) devices, and sensors. These technologies offer substantial benefits by enhancing production processes. Reliance on large-scale production solely through human labor presents significant challenges, especially considering the public health risks associated with consuming food contaminated with excessive antibiotic residues. Artificial intelligence (AI) has emerged as a transformative force in various sectors, including livestock and poultry production. AI technology offers significant opportunities to improve the efficiency, sustainability, and overall management of these industries. By utilizing AI, farmers can better monitor and predict animal growth, optimize feeding and resource use, combat pests and diseases, enhance biosecurity, and oversee both animal welfare and farm operations more effectively. AI-driven systems allow poultry and livestock farm owners to boost production while significantly reducing costs. This is achieved through intelligent data analysis, which can inform decision-making on crucial tasks such as animal activity monitoring, weather control on the farm, weight prediction, early disease detection, and more. AI's ability to identify potential health issues earlier helps ensure healthier animals and more productive farms. Ultimately, AI is paving the way for a more efficient and sustainable future in livestock and poultry production. This text expands on the original by improving clarity, refining the structure, and providing a more in-depth explanation of AI's role in the industry.

Artificial intelligence and poultry industry

Problems such as labor shortages, disease outbreaks, and concerns about animal welfare are a serious threat to production and profitability in the poultry industry. Artificial intelligence has been proposed as a potential solution to address these challenges in the poultry industry. Animal welfare and care are critical components of sustainable animal husbandry, however traditional methods may fall short in addressing various conditions of health, safety, behavior, and stress levels. Artificial intelligence can dramatically improve chicken farming conditions and increase productivity at the same time. This technology allows us to achieve a future where all processes are automated and optimized. AI is used as a powerful tool to collect, analyze and interpret different types of data, including visual and audio data, in studies related to animal behavior, welfare and environmental management. For example, by using artificial intelligence algorithms, it is possible to track the location of animals and identify their behavioral patterns. By rapidly identifying and addressing poultry diseases, we can ensure both bird health and the safety of food products. Therefore, the use of new technologies such as AI and the IoT significantly increases the efficiency and effectiveness of poultry farm management. In the next part, we will mention some of the applications of AI in the poultry industry:

Environmental requirements: Artificial intelligence can help improve the health, well-being and productivity of poultry by monitoring and controlling environmental factors such as temperature, humidity, light, ventilation and air quality.

The heat: Infrared thermal imaging technology is a valuable tool for poultry farms, offering benefits such as temperature control, early disease detection, and improved bird welfare. By monitoring body surface temperature, this technology can identify disease symptoms and facilitate appropriate measures to enhance bird well-being.

The aeration: AI can revolutionize poultry farm ventilation by optimizing air quality, reducing energy costs, and promoting bird health. By leveraging AI sensors and algorithms, it is possible to establish ideal ventilation settings and prevent the accumulation of harmful gases.

Age determination of poultry: AI technology can accurately predict the age of birds using machine learning algorithms. This enables farmers to make informed decisions that optimize breeding and management strategies.

Diseases: Using various data, AI can detect disease symptoms earlier than traditional methods and help predict disease outbreaks. Also, AI can be used in the development of new treatments and vaccines for poultry diseases. Additionally, digital technologies can streamline poultry management operations and enhance animal welfare.

Use of Robot for doing vaccine at Poultry House: Using AI, robots can vaccinate and treatments with high precision. These systems have access to bird health data and can make better decisions to maintain their health. As a result, the use of robots in poultry farms helps to reduce human errors, increase efficiency and improve the welfare of birds.

Artificial Intelligence and the Livestock Industry

The integration of artificial intelligence (AI) in livestock farming has transformed the industry, enhancing productivity, improving animal welfare, and optimizing farm operations. This review explores various AI applications, including disease detection, health monitoring, precision farming, and automation in livestock management. By facilitating early detection of diseases and continuous monitoring of health, AI allows farmers to intervene promptly, ensuring animal well-being and preventing major outbreaks. Key areas where AI is making significant strides include:

Automated Milking and Health Monitoring: AI-driven systems use sensors to monitor milk quality and animal health, providing real-time insights and predictions for potential issues.

Estrus Detection: AI-powered tools accurately detect estrus in dairy cows, optimizing reproduction processes and reducing human errors.

Precision Livestock Farming (PLF): AI helps monitor individual animal health, optimize feed efficiency, and improve farm productivity while addressing environmental sustainability concerns.

Vaccine Delivery and Disease Prevention: AI and robotic systems automate vaccine delivery and utilize pattern recognition from sensor data to prevent disease outbreaks.

AI and Blockchain for Food Traceability: AI enhances blockchain's capacity to track animal products from farm to table, ensuring food safety and transparency.

Drone Assistance: AI-powered drones equipped with sensors monitor large-scale livestock operations, aiding in identifying behavioral issues and pasture conditions.

Facial Recognition for Health Monitoring: AI can identify individual animals within herds and monitor health conditions based on facial expressions, such as pain detection.

Body Weight Prediction: AI systems utilizing 2D and 3D imaging, combined with machine vision, accurately predict animal body weight, reducing the need for traditional scales.

Artificial Meat Production: AI is revolutionizing lab-grown meat by optimizing cell cultures and growth environments, offering a sustainable and ethical alternative to traditional meat production.

The document also addresses the challenges AI faces in animal husbandry, such as data privacy, implementation costs, and the complexity of integrating these advanced technologies. However, as

AI continues to evolve, it promises to further revolutionize livestock farming, making it more efficient, sustainable, and capable of meeting the growing global demand for animal products.

Conclusion

In conclusion, the adoption of Artificial Intelligence in the livestock and poultry industries represents a significant step toward creating more efficient, sustainable, and humane farming practices. By addressing critical challenges such as disease prevention, environmental control, and labor shortages, AI is not only enhancing productivity but also promoting better animal welfare and food safety. As the technology continues to advance, it promises to revolutionize these industries further, ensuring that they can meet the growing global demand for animal products while minimizing their environmental footprint. With AI-driven systems becoming more accessible and refined, the future of livestock and poultry farming is poised to become more intelligent, automated, and sustainable, paving the way for a more resilient agricultural sector.

Keywords: Artificial Intelligence (AI), Animal Welfare, Automation and Sensors, Livestock and Poultry Production, Sustainable Farming

References:

Alipio, M., & Villena, L. V. (2022). Intelligent wearable devices and biosensors for monitoring cattle health conditions: A review and classification. *Smart Health*, 27, pp 100369-100.

Mateti, T., Laha, A., & Shenoy, P. (2022). Artificial Meat Industry: Production Methodology Challenges and Future. *JOM*, 74, No. 9, 2022

Patel, H., Samad, A., Hamza, M., Muazzam, A., & Harahap, M. K. (2022). Role of artificial intelligence in livestock and poultry farming. Sinkron: jurnal dan penelitian teknik informatika, 6(4), 2425-2429.

Taleb, H. M., Mahrose, K., Abdel-Halim, A. A., Kasem, H., Ramadan, G. S., Fouad, A. M., ... & Abd El-Hack, M. E. (2024). Using artificial intelligence to improve poultry productivity–a review. Annals of Animal Science. DOI: 10.2478/aoas-2024-0039

Why does the turbulence in the poultry industry not disappear?

Mostafa Seyed Mostafavi
Chairman of the board of directors of the poultry industry center for knowledge, technology and market
Email: mos1329@gmail.com

More than 70 years of the existence of this profession, which was established next to the farms and gardens, which became industrialized between 1960 and 1980, and even broiler meat and table egg production chains were formed, and the industry grew so much that there was no need to import chicken meat and even chicken meat and table eggs were exported to neighboring countries.

