Evaluation of the protective efficacy of native seed homologous Newcastle disease vaccine candidate against Genotype VII (G7) virus challenge in SPF chickens

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Objective: This study assessed the efficacy of inactivated Newcastle disease virus (NDV) vaccines genotypes I and VII in SPF chickens against a challenged with a local NDV genotype VII strain.

Materials & Methods: Fifty-two one-day-old SPF chickens were divided into 6 groups: Group 1 received a reassortant inactivated NDV VII vaccine; Group 2, an inactivated NDV VII vaccine from local virus seed; Group 3, a bivalent inactivated NDV VII (a local virus seed) plus H9N2 vaccine; Group 4, a bivalent inactivated NDV I (V4) plus H9N2 vaccine; Group 5 and 6 served as a positive and negative controls, respectively. Vaccination was administered subcutaneously at 21 days and the challenge was conducted at 45 days using a local velogenic NDV VII strain (vNDV VII) strain.

Results: Results showed the highest hemagglutination inhibition (HI) titers in groups 1 and 4 (8 and 7.16 $\log_2$, respectively) while Groups 2 and 3 had significantly lower HI titers (5.8 and 5.4 $\log_2$, respectively) three weeks post vaccination. All groups exhibited increased HI titers post challenge. The PC group displays 70% mortality and distinct post mortem lesions, while no mortality occurred in vaccinated groups. Vaccination did not fully prevent the manifestation of clinical signs of the disease. Group 2 showed the least clinical signs and shortest disease duration.

Conclusion: A homologous NDV VII vaccine offered superior clinical protection, while the reassortant vaccine induced higher HI titers. However, the heterologous vaccine (V4) effectively prevented mortality and elicited protective HI titers against NDV VII.

Keywords: Genotype VII, Newcastle Disease, Homologous Inactivated Vaccine

Molecular detection of virulent Newcastle disease virus in industrial poultry flocks in different provinces of Iran

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Objectives: Newcastle disease (ND), caused by the avian paramyxovirus 1 (Newcastle disease virus, NDV), is a highly infectious condition affecting poultry. Despite regular vaccination efforts, ND remains endemic in various regions worldwide. Clinical symptoms of ND can range from respiratory difficulties to neurological manifestations and severe systemic illness, with mortality rates that may approach 100%. Strains of the virus can be categorized into lentogenic, mesogenic, and velogenic types based on the clinical signs exhibited in chickens after experimental inoculation, with these categories reflecting an increasing level of virulence. The aim of this study is the molecular detection of the Newcastle virus in industrial poultry flocks in different provinces of Iran.

Materials & Methods: A total of 30 industrial poultry flocks (broilers, layers, and breeders) suspected of ND from different provinces of Iran were necropsied and subjected to molecular analysis. Depending on the types of lesions and symptoms observed in the flocks, samples were taken from the trachea, lungs, brain, tonsils, proventriculus and spleen of the flocks. RNAs were extracted and evaluated by general ND primers in reverse transcription polymerase chain reaction (RT-PCR). Positive samples in general ND RT-PCR were examined using specific primers of velogenic NDV (vNDV).

Results & Conclusion: The investigation revealed that, out of the 30 poultry flocks suspected of Newcastle disease (ND), 12 flocks (40%) tested positive for general Newcastle disease virus (NDV) with a PCR product of 362 bp, and eight of these (26.66%) were identified as virulent NDV (vNDV) with a PCR product of 254 bp.The presence of vNDV in commercial poultry highlights a significant threat to the poultry industry and underscores the critical need for robust monitoring and control strategies. To enhance our understanding of the dynamics of NDV, it is recommended that positive vNDV samples be sequenced for further analysis.

Keywords: Newcastle disease, RT-PCR, Velogenic NDV, Industrial poultry flocks, Iran

Effects of conditioner temperature and retention time on pellet quality of broiler diet produced by super-conditioner and conventional methods.

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Objectives: Pelleted feed is the most commonly consumed diet for broilers. Its benefits include increased feed intake, weight gain, improved feed conversion ratios, reduced feed wastage, better flock uniformity, and minimized feed selectivity. Additionally, pelleting enhances feed density, facilitates transportation, and improves feed palatability and hygiene. However, the thermal process involved in pellet production can degrade supplements and additives, such as vitamins, amino acids, and enzymes, and affect feed quality. This experiment was carried out to evaluate the effects of conditioner temperatures and retention times on pellet quality parameters and phytase enzyme recovery using both conventional and super-conditioner methods.

Materials & Methods: A starter corn-soy-based diet was formulated according to the NRC 1994 standard for male broiler chickens. Treatments included mash, conventional pellets, and super-conditioner pellets, produced at temperatures of 70, 80, and 90°C with retention times of 3 and 6 minutes. Pellet durability index (PDI), hardness, microbial populations, and phytase activity of treatment samples were measured and compared statistically.

Results and Conclusion: Increasing both temperature and retention time reduced the overall counts of aerobic bacteria. The bacterial total viable count was decreased by 36.7% in conventional pellet treatments and by 39.6 to 98.67% in super-conditioner pellet treatments as compared to that of mash feed. The fungal population in pellet treatments was significantly ($p<0.05$) lower than mash feed, with reductions ranging from 37.71% to 71.42% as conditioning temperature and time were increased. Pellet hardness and PDI were also significantly higher in super-conditioner treatments than conventional pellets. However, the effects of retention time among super-conditioner treatments were not significant. The pelleting process reduced phytase activity by 11.13% to 24.7% as compared to mash feed. No significant relationship was found between increased temperature and retention time and phytase activity in super-conditioner pellet treatments.

The results indicate that thermal processing may positively affect pellet quality in both conventional and super-conditioner methods in which both PDI and hardness were improved linearly as the conditioner temperature increased in super-conditioner treatments. The optimal conditions for pellet production were found to be a conditioner temperature of 80°C and a retention time of 3 minutes when using the super-conditioner method. The super-conditioner method allows for higher temperatures and retention times with no significant effect on phytase activity.