But there was always a place where the people of the industry had to go to discuss the market problems and their needs and the lack of needed liquidity and ask for help. The Islamic Revolution took place and immediately the imposed war broke out that lasted 8 years, but the industry was still growing.

But the need to supply raw materials for food, medicine, and vaccines had also become greater than before, and many companies were established to produce the needs locally for the industry, and many were formed for trade.

However, the owners of large investments in the huge poultry industry still spend most of their time in offices, banks, and customs offices so that they can preserve their capital and compensate for the shortage of foreign exchange and its daily changes, and negotiate with banks to provide liquidity shortages, or to deal with a market that changes product consumption under the influence of national and religious occasions, or to neutralize the inflationary prices that constantly create turbulence in the poultry industry.

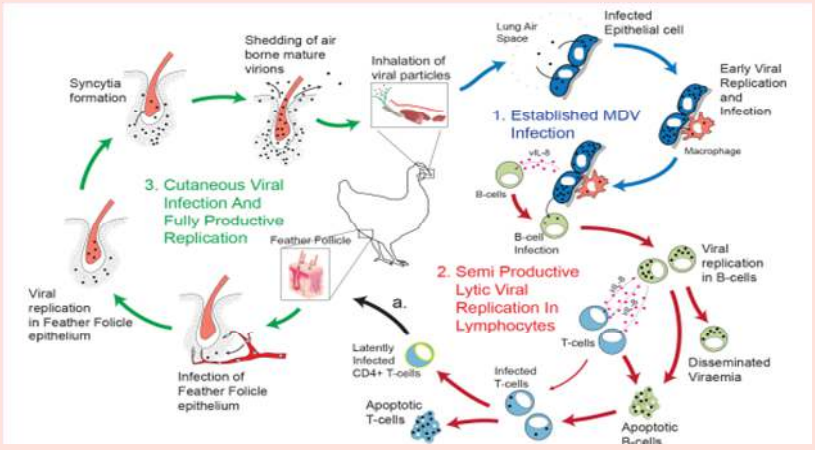
In this article, I will review the five-year country development plans, and especially the Seventh National Development Plan, in order to discuss the causes of management failures and to prevent further turbulence.

Differential diagnosis between marek's disease & avian leukosis,based on pathology

Farhang Sasani
Professor of Veterinary Anatomical Pathology,Faculty of Veterinary Medicine,University of Tehran, Iran
fsasani@ut.ac.ir

Marek's Disease:MD is prevalent all around the world and in fact all flocks are exposed to the effect of the etiological factor. Characterized after its human orthologue (Herpes Simplex Virus; HSV a DNA containing virus), Marek's disease virus (MDV), or Gallid herpesvirus 2 (GaHV-2), the etiologic agent for Marek's disease (MD) is an alpha-herpes virus,in Mardivirus genus, that targets avian species (Gallus gallus domesticus) where it establishes chronic infection. Notably recognized as a multifaceted disease, MD is characterized based on immunosuppression, neurological disorders and neoplastic transformation of CD4+ T cells, localised around peripheral nerves and visceral organs of the host.

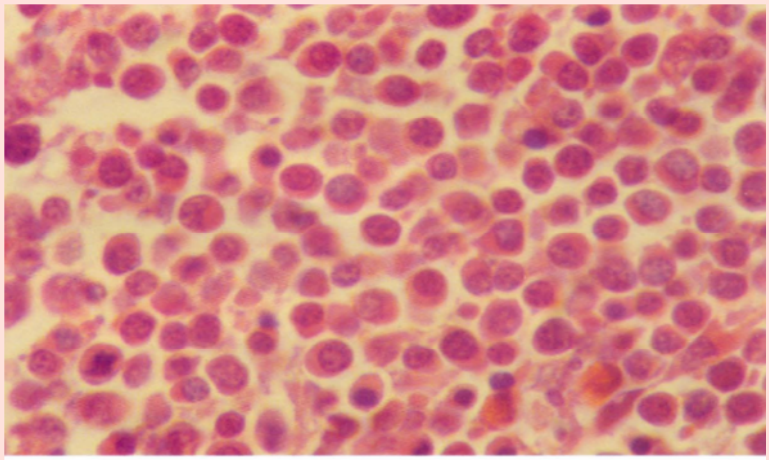
Diagnosis: As a ubiquitous pathogen present in many poultry farms, detection of virus, viral antigens or nucleic acids in the absence of disease does not confirm the occurrence of MD. Clinical disease with signs of paralysis of legs and wings, higher than normal levels of mortality with lesions of tumours in multiple organs, and enlarged peripheral nerves is often sufficient to make a positive diagnosis. Detection of viral- or tumour-specific antigens in tumours by immunohistochemistry is valuable for further confirmation of MD. PCR-based molecular diagnostic tests are increasingly used for the detection and quantitation of virus in clinical/farm materials. Availability of the nucleotide sequences of a number of pathogenic and vaccine strains of MDV has enabled the development of sensitive PCR methods for precise detection and quantitation of pathogenic and vaccine strains.



Model of MDV infectious life cycle in resistant birds

Some T cells, latently infected with oncogenic MDV strains, undergo neoplastic transformation. These transformed cells may multiply to form characteristic lymphoid neoplasms.
In addition to lymphoid neoplasms, Marek's disease virus can also induce other clinical syndromes, including:
transient paralysis
cytolytic infection
early mortality syndrome
atherosclerosis
persistent neurologic disease

Pathological Findings
Nerves: In affected birds enlarged nerves are one of the most consistent gross lesions. Various peripheral nerves, but particularly the sciatic, vagus, and brachial nerves, become enlarged and lose their striations. Goodchild reported that autonomic nerves and especially the celiac plexus are affected at a higher frequency than peripheral nerves, for example, the sciatic and brachial nerves. Diffuse or nodular lymphoid tumors may be observed in various organs, particularly the liver, spleen, gonads, pancreas, adrenal gland, intestine, iris, thymus, heart, lung, kidney, skeletal muscle, and proventriculus. Enlarged feather follicles (commonly termed skin leukosis) may be noted in broilers after defeathering during processing. The cloacal bursa (bursa of Fabricius) is rarely tumorous and more frequently is atrophic. Histologically, the lesions consist of a mixed population of small, medium, and large lymphoid cells plus plasma cells and large anaplastic lymphoblasts. These cell populations undoubtedly include tumor cells and reactive inflammatory cells. When the cloacal bursa is involved, the tumor cells typically appear in interfollicular areas. Some tissues are involved by tumor that are usually not involved in avian leucosis. They are eye, muscle, skin and nerve tissues.



In this histopathological figure, pleomorphic lymphoid cell proliferations, plasma cells and inflammatory cells in affected viscera, nerves or eyes are observed.

Avian Leukosis:

Avian leukosis virus (ALV) infects mainly **chickens** but can also infect other avian species such as pheasants, partridges and quail. The virus is not highly contagious and is readily inactivated by disinfectants.

Avian leukosis is a neoplastic disease of poultry characterized by tumors of hemopoietic tissues and sarcomas, including lymphoid leukosis, myeloid leukosis, and erythroid leukosis, induced by a related group of avian leukosis/sarcoma RNA viruses.

Lymphoid leukosis is a clonal malignancy of the bursal-dependent lymphoid system. Transformation invariably occurs in the intact cloacal bursa, often as early as 4–8 weeks after infection. Tumors are often not detectable until chickens are ~14 weeks old. Death rarely occurs before 14 weeks and is more frequent around the time of sexual maturity.

Pathological Findings

Diffuse or nodular lymphoid tumors are common in the liver, spleen, and cloacal bursa and are found occasionally in the kidneys, gonads, and mesentery.

Involvement of the cloacal bursa has been considered virtually pathognomonic, although bursal lymphomas are also known to be induced by reticuloendotheliosis virus. Sometimes the bursal tumors are small and observed only after careful examination of the mucosal surface of the organ.

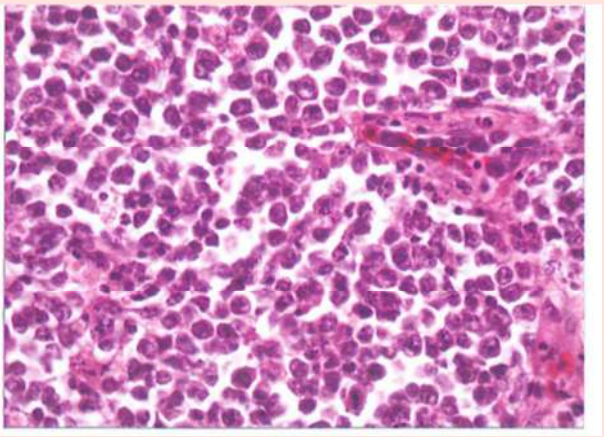
Usually, no enlargement of peripheral nerves is apparent, although such lesions have been noted after experimental inoculation of subgroup J virus.

Microscopically, the tumor cells are uniform, large lymphoblasts. Mitotic figures are frequent.

Outbreaks of neoplasms other than lymphoid leukosis, such as **myelocytomas, hemangiomas, and renal tumors**, have also been noted in meat-type chickens infected with subgroup J avian leukosis virus.

Microscopically, in cases of myelocytomas induced by subgroup J avian leukosis virus, the liver shows a massive intravascular and extravascular accumulation of myeloblasts characterized by the presence of cytoplasmic eosinophilic granules.

Most strains of leukosis/sarcoma viruses also induce nonlymphoid tumors (including sarcomas), erythroblastosis, myeloblastosis, myelocytomas, hemangiomas, nephroblastomas, osteopetrosis, fibrosarcoma and related neoplasms. The nature and frequency of the tumors depend on virus strain, chicken strain, age, dose, and route of infection. Occasional outbreaks of predominantly one type of tumor are observed in the field. The Rous sarcoma virus, a member of this group, has been widely studied in the laboratory. Each strain usually causes a predominantly neoplastic disease and can be distinguished on the basis of pathogenicity. Some viruses (eg, Rous sarcoma and erythroblastosis viruses) contain a viral oncogene that enables the virus to induce neoplasms within a short incubation period; however, such viruses are rare in the field. Avian leukosis virus infection has also been shown to be associated with the **so-called fowl glioma, characterized by cerebellar hypoplasia and myocarditis.**



Spleen. Lymphoid leukosis. 25-week-old broiler breeder

Neoplasms caused by viruses of the leukosis/sarcoma group:

Neoplasm	Synonyms
Leukoses	
Lymphoid leukosis	Big liver disease, lymphatic leukosis, visceral lymphoma, lymphocytoma, lymphomatosis, visceral lymphomatosis, lymphoid leukosis
Erythroblastosis	Leukemia, intravascular lymphoid leukosis, erythroleukosis, erythromyelosis, erythroblastosis, erythroid leukosis
Myeloblastosis	Leukemic myeloid leukosis, leukomyelosis, myelomatosis, myeloblastosis, granuloblastosis, myeloid leukosis
Myelocytoma(tosis)	Myelocytoma, aleukemic myeloid leukosis, leukochloroma, myelomatosis
Connective tissue tumors	
Fibroma and fibrosarcoma	
Myxoma and myxosarcoma	
Histiocytic sarcoma	
Chondroma	
Osteoma and osteogenic sarcoma	
Epithelial tumors	
Nephroblastoma	Embryonal nephroma, renal adenocarcinoma, adenosarcoma, nephroblastoma, cystadenoma
Nephroma	Papillary cystadenoma, carcinoma of the kidney
Hepatocarcinoma	
Adenocarcinoma of the pancreas	
Thecoma	
Granulosa cell carcinoma	
Seminoma	Adenocarcinoma of the testis
Squamous cell carcinoma	
Endothelial tumors	
Hemangioma	Hemangiomatosis, endothelioma, hemangioblastomas, hemangioendotheliomas
Angiosarcoma	
Endothelioma	
Mesothelioma	
Related tumors	
Osteopetrosis	Marble bone, thick leg disease, sporadic diffuse osteoporostitis, osteopetrosis gallinarum
Meningioma	
Glioma	

Differential Diagnosis of Marek's Disease

The major differential diagnosis for MD is lymphoid leukosis (LL). Lymphoid leukosis is a clonal, bursal lymphoma induced by ALV and, under some conditions, by REV in chickens older than 16 weeks of age. Chickens usually have gross tumors in the bursa of Fabricius, and tumor cells are uniform, blast-like, pyroninophilic, and express B-cell markers and IgM. Also, the tumor cells have clonal insertions of proviral DNA near the c-myc gene. Lymphocytes obtained from REV-induced nerve lesions or tumors do not express Meq and have low MDV DNA load, if any. Cells from nonbursal RE lymphomas are negative for MHC class II and predominantly stain for CD8 antigen. Exclusion of ALVs or REVs, where possible, through negative PCR, histochemical assays on tumors, or antibody tests may provide strong support for a diagnosis of MD when other MD-related criteria are positive. However, it is possible to have mixed infections and, in those cases, further diagnostic assays (qPCR for MD and southernblot for retroviruses) are needed. Peripheral neuropathy is a neurological disease of uncertain etiology that causes paralysis and nerve enlargement in a low proportion of commercial chickens 6–12 weeks of age and is characterized by a Th1- to-Th2 shift. Affected chickens lack visceral lymphomas; the nerve lesions are uniformly B-type; and MDV is rarely, if ever, demonstrated. If MDV is present on those chickens, MDV DNA load is low. Other diseases that may present confusing gross lesions or paralytic signs are myelocytomatosis (myeloid leukosis),myeloblastosis, erythroblastosis, histiocytic sarcomas, carcinoma of the ovary, various other nonviral neoplasms, riboflavin deficiency, tuberculosis, histomoniasis, genetic gray eye, Newcastle disease, hepatitis E, and joint infections or injuries. Myeloid leukosis is a common tumor in broiler breeder flocks that superficially resembles MD but **can be differentiated histologically**.The tumor cells are myeloid in nature and lack T cell and MD viral markers.

Differential diagnosis of lymphomas & Avian Leukosis:

In chickens can be difficult. The two most common lymphoid neoplasms, namely MD and LL are particularly confusing. Lymphoid leukosis cannot be differentiated from REV-induced bursal lymphomas on the basis of pathology, immunohistochemistry, and molecular changes in the c-myc region. Virologic, serologic, or PCR tests maybe helpful in establishing infection for one virus and exclusion for the other. However, such assays are not particularly helpful in the diagnosis of virus-induced lymphomas of chickens including LL, as avian oncogenic viruses are widespread, and infection in the absence of tumor formation is common. Detection of proviral DNA and integration junctions by PCR assays has been shown to be useful for tumor diagnosis. Because LL tumors should contain ALV proviral DNA sequences inserted near the c-myc gene, differentiation between LL and REV-induced bursal lymphomas can be made by southern blots and hybridization analysis of tumor DNA for clonal insertion of ALV.Lymphomas in which bursal tumors are lacking or in which the latent period is too short for that of LL can be confused primarily with MD; however, under certain circumstances, REV-induced lymphoma should also be ruled out . In cases in which bursal tumors are lacking, LL and MD can be differentiated only with difficulty, because similar lymphoid tumors may occur in both diseases in the same visceral organs during the same age period. Visceral lesions of these two diseases cannot be distinguished by gross examination. **Diagnosis is possible in most instances on careful microscopic examination; however, considerable experience is necessary**. In coming to a decision, history,signs, gross and microscopic lesions, and cytology should all be considered. Ordinarily, LL does not occur before 14 weeks of age, and most of the mortality occurs between 24 and 40 weeks. Marek's disease,