Keywords: Hardness, Microbial populations, Pellet durability index, Phytase activity, Super-conditioner

"Phylogenetic Study and Annual Rate of Change of the Hemagglutinin-Neuraminidase Gene of Newcastle Disease Virus Sub-genotype VII.1.1 Isolated from Peacock, Turkey, and Broiler Chickens in Iran"

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Newcastle disease virus (NDV), also known as avian orthoavulavirus 1, is responsible for a deadly disease affecting various bird species, including domestic poultry, wild birds, and caged pets, across all age groups (Dimitrov et al. 2019). Newcastle disease has been a persistent issue in the Iranian poultry sector for many years. However, there has been limited exploration of the genetic characteristics of the Hemagglutinin-Neuraminidase (HN) gene among domesticated bird populations in Iran. This study aimed to provide insights into the biological and molecular features of complete HN genes obtained from turkey, peacock, and broiler isolates in Iran from 2018 to 2020. Phylogenetic analysis indicated that these isolates are part of the NDV subgenotype VII.1.1, formerly referred to as VIIL. Further examination revealed the presence of thermostable mutations S315P and I369V within the isolates. The identification of an N-glycosylation site (NIS) at positions 144–146 and a cysteine residue at position 123 may impact the fusogenic properties of the isolates. Additionally, several amino acid changes in both antigenic sites, particularly I514V and E347Q, as well as alterations in the binding sites of the HN protein, raised concerns regarding the pathogenic potential of these isolates. Furthermore, a study analyzed the mutation rate of the HN gene in subgenotype VII.1.1 isolates (n = 30, including those from this investigation) from 2011 to 2020 to understand its evolutionary trends. Using the HKY model in Bayesian analyses, the mutation rate was found to be 1.8088E-3 (relaxed log normal with an Effective Sample Size (ESS) of 643.2). While most regions of the HN and F genes (Molouki et al. 2021) remain largely conserved among NDV isolates, an annual mutation rate of about 2% was observed in both genes among Iranian subgenotype VII.1.1 isolates. In summary, a new variant of NDV with multiple mutations is circulating not only among chickens but also in turkeys and captive birds like peafowls. The ineffectiveness of standard vaccination programs may be linked to differences between the circulating NDV strains and those used in vaccine production. Thus, future measures should focus on expanding vaccination coverage and implementing biosecurity strategies to manage the spread of the NDV strains.

Keywords: Newcastle disease virus (NDV), Hemagglutinin-neuraminidase (HN) gene, Subgenotype VII.1.1, Amino acid substitutions, Annual change rate.

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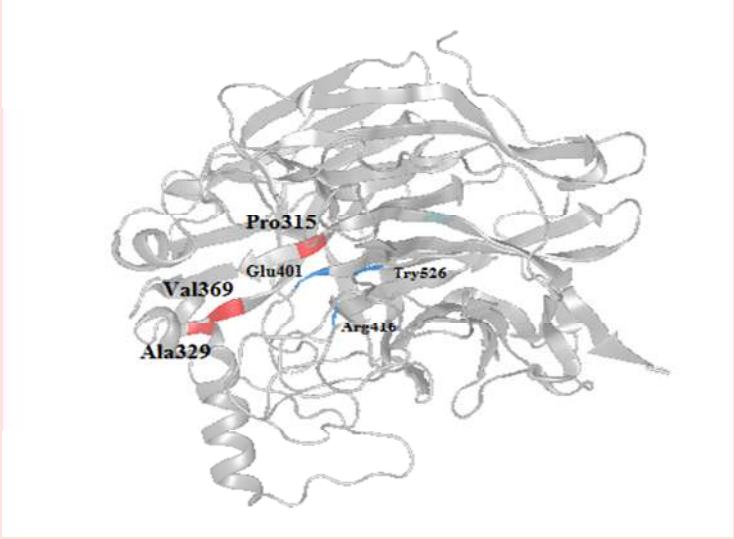
Molouki A, Soltani M, Fallah Mehrabadi MH, Shoushtari A, Abtin A, Mahmoudzadeh Akhijahani M, Abdoshah M, Pourbakhsh SA, Allahyari E, Ghalyanchi langeroudi A, (2021) Predominance of fourth panzootic Newcastle disease virus subgenotype VII. 1.1 in Iran and its relation to the genotypes circulating in the region. Curr Microbiol 78:3068–3078. https:// doi. org/ 10. 1007/ s00284- 021- 02572-z

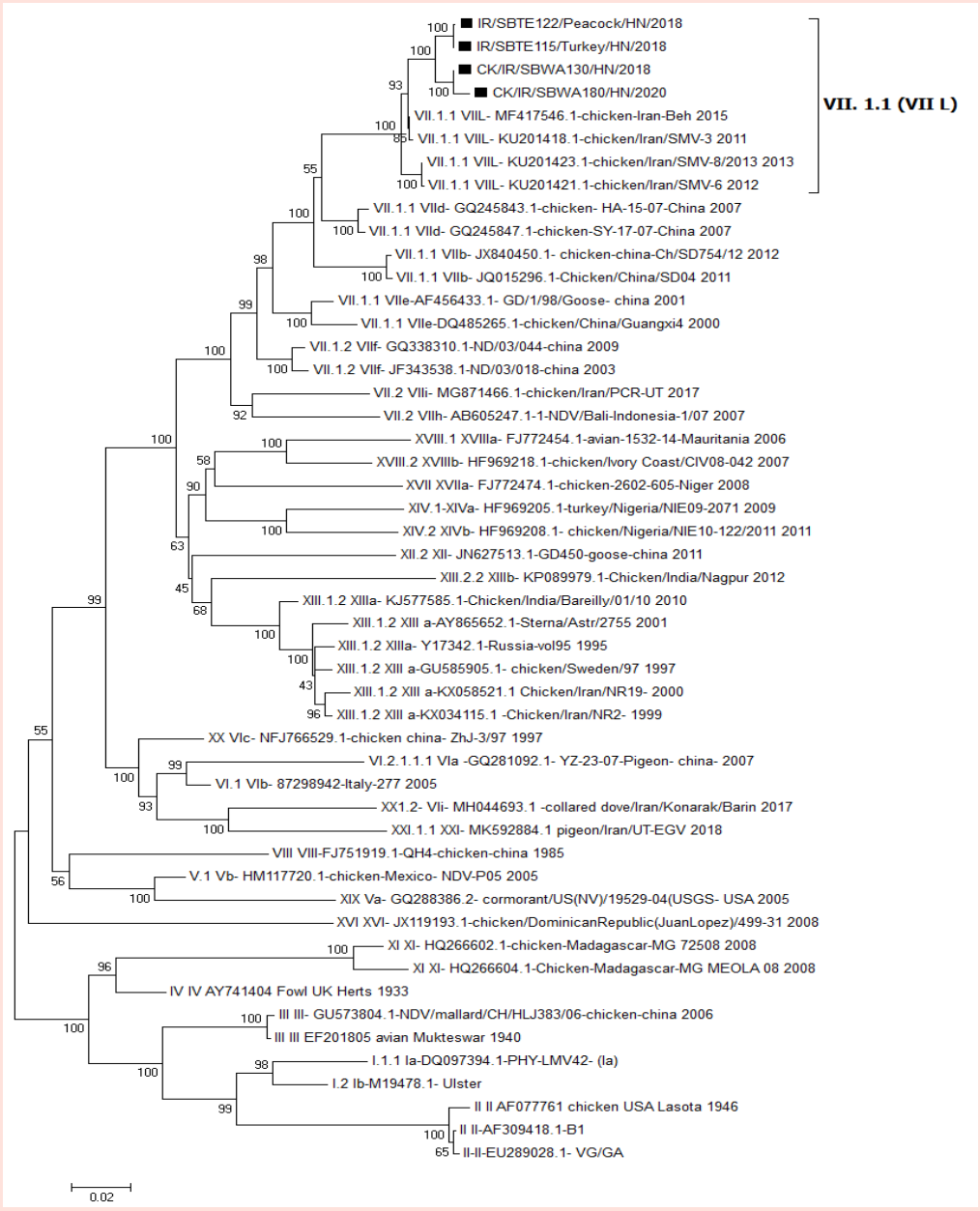
Attachment:

The three-dimensional structure of NDV CK/SBWA180/HN/2020 isolate (accession number: MZ605768) was predicted by the online swiss model software using the homology model of the HN ectodomain belong to the most similar NDV isolate in the NCBI Genbank (PDB ID code: 7BWU-A). Schematic drawing of the HN dimer showing the location of the residues Pro315, Val369, and Ala329 (red residues) compared with the sialic acid receptor binding sites Arg416, Glu401, and Tyr526 (blue residues) edited by the 3D protein software from DNAstar Laser gene package version 8 (DNAStar, USA).



The molecular phylogenetic tree of NDV isolates based on their full length of HN gene ORF was inferred by using the Neighbor-Joining method. The evolutionary history was computed using the Maximum likelihood method based on the

optimum nucleotide method (GTR + G + I) with the bootstrap values of 1000 replicates. This analysis was carried out in MEGA X software.



Relaxed log normal clock rates of complete *HN* gene belong to the Iranian VII.1.1 isolates (n=30, including the isolates of the current study) during 2011 to 2020 using the HKY model of Bayesian analyses from BEAST2 (Bayesian Evolutionary Analysis Sampling Trees 2) software version 2.6.3.0

	HKY
Summary statistic	Relaxed
Mean	1.8088E-3
Standard error	4.1872E-5
Standard deviation	1.062E-3
Variance	1.1278E-6
Median	1.6234E-3
Value range	[6.3039E-4, 0.033]
Geometric mean	1.6901E-3
95% HPD interval	[8.7044E-4, 3.0103E-3]
Auto-correlation time (ACT)	69962.6604
Effective sample size (ESS)	643.2

Cytokine and acute-phase proteins response following vaccination against infectious bronchitis in broilers

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Background: Infectious bronchitis (IB) is an important disease of poultry and vaccination is the best method of preventing IB in the poultry industry worldwide.
Objectives: This study was designed to evaluate cytokine and acute-phase protein (APP) responses and their correlations with antibody titers following vaccination regimes against IB in the broiler.
Materials and methods: Broilers were vaccinated with H120 and 1/96 vaccine strains, and MIX (H120 + 1/96) vaccine strains on days 0 and 14. Heterophils/lymphocyte ratio, acute-phase proteins including chicken serum amyloid A (SAA), chicken pentraxin 3 (chPTX3), chicken interleukin 1β (IL-1β), chicken interleukin 6 (IL-6) levels, and antibody titers were measured.
Results: An increase in the H/L ratio, SAA, chPTX3, IL-1β, and IL-6 levels in vaccinated groups was observed one day after the first (highest rates) and second (lower levels) vaccination up to 3 days in three different patterns, then started to decrease. The results showed an immediate, short-lived response and moderate increases in all criteria. Changing patterns of APPs were different but in similar patterns after the first and second immunization in vaccinated groups. A positive correlation between all criteria values on days 1 and 15 with antibody titers on day 28 may indicate agonistic cross-regulation.
Conclusion: Different types of IB vaccines could induce different patterns of APPs responses, which can be used to evaluate immune response outcomes in vaccine design, development, and administration. The IL-6 with the highest increase can be a sensitive parameter and chPTX3 with the high increase could be an important criterion.
Keywords: APP, broilers, cytokines, IB, Vaccination

Molecular detection of Q1 infectious bronchitis, a new pathogenic genotype in a broiler flock from eastern Iran

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Abstract

Introduction: Infectious bronchitis (IB) is a highly contagious respiratory disease with a global prevalence that results in considerable economic losses for the commercial poultry sector. IBV is categorized into six genotypes, comprising 32 distinct viral lineages and several inter-lineage recombinants. The S1 gene of the IBV virus contains three hypervariable regions (HVRs), with the third HVR commonly utilized for viral genotyping. The Q1 genotype of IBV is known to be pathogenic, causing respiratory disease, proventriculitis, significant reductions in egg production, and nephropathogenic disease. This study presents the second documented case of the Q1 IBV genotype, isolated from a broiler farm located in eastern Iran.

Material and Methods: During an outbreak of respiratory disease among commercial broiler flocks in South Khorasan province, eastern Iran, 36 flocks exhibited respiratory symptoms and irregular mortality. For each flock, pooled samples were prepared by homogenizing trachea, lung, and kidney tissues. Following the isolation of viral RNA and synthesis of complementary DNA (cDNA), primers specific to the S1 gene were employed for reverse transcription (RT). Nested PCR was then conducted using primers common to most known strains of IBV to amplify the hypervariable region 3 (HVR3) of the S1 gene. The products of the nested PCR were sequenced and aligned for phylogenetic analysis using data from NCBI GenBank.

Results and Discussion: Q1 IBV was identified in one of the flocks examined in this study. Prior to this investigation, Q1 IBV infection had been reported in northern Iran in 2020 by Ghalyanchilangeroudi et al. The Q1 IBV strain detected in South Khorasan province was found to be 100% similar to the previously reported Q1 genotype in Iran. Q1 IBV is known to be a pathogenic genotype associated with respiratory disease, proventriculitis, severe egg drop, and nephropathogenic conditions. Given that this is only the second detection of the Q1 genotype in Iran over a three-year period, it is hypothesized that the dominance of variant 2 in the country may have hindered the spread of the Q1 genotype. Nevertheless, the detection of the Q1 virus in this study suggests that this genotype has remained present but previously undetected in the country.

Keywords: Infectious bronchitis, Broiler, Genotype Q1, South Khorasan

Evaluation and effectiveness of prime-boost strategy of vaccination against avian infectious bronchitis in laying hens

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Objectives: Avian Infectious bronchitis (IB) is a highly contagious poultry disease that negatively affects the respiratory, renal and reproductive systems of chickens, and its greatest economic importance in laying hens is due to the harmful effect of the virus on production and quality of egg. Vaccination is the most important way to prevent the spread of this disease in poultry. The emergence of new variants of IBV in laying hen flocks is more frequent than in meat flocks, and the escape from the immune system increases the probability of the emergence of new varieties of IBV. The general purpose of this research is to investigate the effectiveness of using homologous and heterologous vaccines with prime-boost strategy against the dominant and circulating variants and strains of IBV in laying pullets.

Material and Method: Two separated houses with capacity of 30,000 pullets in each house in a layer farm in Qazvin province was chosen. All the birds were LSL strain. In one house chickens were vaccinated as prime-boost strategy which received H120 and 793/B strain as a prime vaccine in day one through spray route and variant2 vaccine as a booster in 8 and 14 days of age through eye-drop and spray route respectively. In another house all day-old chicks were vaccinated with H120 and 793/B through spray route. Number of samples for serology was calculated with Connone & Roe formula. All the samples tested before and after vaccination for determination of amount of antibody against IB in the flock.

Result & Conclusion: The antibody level of the flocks was evaluated by ELISA test with the IDVet commercial kit in both layer houses the age of 14 days of age 28 days of age. At 28 days, the average antibody titer against infectious bronchitis (IB) in the house where the prime-boost strategy was implemented was 6490 and the coefficient of variation was 26 and the difference was significant ($P<0.05$) but another house had an antibody titer of 5150 and a coefficient of variation of 38. According to the prevalence of infectious bronchitis variant 2, it is suggested that in order to control this disease, vaccinate flocks with prime-boost strategy.

Keywords: Infectious bronchitis, IB, Vaccination, Variant 2 bronchitis, ELISA, Laying hens

Molecular detection of avian influenza virus H9N2 in industrial poultry flocks

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Objectives: The H9N2 subtype of the avian influenza virus (AIV) is classified as a low pathogenic avian influenza virus (LPAIV) and is widely prevalent among poultry in Asia. This subtype can cause severe respiratory diseases in immunocompromised chickens. Additionally, it contributes to increased mortality rates in young chicks and significantly reduces egg production in laying hens, ultimately leading to considerable economic losses for poultry producers. This study investigates molecular detection of the H9N2 influenza virus in industrial poultry flocks in Iran.

Materials & Methods: A total of 25 industrial poultry flocks, including broilers, layers, and breeders, suspected of H9N2 infection from various provinces in Iran, were necropsied and subjected to molecular analysis. Between April and December 2024, samples were collected from the trachea, lungs, and cecal tonsils of the birds. All samples were analyzed using reverse transcription polymerase chain reaction (RT-PCR) targeting the haemagglutinin (HA) gene, with a product length of 549 bp to detect H9N2.

Results & Conclusion: The findings indicate a significant prevalence of the H9N2 avian influenza virus among industrial poultry flocks in Iran, with 48% (12 out of 25) of samples testing positive. Notably, two flocks showed negative results in trachea and lung samples but tested positive in cecal tonsil samples, underscoring the need for comprehensive sampling strategies. Given the extensive circulation of the H9N2 virus in industrial poultry settings, it is crucial to implement stringent biosecurity measures and enhanced surveillance protocols to effectively control this disease. Furthermore, incorporating tonsil sampling alongside traditional respiratory tract sampling is recommended to improve diagnostic accuracy and gain a deeper understanding of the epidemiology of H9N2 in poultry populations

Keywords: H9N2, RT-PCR, Avian influenza virus, Industrial poultry flocks, Iran

Genetic design and virtual cloning of recombinant H9- RBD-pPIC9k plasmid for the production of a novel recombinant influenza H9N2 vaccine for poultry

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Objectives: One of the most common diseases in poultry is influenza A virus subtype H9N2 (H9N2 influenza), which is the most important cause of disease and mortality in chickens. Due to its economic importance and zoonosis potential, this virus has attracted a lot of attention, and many efforts are underway to develop its recombinant vaccines. The aim of the present study was to design a recombinant plasmid (H9-RBD-pPIC9k) based on the pPIC9k vector for the genetic engineering of *Pichia pastoris* yeast as a host. This study was derived from the doctoral dissertation of Dr. Amir Asghari Baghkheirati and resulted in the production of the first H9N2 influenza recombinant vaccine in Iran.

Materials and methods: The hemagglutinin protein receptor binding domain sequence was obtained from the A/chicken/Iran/D7/1998 (H9N2) virus, an endemic strain, and used in the present study. Then, the recombinant pPIC9K plasmid was designed. This plasmid contains signal peptide sequence, His-tag, H9 antigen, alpha agglutinin anchor, and vector backbone sequence, including selectable marker, origin of replication, AOX1 promoter, and terminator. *In silico* cloning was performed using SnapGene software, and the sequence was codon optimized for proper expression in *P. pastoris* GS115 strain.

Results: The tag, receptor binding domain of H9 antigen, and anchor encoding sequences were virtually cloned, and the H9-RBD-pPIC9k plasmid was designed successfully. A signal peptide was designed to put the recombinant protein into the secretory pathway. Finally, the fully mature hemagglutinin protein will bind to the cell's surface due to the alpha-agglutinin anchor.

Conclusion: The recombinant plasmid designed in this study was successfully used in subsequent steps to engineer the *P. pastoris* and produce recombinant influenza H9 antigen as a subunit vaccine for poultry.

Keywords: recombinant plasmid, *in silico* cloning, influenza vaccine, *P. pastoris*.