however, may occur as early as 4 weeks, and the mortality peak varies from 10–20 weeks. Occasionally, losses continue and may reach a peak after 20 weeks. Nodular tumors of the bursa can often be palpated through the cloaca in birds infected with ALV. Paralysis associated with gross lesions in autonomic and peripheral nervous systems and gross lesions of the iris (“gray eye”) are specific for MD. As stated previously, the bursa of Fabricius plays a central role in development of LL. When distinct focal or nodular lymphoid tumors are present in the bursa, a diagnosis of LL can be made; however, REV-induced bursal lymphomas must be ruled out. Such tumors are sometimes quite small and may be overlooked. In some birds, MD induces a premature atrophy of the bursa. In others, the bursa may be tumorous, in which case the walls and the plica may be thickened from interfollicular infiltration with pleomorphic lymphocytes. In contrast, intrafollicular tumors of the bursa consisting of uniform large lymphocytes are usual with LL. Microscopic lymphoid infiltration in nerves, cuffing around small arterioles in the white matter of the cerebellum, and the feather follicular pattern of lymphoid cell infiltration in the skin, which are characteristic of MD, are not seen with LL. Cytologically, LL tumors generally are composed of a homogeneous population of lymphoblasts. In contrast, tumors of MD usually contain lymphoid cells varying in size and maturity from lymphoblasts to small lymphocytes, and plasma cells may also be present. Special stains such as methyl green pyronin are helpful for cytology. Immature lymphoblasts characteristic of LL tumors are highly pyroninophilic; whereas the medium and small lymphocytes that predominate in tumors of MD do not stain with pyronin. Lymphoid leukosis tumors are composed almost entirely of B cells and have surface IgM markers; whereas 60–90% of MD tumor cells are T cells that lack IgM markers and only about 3–25% are B cells. In addition, from 0.5–35% of MD tumor cells have a tumor-associated cell surface antigen (MATSA), which is absent from LL tumor cells. Recently, Witter et al. introduced a diagnostic strategy for the differential diagnosis of viral lymphomas in chickens. Other diseases that may be confused with LL are erythroblastosis, myeloblastosis, myelocytomatosis, pullorum disease, tuberculosis, enterohepatitis, Hjarre’s disease, and fatty degeneration of the liver.For erythroblastosis a firm diagnosis must be based on finding large numbers of erythroblasts by microscopic examination of a blood smear and sections or smears of liver and bone marrow. Chickens in early stages of disease or without obvious signs may be missed easily unless microscopic examination is made. Erythroblastosis with concurrent anemia is often difficult to differentiate from anemia resulting from nonneoplastic causes. In erythroblastosis, there is usually a defect in maturation of erythroblasts, resulting in the presence of large numbers of them and very few polychrome erythrocytes. In anemia, the reverse usually occurs. Extramedullary erythropoiesis and stasis of erythroblasts in the sinusoids are usually more prominent in erythroblastosis than in anemia.Erythroblastosis can be distinguished from myeloblastosis on the following grounds. In myeloblastosis, the liver is usually pale red and the marrow is whitish; whereas in erythroblastosis, the liver and marrow are usually cherry red. In myeloblastosis, the cells accumulate intravascularly and extravascularly, whereas in erythroblastosis they are always intravascular. The erythroblast and myeloblast may be difficult to distinguish. Erythroblasts have a basophilic cytoplasm and perinuclear halo; myeloblasts often have some granules.Erythroblasts are cells of the erythropoietic system and can be differentiated from cells of the myelopoietic system on the basis of the presence of certain markers. Thus, erythroblasts have erythroid markers including hemoglobin, chicken erythrocyte-specific histone H5, and chicken erythrocyte-specific cell surface antigens detected by immunofluorescence. Myeloblasts and myelocytes have myeloid markers including adherence and phagocytic capacity, Fc receptors as determined by rosette formation,

macrophage- and granulocyte-specific cell surface antigen as detected by immunofluorescence, and dependence of colony formation on colony-stimulating factor. Erythroblastosis can be distinguished from LL by the nature and distribution of lesions. Microscopically, the cytoplasm of lymphoblasts is somewhat less basophilic than that of erythroblasts, and there is also a larger nuclear<thin: cytoplasmic ratio than in the latter cells. Lymphoblasts are more variable in size and shape than erythroblasts, but they are all at the same primitive developmental stage. Lymphoblasts tend to have an ovoid rather than spherical nucleus and a finer, more delicate-looking chromatin network. Myelocytomas and erythroblastosis can be distinguished histologically. Myeloblastosis as in erythroblastosis, a tentative diagnosis may be based on gross lesions; however, these are often so similar to those of LL that specific diagnosis cannot be made without examination of a blood smear. Examination of liver or bone marrow sections is helpful when identity of the cell type is in doubt. The myeloblast is, on the average, smaller than the erythroblast or lymphoblast; its cytoplasm is more acidophilic and is polygonal or angular. The nucleus is less vesicular; the nucleolus, while present, is not nearly so frequently seen or conspicuous as in the other two leukoses. Myeloblasts also have physiologic markers that identify them as members of the myeloid series. Myelocytomatosis the distinctive character and location of tumor provide the basis for diagnosis, which can be verified by examination of a stained smear or tumor section. Gross tumors must be differentiated from myeloblastosis, LL, osteopetrosis, and necrotic and/or purulent processes occurring in tuberculosis, pullorum disease, and mycotic infections. In recent outbreaks of ALV-J-induced tumors, myelocytomatosis was diagnosed primarily on the basis of presence of characteristic microscopic feature of tumor cells .

Hemangioma:Hemangiomas on the skin should be differentiated from wounds, bleeding feather follicles, and cannibalism. Those in the visceral organs should be differentiated from hemorrhages and sarcomas.

Renal Tumors: Renal tumors should be suspected when tumor nodules or large masses are found only in the kidney or are encountered suspended from the lumbar region. Diagnosis can be verified by microscopic examination. Tumors should be differentiated from other causes of kidney enlargement including hematomata, LL, and accumulation of urates.

Osteopetrosis: Bone lesions of advanced cases are sufficiently distinctive to present no difficulty in diagnosis. Cross- and longitudinal-sectioning of long bones is helpful in detecting slight exostoses and endostoses, particularly in early stages.

Scientific significance and development of poultry mRNA vaccine

Shahla Shahsavandi

Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization, Karaj, Iran

S.SHAHSAVANDI@rvsri.ac.ir

Managing existing and newly emerging infectious diseases is a challenge for the poultry industry. To minimize economic loss or avoid the emergence of clinical infectious diseases, industrial poultry is heavily vaccinated against infectious agents. Conventional vaccines based on live-attenuated or inactivated viruses, and recombinant subunit vaccines are useful tools for disease prevention and control. With the rapidly increasing demand for poultry products and the current challenges in disease control facing the poultry industry, the application of new vaccine production platforms to improve poultry health and production efficiency has gained growing significance. In this regard, new research has been launched to advance mRNA vaccine development against poultry pathogens.

The mRNA ability to direct immunity and induce CD4+ and CD8+ T cell responses was demonstrated in the early 1990s by liposome-entrapped mRNA encoding influenza virus nucleoprotein. In contrast, its potential as a vaccine has successfully been presented against the human pandemic COVID-19 for more than three decades. The mRNA vaccine production method involves *in vitro*-transcribed (IVT) followed by 5′ capping and polyadenylation at the 3′ end mimicking the natural process of mRNA maturation in the cytoplasm. Incorporating modified nucleosides instead of uridine, optimizing coding sequences, and attentive IVT- mRNA purification improve biological stability and translational capacity. To function *in vivo*, mRNA requires appropriate delivery systems due to its large size, dense negative charge, and instability. Lipid nanoparticles (LNPs) are the most clinically advanced mRNA delivery vehicles and offer numerous benefits including ease of formulation, biocompatibility, large mRNA payload capacity, enhanced efficient cellular uptake, and increased immunogenicity. The delivery platform is mainly based on ionizable cationic lipid, cholesterol, helper phospholipid, and polyethylene glycol derivatives together encapsulate and protect the fragile mRNA.

The success of mRNA vaccine use in the COVID-19 pandemic has led to great interest in the research and development of poultry mRNA vaccines. We are facing the question of whether the newly developed vaccine platform affects positively the protection of chickens against viral

pathogens that kill millions of poultry worldwide. Recent research in mRNA vaccine technology is demonstrating promise for the prevention and control of diseases that pose a great threat to the poultry industry and public health. Most of the effort is regarding the development of Marek's disease (MD) and highly pathogenic H5 avian influenza A virus (AIV) vaccines.

MD serves as a valuable model for investigating oncogenic virus patterns of the viral latency phase, which is related to human and poultry health. MD caused by a chicken alphaherpesvirus is a highly contagious malignant T-cell lymphomatosis in chickens. Current vaccines can limit tumor growth and death but not virus replication and transmission. The efficacy of a bivalent mRNA-LNP vaccine encoding MDV's glycoprotein B and phosphoprotein 38 antigens was demonstrated by the expression of type I and II IFNs, a panel of interferon-stimulated genes, and two key antiviral cytokines in spleen and lungs of the vaccinated chickens. The transcriptional analysis showed significant increases in the expression of MDA5, MyD88, IFN-α, IFN-β, IFN-γ, IRF7, OAS, Mx1, IL-1β, and IL-2 within the first 36 h of immunization. The spleen showed significant upregulation of IFN-γ, MDA5, MyD88, Mx1, and OAS after the secondary immunization. The data demonstrate the potential of the MDV mRNA vaccine to activate innate and adaptive responses and induction of an antiviral state in chickens.

H5 AIVs are a significant public health risk due to their widespread circulation in avian species and the potential to cause a future pandemic. Vaccination of poultry is the most effective countermeasure against these zoonotic viruses will reduce the excretion of the virus in infected animals and consequently avert human future epidemics/pandemics. Rapid AIV mutations and rearrangement are challenging the efficacy of current vaccines, hence, the role of mRNA vaccines in managing existing and newly emerging strains has been highlighted. H5 viruses of the Gs/Gd lineage emerged in Southeast Asia in 1996 and have rapid transcontinental spread as evidenced by outbreaks in 2005–2006, 2014–2015, and 2016–2017. Upon re-emerging in 2020, a wider range of wild and domestic bird species have been affected by the spread of clade 2.3.4.4b H5 viruses resulting in occasional human infections and mammalian adaptation, indicating the potential risk an expanded host range can pose. Recently, the WHO announced plans to develop an H5 mRNA vaccine to protect people in low-income and middle-income countries. Self-amplifying mRNA-LNP vaccine encoding the membrane-anchored full-length HA protein of H5N1 clade 2.3.4 can potentially induce sufficient protective antibody levels and cellular responses in chickens and mice. The platform has significant potential for application in domestic poultry and humans to prevent future epidemics/pandemics. Researchers hope the mRNA vaccine based on conserved elements could target more strains of AIV at once.

Pathological Changes in the Liver of Birds

Dr. Nariman Sheikhi
Department of Clinical Science, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran
Pasteurlab78@yahoo.com

The liver is a vital organ in any vertebrate, playing key roles in metabolism, detoxification, and the synthesis of important proteins. In birds, the liver has unique structural and functional characteristics that distinguish it from the livers of mammals. Understanding the pathological changes that occur in avian livers due to various diseases is essential for avian health management and developing effective treatment strategies.

The liver is responsible for metabolizing carbohydrates, fats, and proteins. Unlike mammals, birds have unique metabolic pathways that allow for efficient utilization of their high-energy diets. The liver converts excess carbohydrates to fat, which is stored for energy use during periods of fasting. The liver detoxifies harmful substances, including drugs and toxins, producing bile which aids in digestion and excretion. Also, this organ synthesizes critical proteins, including clotting factors and albumin, which maintains osmotic pressure in the blood. The liver is involved in the immune response by producing immune factors and filtering pathogens from the blood. The liver of birds is divided into distinct lobes, with a more compact cellular organization compared to the lobular structure in mammals. Birds also possess a unique arrangement of hepatocytes and sinusoids facilitating efficient blood flow and nutrient exchange. Inflammatory liver disease can result from viral infections, bacterial infections, or toxins. Pathological changes include hepatocyte necrosis, inflammatory cell infiltration, and hepatic fibrosis. Fatty Liver Disease is characterized by an excessive accumulation of fat within hepatocytes. It can arise from overfeeding, inadequate nutrition, or stress. Pathological changes include pale, enlarged livers with a greasy appearance. Affected birds often display decreased activity and lethargy. Chronic damage due to various factors can result in cirrhotic changes, where healthy liver tissue is replaced by scar tissue. This alteration can impair liver function significantly and lead to portal hypertension. Symptoms may include weight loss, diarrhea, and ascites. Liver tumors, though less common, can affect avian species. Hepatic neoplasia may lead to abnormal growths, impacting liver function. Exposure to toxic substances, such as aflatoxins or heavy metals, can lead to necrosis and liver failure. Pathological changes include diffuse necrosis and cellular degeneration. Liver diseases in birds can result from a variety of causes, including infections, nutritional imbalances, toxins, and underlying health issues.

Viruses such as adenoviruses, herpesvirus, certain bacteria (like *Salmonella* or *Escherichia coli*) can lead to liver inflammation or necrosis. Fungi like *Aspergillus* can cause systemic infections affecting the liver. Overfeeding or an imbalanced diet can lead to hepatic lipidosis, where fat accumulates in the liver. Exposure to chemicals (pesticides, herbicides) can lead to liver damage. Some drugs, if overdosed or improperly administered, can affect liver health. Physical Trauma to the liver (from accidents or aggressive behavior) can cause hemorrhage or rupture. Infestations by liver flukes or other parasites or protozoa can lead to liver damage.