Identification, molecular characterization and phylogenetic study of *Mycoplasma Gallisepticum*

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Objectives: *Mycoplasma gallisepticum* (MG) is significant poultry pathogen worldwide, causing chronic respiratory disease in chickens and turkeys, reducing poultry production, and substantial economic losses in the poultry industry. Gold standard test for the detection of MG is isolation and identification of the organism, which typically requires 2 to 3 weeks. However, PCR has been employed for the rapid detection and identification of MG from cultures or directly from clinical specimens. This study aimed to isolate and molecularly identify MG isolates and investigate the presence of a partial sequence of the mgc2 gene-one of the most important binding proteins involved in the bacterial pathogenicity process-in samples taken from a breeder flock.
Materials and Methods: Swab samples were collected from a 25-week-old Ross breeder flock exhibiting clinical symptoms, including respiratory sound, along with a low mortality rate. The samples were manually extracted. PCR amplification was performed to confirm the presence of the bacteria through targeting a partial sequence of mgc2 gene. PCR products were sequenced with Sanger Sequencing method and then phylogenetically analyzed using MEGA software version 7, by the maximum-likelihood method with a bootstrap of 1000.
Results and Conclusion: The PCR amplification results confirmed the presence of a partial sequence of the mgc2 gene. Phylogenetic analysis of the sequenced PCR products revealed the highest similarity to two previously submitted sequences from 2015: one from Iran (MK984183) and one from India (KP279743). Due to the difficulty of MG detection through isolation, PCR amplification is the most accurate method, detecting active infection in the early stages of the disease. MG is a significant pathogen that severely impacts the poultry industry economically, necessitating continuous monitoring through both serological and molecular methods, along with restricted biosecurity measures to control its spread.

Keywords: *Mycoplasma Gallisepticum*, Breeder Flock, PCR, Phylogenetic Analysis, mgc2 gene.

Co-infections of *Ornithobacterium rhinotracheale* and other respiratory pathogens in poultry: A review of experimental and natural cases in Iran and the world

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Objectives: The pathogenicity of *Ornithobacterium rhinotracheale* (ORT) isolates varies from mild to high, but most isolates, including the isolates tested in Iran, show mild pathogenicity and are considered secondary pathogens and cause severe disease only in infections with other pathogens. However, a naturally non-virulent strain of ORT has not been found. Mildly pathogenic ORT alone has failed to cause mortality, clinical signs, and sometimes even obvious necropsy signs in commercial or specific pathogen-free (SPF) poultry. There are also few reports of highly pathogenic ORT isolates with the ability to produce disease alone and as primary pathogen. The aim of this study was to investigate the experimental co-infections of Iranian ORT isolates with several other poultry respiratory pathogens, which also showed mild pathogenicity alone. Another aim was to investigate the natural co-infections of ORT and other pathogens reported from poultry farms submitted to the Razi Institute.

Materials and Methods: SPF chickens and equipped isolation chambers at the Razi Institute allowed researchers to study single and double infections of ORT with three viruses and another pathogenic bacterium of the avian respiratory tract in experimental infections. For this purpose, SPF chickens were divided into experimental groups. Four pathogens isolated from the poultry industry, including ORT, infectious bronchitis virus (IBV) serotype 793/B, avian influenza virus (AIV) H9N2, and *E. coli* serotype O2, as well as Newcastle disease virus (NDV) Lasota vaccine strain were used individually as single infections. In other experimental groups, dual infections of ORT with each of the other 4 agents were induced in SPF chickens. The chickens in each group were examined for mortality, clinical signs, necropsy, histopathology, bacterial and viral culture and PCR identification. In addition, natural concurrent infections of ORT with other poultry pathogens in the country's poultry farms, as well as similar studies in other countries, were also reviewed.

Results and Conclusions: In groups with single infections or controls, no severe clinical signs were observed. However, dual infections of ORT with H9N2 avian influenza showed significant clinical and necropsy findings and also mortality. Exacerbation of necropsy signs was noted in the ORT + LaSota group, while no such exacerbation occurred with ORT co-infected with IB virus or *E. coli*. Similar studies in the world have indicated that co-infection of ORT+IBV or ORT+*E.coli* can worsen clinical signs due to probably varying virulence of agents or avian susceptibility. In 2002, Banani et al. linked high mortalities in poultry industry of Iran to concurrent H9N2 and ORT infections. The economic loss from ORT infections in the US poultry industry has been reported to

be in the hundreds of millions of dollars per year, and this loss is largely due to disease exacerbation and mortality in co-infections. Therefore, the hidden economic loss from ORT infection, especially due to disease exacerbation in co-infections with other respiratory pathogens, should not be underestimated.

Keywords: *Ornithobacterium rhinotracheale* (ORT), respiratory pathogens, experimental co-infection, natural co- infection, poultry industry, SPF chickens, Iran.

A brief review on Avian Nephritis Virus pathology and a report of detection from Mashhad, Iran

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Abstract:

Objectives: Avian nephritis virus (ANV) is an etiological agent responsible for growth retardation in young chickens by inducing interstitial nephritis. The virus was initially isolated from young chickens. The capsid protein is crucial for virus pathology and antigenicity. This study aimed to conduct nucleotide sequence and phylogenetic analysis of the capsid gene in the Avian Nephritis Virus (ANV) detected in Iran.

Materials & Methods: Samples were collected from broiler chicken flocks exhibiting symptoms of diarrhea, undigested feed in feces, growth retardation, poor uniformity, lethargy, and depression. Additionally, some samples were obtained from chickens without specific clinical symptoms. Briefly, fecal samples were homogenized and RNA was extracted. Utilizing various specific primers, cDNA was synthesized, followed by polymerase chain reaction (PCR), and subsequently, the PCR product was visualized via agarose gel electrophoresis. The PCR products were purified and used for sequencing. The chromatogram of nucleotide sequences and deduced amino acid sequences were analyzed using relevant software, and the complementary data were obtained from the National Center for Biotechnology Information (NCBI). The nucleotide sequence of the capsid gene was submitted to GenBank, and the phylogenetic tree was constructed.