Pathogenicity of Avian Influenza Viruses: Beyond the Hemagglutinin Cleavage Site

Abdolhamid Shoushtari
Department of avian viral diseases, RVSRI
hamid1342ir@yahoo.com

Shoushtari 1, A, , 1 , Abtin 1, A, , Eshratabadi1, F

Conventionally, the pathogenicity level of avian influenza viruses is measured by their ability to cause lethality in the chicken, as all avian influenza viruses are categorized as either low or high pathogenicity. It should be emphasized that this scale is applicable only to poultry, and cannot be generalized to, for example, wild aquatic birds that are known to be the reservoir of these viruses. However, the observed widespread mortality in various species of wild aquatic birds in recent years has challenged this concept. On the other hand, unusual losses in infection with influenza viruses with low pathogenicity are another challenge to this classic concept. Although the initiation of the infection at the cell surface begins with the binding of hemagglutinin glycoprotein(glp) to its specific receptor, an effective infection also requires the action of another glp, the neuraminidase. Therefore, there is a functional balance between these two glycoproteins, maintained by an epistatic relationship. If this relationship is not established and the proper functional balance between these two glycoproteins is not achieved, a dynamic infection will not occur. If the viral genome does not enter the cytoplasm, an abortive infection occurs. For a productive infection, the fusion of hemagglutinin with the lysosomal membrane is required after the virus enters via endocytosis. The fusion process occurs at an acidic pH and is highly dependent on the stability of hemagglutinin in this acidic environment. After the virus genome enters the cytoplasm, the cellular mechanisms for virus replication must be taken over, and the intracellular defense system, i.e., innate immunity, must be neutralized. Although a set of viral factors, including most structural proteins and accessory proteins such as PA-X and PB1-F2, can participate in distinct stages of this process, the most critical role is played by the NS1 protein. Variations in the amino acid composition of this protein can significantly influence the quantity and quality of viral replication and pathogenicity. Although a set of viral factors, including most structural proteins and accessory proteins such as PA-X and PB1-F2, can participate in distinct stages of this process, the most critical role is played by the NS1 protein. Variations in the amino acid composition of this protein can significantly influence the quantity and quality of viral replication and pathogenicity. As mentioned, accessory proteins such

as PA-X and PB1-F2 can also influence this process, both in terms of their presence and differences in amino acid composition. This review aims to demonstrate that the pathogenicity determinants of avian influenza viruses are not solely related to the amino acid composition at the hemagglutinin cleavage site. Other factors and various viral proteins also contribute to the quantity and quality of their pathogenicity.

Exploring the Chick Embryo Model: A Revolutionary Approach in Biomedical Research for Cancer Treatment and Public Health Enhancement

Hadi Tavakkoli
Department of Clinical Sciences, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
tavakkoli@uk.ac.ir

Objectives :

The use of chick embryos as a model for biomedical research has gained significant attention due to their unique advantages in studying various biological processes and disease mechanisms. This study aims to explore the potential of the chick embryo model in evaluating human breast cancer cell lines (MCF7) and the efficacy of silver nanoparticles (AgNPs) and fosfomycin in cancer treatment. The primary objectives include assessing the anti-cancer properties of AgNPs and investigating the effects of fosfomycin on angiogenesis in cancer cells.

Materials & Methods:

Chick Embryo Model

The chick embryo model provides an accessible and ethical platform for in vivo studies. Fertilized eggs were incubated under controlled conditions until reaching the desired developmental stage. The MCF7 human breast cancer cell line was introduced into the chorioallantoic membrane (CAM) of the chick embryos to simulate tumor growth.

Silver Nanoparticles Treatment

Silver nanoparticles were synthesized and characterized for their size, shape, and stability. A series of concentrations were administered to the MCF7 cells implanted in the CAM. The effects on cell proliferation, apoptosis, and overall tumor growth were evaluated using histological analysis and molecular assays.

In Silico Analysis of Fosfomycin

To assess the impact of fosfomycin on angiogenesis, an in silico approach was employed. Molecular docking simulations were conducted to predict the interaction between fosfomycin and

key proteins involved in angiogenic pathways. The results were validated through experimental assessments in the CAM model.

Results:

Efficacy of Silver Nanoparticles

The treatment with silver nanoparticles demonstrated a significant reduction in the proliferation of MCF7 cells. Histological analysis revealed decreased tumor size and altered cellular morphology indicative of apoptosis. Quantitative PCR assays showed an increase in pro-apoptotic markers (BAX) and a decrease in anti-apoptotic markers (Bcl-2), confirming the induction of apoptosis by AgNPs.

Impact of Fosfomycin on Angiogenesis

In silico simulations indicated that fosfomycin interacts effectively with angiogenic proteins, suggesting its potential to inhibit vascular growth necessary for tumor progression. Experimental results corroborated these findings, showing a marked reduction in blood vessel formation within the CAM model treated with fosfomycin.

Conclusion:

This study highlights the chick embryo model as a promising platform for investigating novel anti-cancer therapies. The findings indicate that silver nanoparticles can effectively reduce human breast cancer cell proliferation, while fosfomycin shows potential as an angiogenesis inhibitor. These compounds may serve as new therapeutic agents in cancer treatment strategies.

The implications of these results extend beyond oncology, offering insights into public health enhancement through innovative biomedical research methodologies. Future studies should focus on optimizing dosages and exploring combination therapies to maximize therapeutic efficacy while minimizing potential side effects.

Keywords: Chick embryo, Breast cancer, Silver nanoparticles, Fosfomycin, Angiogenesis, In silico modeling.

Review of diagnostic, preventive and control measures in the Hepatitis-Hydropericardium Syndrome (HHS) epidemic in northeast Iran

Toroghi Reza

Mashhad Branch, Razi Vaccine & Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Mashhad, Iran; MAAD Veterinary Diagnostic Laboratory, Mashhad, Iran

r.toroghi@rvsri.ac.ir

HHS is the most significant disease caused by avian adenoviruses, previously considered an exotic viral disease in the Iranian poultry industry. The first reported outbreak in the country occurred in Khorasan Razavi province. During this outbreak, rapid diagnosis was achieved using molecular, serological, and virus isolation methods, and the disease was successfully controlled through a stamping-out program. In the second wave, the disease was reported simultaneously in multiple locations through the progeny of a broiler breeder flock. Despite employing similar diagnostic and control strategies as in the first wave, the slower response and financial constraints related to compensating poultry farmers hindered the effectiveness of control measures compared to the first wave. During the third wave, the disease spread to more broiler flocks via flock-to-flock transmission. Mass vaccination against fowl adenoviruses (FAdVs), including FAdV-4, was implemented for all broiler breeder flocks in the province. Combining ELISA results with PCR testing for adenovirus detection in eggshells and yolks during the peak production period provided valuable insights. These included evaluating vaccination efficacy, assessing flock field challenges, and categorizing offspring flocks for distribution to the province's low- and high-risk areas. Although categorizing offspring based on geographical risk levels was innovative, it was not fully implemented due to logistical challenges and a lack of coordination among relevant governmental organizations. The hyperimmunization of broiler breeder flocks reduced the occurrence of disease in chicks under 10 days old; however, cases in broilers older than 30 days became more prevalent. Moreover, a peptide-based ELISA was developed as a differentiation test (DIVA) to distinguish between infected and vaccinated animals. Sera collected from broiler, layer, and broiler breeder flocks in the province are being analyzed using this method. Over the past three years, HHS appears to have transitioned from an exotic viral disease to an endemic one in Khorasan Razavi province. Several factors may have contributed to this endemicity, including a lack of transparent reporting and information dissemination among experts, limited scientific capacity, insufficient coordination among governmental organizations, a politically driven perspective on pathogens rather than a scientific approach, the existence of multiple decision-making centers, the absence of a clear action plan, excessive focus on disease diagnosis rather than prevention and control measures, and inadequate quality control of imported adenovirus vaccines. It is concluded that introducing new methods or approaches for disease diagnosis, prevention, and control alone cannot guarantee success. The accurate measure of success lies in the degree to which these approaches are operationalized under existing conditions.

Keywords: Hepatitis-hydropericardium syndrome, FAdV-4, ELISA, control measures, Iran

AI-Powered Poultry Health Management: Innovations in Early Disease Detection and Control

Farzad Yavari
Sadid Company, Tehran, Iran 2CTO, Sadid Company
yavari.frd@gmail.com

Farzad Yavari 1 , Fatemeh Mohseni 2 1CEO, Sadid Company, Tehran, Iran 2CTO, Sadid Company, Tehran, Iran

Objectives: The poultry industry faces significant challenges due to diseases that result in economic losses and reduced productivity. Early detection and effective management of poultry diseases are critical to maintaining flock health. This study explores the integration of artificial intelligence (AI) in poultry health management, highlighting its transformative potential in early disease detection, monitoring, and control.