Results & Conclusion: ANV-1 was detected in 2 different broiler chicken flocks. Also, 3 different groups of ANV2 in one flock and one ANV2 in a different flock were detected in this study. Despite numerous attempts, we were unable to sequence the entire capsid genome of ANV1. This failure reinforces the variability in the capsid nucleotide sequence of the virus, as previously reported for various Astroviruses. Three sections of the ANV-2 capsid genome were amplified and sequenced. We tried to sequence the capsid genome of ANV-2 by aligning the sequences of samples with available data from GenBank. The phylogenetic analysis of ANV-2 isolates was evaluated in comparison with reference strains of AsTVs, serotype 1 ANV and Pigeon ANV. In conclusion, to conduct a comprehensive sequence analysis of the ANV1 capsid, the virus must be isolated and purified. Subsequently, the virus genome should be amplified using random primers, followed by cloning, sequencing, and assembling the contiguous sequences to obtain the complete sequence. This study provides valuable information on the molecular characterization of ANV in Iran, which can contribute to the development of effective control strategies and diagnostic tools for this virus.

Keywords: Astrovirus, Avian Nephritis Virus, Capsid Gene, Phylogenetic Analysis, Polymerase Chain Reaction (PCR)

Phylogenetic analyses on Marek's disease virus circulating in Iranian backyard and commercial poultry indicate viruses of different origin

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Objectives: Gallid alphaherpesvirus 2, commonly known as serotype 1 Marek's disease virus (MDV-1), is an avian virus that is mostly reported from poultry. Typical clinical signs of MDV-1 include leg and wing paralysis, weight loss, reduced egg production in layer chicken, grey iris, and enlarged feather follicles. Necropsy signs include nodular and diffuse visceral lymphoid tumors in organs such as the spleen, liver, and kidney. The first step in prevention may therefore be to genetically characterize and systematically identify their source and origin.

Materials & Methods: Two birds showing typical late-stage neoplastic diseases were delivered to the Department of Avian Disease Research and Diagnostic of Razi Institute. The first subject was an unvaccinated 32-week-old backyard turkey in Mazandaran in September 2020, which suffered from depression, weight loss, enlarged feather follicles, and paralysis. Necropsy revealed severe nodular neoplastic growth liver and spleen. The second subject, a vaccinated 36-week-old flock of commercial breeders from the city of Gorgan in June 2021, had similar symptoms before and after necropsy. All the affected organs, including the spleen, liver, sciatic nerve, kidneys, proventriculus, and feather follicles, were collected separately and stored at -70 °C. Meq gene PCR amplification was performed using OIE standard primers Fwd: 5′-GAG-CCA-ACA-AAT-CCC-CTG-AC-3′and Rev: 5′-CTT-TCG-GGT-CTG-TGG-GTG-T-3′. The primers are able to amplify any meq size. The FASTA dataset was used to study the phylogenetic relation between the strains of the current study and international strains. BLAST was also used to identify the matching strains. MEGA X was used to study phylogenetic relationships.

Results & Conclusion: The backyard turkey of the current study was positive for MDV-1. The sequencing results showed an ORF of 897 bp, which encoded a protein product of 298 aa. This size has never been previously reported in any turkey species. According to phylogenetic analysis, a close genetic relationship (0.68%) to several chicken-origin isolates such as the American vv+648A strain was found. In addition, we identified a standard meq protein from an MDV-1-infected

commercial chicken farm. It is likely that the highly identical MDV-1 viruses currently circulating in Iranian chicken farms, which may be indicative of human role in the spread of the virus, have similar Eurasian origin. Our data suggest that regardless of the meq size, MDV-1 circulating in Iran is from different origins. On the other hand, meq sequences from bird species other than chicken have been reported but are very few. Our investigation suggests MDV-1 circulating in turkey does not have species-specific sequences.

Keywords: Marek's disease, Backyard turkey, Chicken farms, *Meq* gene, MDV-1

Analysis of global climate change and its future research on bird migration and behavior (case study: International Anzali Wetland)

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Objective :Global climate change and global warming are the focus of planners at national, regional and international levels. The International Conference on Climate Change (IPCC) announced that human activity is responsible for 99.9% of the changes that have occurred in the world. Considering the geographical location of Iran, which is located in a dry and semi-arid climate, it is very vulnerable to climate change. Wetland which are the habitats of migratory and native birds are also affected by these changes.

The aim of the research is to analyze the global climate change and its future research on the migration and behavior of birds in Anzali International Wetland.

This Wetland, which is the wintering and breeding habitat of birds in the migration corridor of West Siberia, Caspian and Nile in the Middle East, was chosen as a case study.

Materials and methods: Geographical location of the area: Anzali Wetland is located in the vicinity of Anzali free zone in Gilan province. This natural Wetland has fresh water and is a bird watching center located on the southern edge of the Mazandaran sea and in the humid and temperate Caspian climate.The lenghth of this Wetland is 33 km and its width is 11 km.

The method of library studies and field studies (interview and observation) has been used. The resulting research is applied in terms of purpose and from the point of view of descriptive-analytical method.

Results and conclusion:The average temperature of the earth's surface in 2022 is 0.89 degrees Celsius, higher than the average of the last 40 years. According to the Bird Protection Organization, climate change has had a negative impact on a quarter of the 5700 species of birds studied in the world, and only 13% of these species have have found compatibility now. The geographical location, shape and size of Anzali Wetland have made it a stop on 3 out of 9 global bird migration routes. It is predicted that every year about 100 to 120 thousand water birds spend the winter in the wetland and it has 243 species of birds including 51 aquatic species, 61 aquatic species and 131 species It is dry. 9 species, including the black-bellied gypsy rooster, are on the critical list, 17 species are on the endangered list, including the Saker Falcon and the Sea eagle, and the species of

Common pochard and Podiceps Auritus, etc are in the vulnerable category of the International Union for Conservation of Nature and Natural Resources (IUCN).

conclusion Global climate change and global warming have had a negative impact on the reproduction, behavior of birds and the distribution and abundance of their diet. So that the number of birds has decreased significantly in the last two decades. Some studies show a 30% decrease in the landing of migratory birds in the last two decades. The main reasons are the increase in greenhouse gas production, the decrease in water depth and level, and the decrease in oxygen due to the growth of invasive and non-native plant species such as azolla and water hyacinth, forests. He pointed out the reduction of the water level of the Caspian Sea, non-observance of the basic hydrological principles, the weakness of the watershed operation, the entry of sewage from the two rivers Gohar Rood and Zarchob and Shekarashare. Considering that this Wetland has been registered in the Montreux list and according to the studies of the American Geophysical Union, it may dry up completely by 1438. Therefore, in the absence of comprehensive planning and foresight, no more migratory birds will fly in the sky of this area. The Wetland will become a center of dust, which will endanger the health of the inhabitants.