Materials & Methods: We developed an AI-powered system comprising autonomous mobile robots equipped with sensors, cameras, and disease detection algorithms. The system Analysis data from poultry farms, including behavioral patterns, environmental conditions, and historical flock data. Advanced deep learning models analyze this data to identify early signs of diseases.

Results & Conclusion: The AI-driven system demonstrated high accuracy in detecting early signs of common poultry diseases, significantly reducing mortality rates and improving feed conversion ratios (FCR). The integration of autonomous robots for farm navigation and disinfection further enhanced biosecurity and minimized human intervention. This study underscores the potential of AI in revolutionizing poultry health management, offering scalable solutions for disease control and sustainable farming practices.

Keywords: Artificial Intelligence, Poultry Health Management, Early Disease Detection, Autonomous Robots, Disease Control, Biosecurity, Poultry Behavior

Uncertainty in the use of synthetic amino acids in poultry feed

Mojtaba Zaghari,
Emeritus Professor of Poultry Nutrition, Department of Animal Science, University of Tehran
mzaghari@ut.ac.ir

Objectives

Inclusion of crystalline-free amino acids (AAs) in the poultry diets reduces growth and nitrogen retention, possibly caused by significantly lower utilisation of synthetic-free AAs compared to those bound to protein.

Introduction

Single-meal-fed, meat-type birds, such as broiler and turkey breeders, are subjected to feed restrictions in the rearing period, and must consume their meal in 20–30 min once a day. It is likely that, during single-meal feeding, free AAs are rapidly absorbed and metabolised before intact protein. Consequently, the efficiency of free AAs utilisation is reduced. Research results indicated that the rate of lay and egg output of broiler breeder hens subjected to dietary supplementation with synthetic methionine (Met) and lysine (Lys), for each extra gram of dietary free AA content per kg diet, decreased by 3.0% and 2.5 g/day, respectively, and the efficiency of Met utilisation decreased by 4.3%. Given the debate over the use of crystalline AAs, a solution is needed. One approach can be to balance the arrival of free and protein-bound AAs (PB-AAs) at the site of absorption by developing a slow-release AA compound, which would establish an equilibrium between the gut, blood and tissues with regard to utilisation for protein synthesis. An alternative approach would be to use the potential benefits of protein sources with a high concentration of essential AA to substitute for crystalline-free AAs in practical diets.

Overview

Except for the research of Nonis and Gous (2006) and Zamani et al. (2021), there is a lack of data *in vivo, in vitro* or meta-analysis studies investigating the efficiency of free and protein-bound AA utilisation in single-meal-fed, meat-type birds. Thus, as Met is the first limiting AA in poultry, in the series of experiments potential of feeding protein-bound Met in comparison with crystalline-free Met on performance, intestinal absorption, absorption kinetics and efficiency of protein and AA utilisation in single-meal-fed, meat-type birds (breeder pullet) were evaluated.

The significant increase in BW and WG and the decrease in FCR for the single-meal meat-type chickens fed the DL-Met, and PB-Met supplemented diets compared to birds fed the basal diet

indicated that Met was required by chickens at the level higher than that found in the basal diet. The comparison of different Met sources showed higher performance responses to the PB-Met than for the synthetic Met sources. The data demonstrated that the PB-Met source was better utilised by single-meal-fed birds than the synthetic-free Met sources. However, the chickens fed DL-Met added diets showed higher performance compared with those fed the basal diet. This could be because the Met level in the basal diet was lower than requirement for chickens.

The early peak concentration of the plasma Met at 1 h after feeding with the DL-Met supplemented diets compared to 1- and 2 h postprandial levels with the PB-Met substituted diets indicated that the crystalline-free Met was absorbed more rapidly than the protein-bound Met.

Yen et al. (2004) showed that plasma AA concentration attained the highest level at 1 h postprandial in birds fed a 12% CP + AA diet, but peaked at 2.5 h after feeding a 16% CP diet. This was because of the difference in absorption rate of the free and protein-bound AAs and an imbalance of AAs at the site of protein synthesis when feeding crystalline AAs diets. The results from research on different types of animals such as fish, pigs and poultry demonstrated that the protein-bound AA were utilised more efficiently than those provided in the crystalline-free form, probably because the free AA were absorbed and catabolised more rapidly than the protein-bound ones.

Serum GSH/GSSG increased with incremental doses of all Met sources compared with the basal diet. This could be because the Met level in the basal diet was lower than the minimum requirement for meat-type chickens. Dietary inclusion of the synthetic Met sources (DL-Met) from 0.4 to 2.0 g/kg increased the plasma HCY concentration from 26.60 to 34.38 μmol/L and 20.83 to 40.43 μmol/L, respectively. Conversely, the substitution of protein-bound Met in the diets decreased the plasma HCY content from 36.25 to 25.68 μmol/L. This result could have been due to the positive role of the protein-bound Met for balancing intestinal absorption of Met to improve protein and AA utilisation. HCY is produced by demethylation of the dietary Met and is converted to cystathionine during transmethylation and, subsequently, to Cys in the trans-sulphuration pathway in the interaction with cystathionine β-synthase (CβS) and cystathionine-γ-lyase (CγL). Excessive blood Met levels inhibit the activity of CβS enzyme and block the trans-sulphuration pathway, resulting in an accumulation of HCY. When the balance between HCY production and consumption is disrupted, it accumulates in intracellular and extracellular fluids and causes an increase in blood plasma, which called the hyperhomocysteinemia. When feeding protein-bound Met, Met is directed to the protein synthesis pathway and there is no excess to inhibit the activity of the CβS enzyme and increase in the plasma HCY level.

The efficiency of Met utilisation declined in chickens fed the DL-Met supplemented diets. Conversely, Met utilisation significantly increased with the addition of PB-Met in the diets. It would appear that the synthetic-free form of Met is not utilised in single meal meat-type birds, and should not be used in their feed. Regarding other AAs, the efficiency of utilisation was similar to that measured for Met. In fact, the decrease in AAs utilisation with dietary DL-Met supplementation and the increase with dietary PB-Met substitution were observed. This result indicated the positive role of the protein-bound Met source for improving absorption and creating a balanced AA supply at the site of protein synthesis.

Comprehensive research results on rate of intestinal absorption of synthetic crystalline methionine and methionine present in the protein structure showed that methionine bound to protein (BP-Met)

could improve methionine efficiency, increase villus height, and improve liver health by balancing the absorption pattern.

Conclusion

Due to the lack of synchronous absorption of the synthetic-free and protein-bound AA in single-meal-fed animals, free AA is used with the low efficiency of utilisation. Therefore, until a suitable source of the free AA is introduced for co-absorption, the protein-bound AA can be suggested as a preferred source of supplemental AA in the single-meal-fed, meat-type birds.

Keywords: Protein efficiency, excreta nitrogen content, amino acid imbalance, homocysteine

Molecules of shRNA: A Novel Approach in the Control and Treatment of HHS

Abdolkarim Zamani Moghaddam
Professor of Avian Medicine, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahkrekord University, Shahrekord, Iran
azamani2@yahoo.com

In collaboration with:
Dr. Azam Mokhtari , Dr. Ali Lotfivand & Dr. Seyed reza abtahi

Introduction

Avian adenoviruses of the family adenoviridae are found all over the world, infecting a wide variety of poultry hosts and ages, including falcons, raptors, ostriches, psittacine, and parrots. Chickens are the primary host for some avian adenovirus diseases such as inclusion body hepatitis (IBH), hepatitis hydropericardium syndrome (HHS), adenoviral gizzard erosion (AGE), avian adenoviral splenomegaly (AAS), and egg drop syndrome (EDS). All of these diseases are caused by different virus types belonging to different adenovirus genera. Recently, IBH and HHS have been widely distributed in broiler flocks in several countries, and there has been a trend toward more epidemic breakouts rather than sporadic epidemics. Furthermore, the significant mortality and growth retardation associated with IBH, and HHS have resulted in enormous economic losses.