Keywords: Climate change, global warming, future research, migration, bird behavior, Anzali Wetland

Molecular detection of Fowl Adenovirus associated with Inclusion Body Hepatitis (IBH) in Iranian chickens

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Objectives: *Fowl adenoviruses (FADVs)* encompass 12 serotypes (1-7, 8a, 8b, 9,10, and 11) and five genotypes (A-E), which are associated with various fowl diseases, including Hyperpericardium Hepatitis Syndrome, Gizzard Syndrome, and Inclusion Body Hepatitis. Inclusion body Hepatitis (IBH), caused by all Fowl Adenovirus Serotypes except Serotype 1 and 4, is classified under genotypes B, C, D and E. While often asymptomatic, FADV-IBH Serotypes especially Serotypes 11, 8b, and 2 can lead to sudden increases in flock mortality. Gross lesions associated with the disease include liver swelling and hemorrhagic and pale foci in the liver. In some cases, mild hepatitis may develop; however, when compounded by secondary immunosuppressive infections, mortality rates can soar to 40%. Both vertical and horizontal transmission significantly contribute to the propagation of this disease. This study aimed to monitor and assess the prevalence of FADV Serotype 11, 2, and 8b in unvaccinated poultry flocks suspected of IBH.

Materials and Methods: Tissue samples were collected from suspected poultry flocks across 15 different farms in 10 provinces of Iran (including broiler, layer, breeder, and pullet flocks) that had been referred to Sana Specialized Poultry Hospital since fall 2024. Conventional PCR was performed using a general primer and three specific primers targeting the Hexon gene of serotypes 2, 11, and 8b that amplified 260 bp,112 bp, and 560 bp fragments respectively. The sampled birds, comprising both live and dead individuals, exhibited signs of hepatitis, and their ages ranged from one-day-old chicks to 85-week-old birds.

Results & Conclusion The results indicated that 60% of the cases were positive for fowl adenovirus using the general primer. Among these positive cases, the distribution of serotypes was as follows: 78% serotype 11, 22% serotype 2. Interestingly all positive cases of serotype 8b were co-incided with serotype 11. Furthermore, molecular detection of fowl adenovirus was performed on various tissues, including the liver, kidney, tonsil, heart, lung, and spleen, at different stages of the disease and across different ages of the birds. Notably, liver and cecal tonsil samples yielded contradictory results regarding viral presence.

Our data confirmed that serotype 11 is the dominant strain in these 9 provinces, which are predominantly located in the central regions of Iran. This highlights its significance in the region. To achieve a definitive and rapid diagnosis, it is recommended to sample both liver and cecal tonsil tissues and pool these two tissues for PCR analysis. This pooled sampling approach may enhance detection rates and provide a more comprehensive understanding of the viral burden in affected flocks.

Keywords: Fowl Adenovirus, IBH, Serotype 2, Serotype 11, Serotype 8b, PCR, Molecular detection

Detection and identification of Novel Ostrich parvovirus in Iran

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Objectives: In recent years, a novel disease affecting ostriches has emerged in several regions of Iran, characterized by sudden death, diarrhea, and paralysis. This outbreak has significantly impacted the Iranian ostrich farming industry, leading to substantial economic losses. This study aims to characterize and identify a novel ostrich parvovirus (OsPV) originating from chicks that exhibited paralysis and diarrhea in ostrich flocks in three provinces in Iran.
Materials & Methods: Samples were collected from paralyzed ostriches and evaluated through molecular detection and phylogenetic analysis. Conventional PCR was performed to amplify the VP1 gene, and one positive sample, named as SANA/OsPV/001/Ostrich/IR/2024, was sent for sequencing using both forward and reverse primers. A comprehensive analysis of the VP1 gene of OsPV was conducted to elucidate its genetic characteristics.
Results & Conclusion: Throughout 2024, three distinct isolates of OsPV were identified in three provinces: Tehran, Razavi Khorasan, and Isfahan. A 659 bp fragment of the VP1 gene of OsPV was detected in various organs, including the intestine, liver, brain, bursa of Fabricius, articular content, spleen, kidney, pancreas, sciatic nerve, and esophagus, indicating that the new OsPV variant has multiple organ tropism. Comparative sequence analysis of the VP1 gene revealed a high degree of homology between the SANA/OsPV/001/Ostrich/IR/2024 isolate and strains from Turkey, the United Kingdom, and Russia. Notably, the SANA/OsPV/001/Ostrich/IR/2024 isolate was found to be closely related to goose parvovirus and other ostrich parvovirus strains.
This study represents the first documented molecular characterization of ostrich parvovirus in Iran. Our findings contribute to a better understanding of the virus's epidemiology and its impact on the ostrich farming sector, underscoring the need for ongoing surveillance and control measures in affected regions.
Keywords: Ostrich parvovirus (OsPV), Phylogenetic, Molecular Detection, Iran, Histopathology, NSP-VP1 gene

Serological screening for Salmonella pullorum in broiler breeder farms across different provinces of Iran

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Objectives: *Salmonella* Pullorum is a significant pathogen in the poultry industry, resulting in considerable economic losses due to decreased productivity and increased mortality. The rapid serum agglutination (RSA) test is a widely used serological method for detecting antibodies against *Salmonella* Pullorum in poultry, offering a quick, reliable, and cost-effective diagnostic approach. This study aimed to serological screening for *Salmonella* Pullorum in broiler breeder flocks in different provinces of Iran using the RSA method.
Materials & Methods: Between June and December 2024, 1053 serum samples were collected from 25 broiler breeder flocks across various provinces of Iran. The presence of *Salmonella* Pullorum antibodies was detected using the RSA test in accordance with existing standards to ensure optimal sensitivity and specificity. For the RSA test, 50 µL of serum was mixed with 50 µL of colored *Salmonella Pullorum* antigen on a clean microplate. The mixture was incubated for 2 minutes at room temperature, after which agglutination was observed.
Results & Conclusion: Among the 1053 serum samples, 43 (4.09%) tested positive for *Salmonella* Pullorum antibodies. The findings of this study highlight the presence of *Salmonella* Pullorum antibodies in Iranian broiler breeder flocks. Given that the flock is culled if infected with *Salmonella* Pullorum infection, strict biosecurity measures are essential to prevent and control outbreaks. Additionally, regular surveillance using reliable diagnostic tools like the RSA test is crucial for early detection and control of the infection.
Keywords: *Salmonella* Pullorum, Rapid serum agglutination, Broiler breeder flock, Serological screening Iran