Hydropericardium hepatitis syndrome (HHS), also known as Angara disease, is a poultry disease for which, unfortunately, no effective or efficient treatment has been identified. The proposed therapeutic approaches have demonstrated only partial efficacy. In a series of studies conducted by the research team at the Faculty of Veterinary Medicine, Shahrekord University, under the supervision of the author, novel molecular genetic methods have been explored to control and treat this disease. One of the emerging strategies in disease control and treatment is RNA interference (RNAi). RNAi is a pathway that falls under the broader category of RNA silencing mechanisms. In these pathways, small RNAs serve as guides to specifically silence complementary sequences in mRNA molecules. RNAi was first discovered in Caenorhabditis elegans and was initially defined as the sequence-specific degradation of mRNA triggered by a long double-stranded RNA (dsRNA). The RNA molecules involved in RNA silencing pathways exhibit diverse structures. These include dsRNA with blunt ends, short and long hairpin RNAs (shRNAs) with varying degrees of complementary base pairing within their structures, sense and antisense RNA (either paired or unpaired), and single-stranded "aberrant" RNA, which is converted into dsRNA or directly into

small RNAs by RNA-dependent RNA polymerases (RdRPs). Short hairpin RNAs (shRNAs) are RNA sequences that form a tightly structured hairpin based on their sequence, comprising a specific target region, a spacer, and an inverted complementary sequence of the target. To achieve more stable gene expression suppression, the design of endogenous pre-miRNA has been utilized for shRNA development. Typically, shRNA sequences are delivered into cells via vectors (e.g., plasmids) and must be transcribed in the nucleus to generate structured hairpin-containing shRNA.

Design and Application of shRNA Molecules Against Fowl Adenovirus Serotype 4 (FADV-4)

The DNA polymerase (DNA pol) gene of Fowl Adenovirus Serotype 4 (FADV-4) was selected as the target for shRNA molecule design due to its critical role in viral replication and propagation. Based on literature review, the standard strain of FADV-4 was identified, and the oligonucleotide sequence of the DNA pol gene from this strain was retrieved from the NCBI database. Subsequently, all known sequences of this gene from other FADV-4 strains were extracted from the NCBI database. These sequences were aligned with the oligonucleotide sequence of the standard strain's DNA pol gene using bioinformatics software to identify conserved regions. Additionally, the oligonucleotide sequence of the standard strain's DNA pol gene was analyzed using three online shRNA design tools: BLOCK-iT RNAi Designer, WI siRNA Selection Program, and InvivoGen's siRNA Wizard. Each software proposed multiple shRNA molecules based on different algorithms, and approximately 30 shRNA candidates were selected. These candidate sequences were aligned with the DNA pol gene sequences of all known FADV-4 strains, and only those that showed full homology with conserved regions were retained, while others were excluded. The secondary structure of the RNA molecule encoded by the DNA pol gene was predicted using CLC Genomics Workbench. The previously selected shRNA molecules were then BLASTed against this predicted RNA secondary structure, and only those located in the loop regions of the RNA structure were retained. At this stage, additional shRNA molecules were eliminated. To remove shRNA molecules that might target the chicken genome and cause off-target effects, the remaining candidates were BLASTed against the avian genome using NCBI's Nucleotide BLAST. Sequences that exhibited 15 or more nucleotide matches with mRNAs in the avian genome were discarded. Finally, the remaining shRNA molecules were ranked based on sequence specificity, off-target effects (as predicted by the WI siRNA Selection Program), and GC content. The three highest-ranking shRNA molecules were selected for further experiments. **Lentiviral Plasmid Construction and Bacterial Transformation**

To facilitate the cloning and amplification of lentiviral plasmids, the competent E. coli DH5α strain was used. Artificial transformation was performed via heat shock, and large-scale plasmid purification was carried out using a plasmid extraction and purification kit. To obtain a linearized vector for ligation, a double digestion reaction was performed using EcoRI and BamHI restriction enzymes. Digested plasmid fragments were purified via agarose gel electrophoresis, and

undigested plasmids were removed. Following the design and synthesis of single-stranded complementary DNA oligonucleotides, these were annealed under optimal conditions to generate double-stranded oligonucleotides for cloning into the desired vector. The synthesized shRNA molecules were then ligated into the digested pCDH-CMV-MCS-EF1-cGFP-T2A-Puro plasmid using T4 DNA ligase.

Transformation and Verification of Recombinant Plasmids

The ligated plasmid containing shRNA molecules was transformed into competent DH5α cells via heat shock. Positive and negative controls were included to confirm successful transformation. To verify the correct insertion of the recombinant gene into the plasmid, colony PCR was performed. After confirmation, recombinant plasmids were extracted using alkaline lysis.

Lentivirus Production and Functional Analysis

For the production of lentiviruses expressing shRNA molecules, HEK293T cells were co-transfected with the transfer plasmid and other essential packaging plasmids using the calcium chloride transfection method. The titer of the lentiviruses expressing shRNA molecules was determined using the TCID50 assay (Tissue Culture Infectious Dose 50%).

To assess the impact of shRNA on FADV-4 replication, Chicken Embryo Fibroblast (CFE) and or LMH cells were infected with the lentiviruses expressing shRNA and subsequently inoculated with FADV-4. The viral titer was determined using the TCID50 assay post-infection. The observed reduction in FADV-4 titer following treatment with lentiviruses expressing shRNA molecules demonstrated the inhibitory effect of RNA interference (RNAi) on viral replication and activity.

Challenges in the monitoring of *Mycoplasma gallisepticum* infection in broiler breeder flocks

Hadi Haghbin Nazarpak
Department of Clinical Sciences, Garmsar Branch, Islamic Azad University, Garmsar, Iran
hhaghbinn@yahoo.com

Mycoplasma gallisepticum (MG) is a major bacterial pathogen in the poultry industry, primarily due to its economic impact, including reduced egg production, drops in hatchability, impaired feed conversion efficiency, increasing contaminations at processing and the exacerbation of concurrent viral and bacterial infections. Breeding flocks, including line, grandparent, and parent stocks, must undergo continuous monitoring for MG infection, as it can spread via both horizontal and vertical transmission. This becomes particularly critical when the primary goal is the early and precise detection of infection within a flock. For routine surveillance, serological tests such as the rapid serum agglutination (RSA) test and enzyme-linked immunosorbent assay (ELISA) are widely used. However, test results from different RSA antigens, as well as variations between ELISA kits, may not always be identical and may have different results. Regardless of the reasons for these findings, in cases of inconsistency, the hemagglutination inhibition (HI) test can be employed as a confirmatory assay to support decision-making. While bacterial culture remains the gold standard for MG detection, it is a time-consuming method that may take up to four weeks to yield definitive results. Molecular diagnostic techniques, such as PCR-based assays, have gained widespread adoption as rapid and reliable alternatives. However, strict adherence to scientific protocols at every stage of testing is crucial to ensure accuracy and reliability. Ultimately, it is important to understand that making the right decision about the status of a herd requires the combination of laboratory results with a logical and scientifically grounded interpretation. Premature or inexperienced decisions should be avoided until confirmatory results are obtained.

Keywords:*Mycoplasma gallisepticum*, Poultry disease, Flock monitoring, Serological tests, Molecular diagnostics, Horizontal and Vertical transmission