In vitro evaluation of different doses of specific egg yolk antibodies against *Salmonella Typhimurium* in inhibiting bacterial growth

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Objectives: *Salmonella Typhimurium* is one of the main causes of zoonotic diseases associated with food consumption worldwide, primarily leading to gastrointestinal infections. Poultry acts as a primary reservoir and transports bacteria into the human food chain. The increase in antibiotic resistance, caused by the excessive use of antibiotics, has led to the demand for alternative treatments. Egg yolk-derived specific antibodies (IgY) have emerged as a promising immunotherapy option due to their affordability, high efficacy, and potential for mass production. This study aimed to investigate the inhibitory effects of egg yolk specific antibodies (IgY) against *Salmonella Typhimurium* in different doses invitro.

Materials & Methods: 16 Light Lohman LSL-LITE laying hens were randomly divided into control and experimental groups. The experimental group was immunized with formalin-inactivated *Salmonella Typhimurium* whole-cell antigen using complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) in five doses at two-week intervals. Specific antibody activity was measured in both serum and egg yolk during experimental period by ELISA. To evaluate the antimicrobial properties of specific antibodies, in vitro assays were conducted to assess their ability to inhibit bacterial growth in different doses (50, 100,150 & 200 mg/mL). In addition, the bactericidal activity with and without supplementation and the capacity of antibodies to prevent bacterial adhesion to the intestinal mucosa and jejunum were evaluated through colony counting.

Results & Conclusion: Egg yolk-derived specific antibodies effectively inhibited the growth of *Salmonella typhimurium* in vitro and showed a dose-dependent response with the highest inhibition observed at 200 mg/mL ($p<0/05$). These antibodies significantly reduced the adhesion of bacteria to jejunum and intestinal mucosa in a dose-dependent ($P<0/05$). However, regardless of the presence or absence of supplementation, no significant difference in bactericidal activity of specific egg yolk antibody (IgY) was observed between treatments ($P>0/05$). The results demonstrated that specific (IgY) exhibit significant inhibitory effects on the growth of *Salmonella Typhimurium* at high doses. These antibodies also dose-dependently reduce bacterial adhesion to the intestinal mucosa. As an effective alternative to antibiotics.

Keywords: specific egg yolk antibody, IgY, *Salmonella Typhimurium*, Invitro, growth inhibitor

Application of ML to predict the occurrence and risk analysis of avian influenza based on important ecological factors

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Objectives: Highly Pathogenic Avian Influenza (HPAI) occurrence pose a significant threat to the poultry industry, animal health, and public health due to their potential for rapid transmission and economic impact. This study applies Machine Learning models to predict the occurrence of AI outbreaks based on farm, bird, and environmental determinants, with a focus on Mazandaran province, a northern region in Iran with a high density of poultry farms. By incorporating diverse ecological and environmental data, the aim of this study was to investigate AI outbreaks in Mazandaran province, identifying and prioritizing key ecological and farm-specific factors that contribute to the risk of outbreaks.

Materials & Methods: Data were collected from multiple sources, including BirdLife International for migratory and native bird presence, the Cornell Lab of Ornithology for avian species' classification and FAO for avian species' health histories, local authorities for poultry production records, and Open-Meteo for weather conditions. Key features included farm-specific data (e.g., farm history, previous infections), bird-related variables (e.g., migratory patterns, species health status), and weather metrics (e.g., temperature, humidity, wind). The study faced challenges of data imbalance and underreporting, which were managed using techniques like class weighting and cost-sensitive learning rather than oversampling to maintain data integrity. The data were fed to three ML models: Logistic Regression, Random Forest, and XGBoost and then we analyze the outputs.

Results & Conclusion: Random Forest and XGBoost demonstrated strong performance, with farm-related factors emerging as the most influential predictors across all models. Logistic Regression highlighted bird-related features, underscoring a potential linear relationship with infection risk. Weather-related factors, while significant, were less impactful than anticipated, suggesting that micro-level farm and bird variables play a more direct role in AI occurrence within Mazandaran's specific environment. This study utilized SHAP values to analyze feature importance to examine the impact of each feature on the model outputs more accurately. Overall, the results of this study demonstrated that farm-related factors, particularly disease history and structural characteristics of

farms, play a significant role in predicting AI occurrence. Additionally, findings indicated that bird-related factors, such as the number of migratory and presence of species with morbidity history in previous weeks, were also important in some models, though their impact was less than that of farm-related factors. Weather factors, especially temperature and seasonal patterns, also played a role in more complex models but were less influential compared to farm-specific factors.

Keywords: Highly Pathogens Avian Influenza, Disease modeling, Machine Learning, Environmental determinants, Mazandaran

Investigating the correlation of Inflammatory cytokines and Serum amyloid A gene expression level in broiler chickens

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Objectives: Factors such as trauma, infections, and vaccinations that trigger inflammation and initiate the acute phase response start with the release of pro-inflammatory cytokines, resulting in the production of acute-phase proteins (APPs) such as serum Amyloid A(SAA). In this discussion, we explore the correlation between, pro-inflammatory cytokines, and one of the important APPs (SAA) following the immunization of broiler chickens as a stress trigger.

Materials and methods: Ninety-one-day-old chicks were divided into two groups: a treatment group of 60 chicks and a control group of 30 chicks. The treatment group was vaccinated against Newcastle disease (NDV) to introduce a stress factor. Subsequently, the liver and spleen were extracted from the samples, and after that Total genomic RNA was extracted from liver and spleen tissue using TRIzol (Invitrogen, US) and reverse transcription was carried out by M-MLV Reverse Transcriptase kit (Fermentas, Germany) to assess the expression levels of the pro-inflammatory cytokine IL-6, IL-1, LITAF genes, and SAA gene.

Results and conclusion: Analysis of the correlations between SAA mRNA in liver and mRNA expression of IL-1, IL6 and LITAF cytokines in spleen showed significant positive correlation. In summary, vaccinating broiler chickens can lead to stress and trigger an inflammatory response. This inflammation results in elevated levels of pro-inflammatory cytokines, such as IL-1, IL-6, and LITF, as well as acute phase proteins like SAA. Our findings indicate a significant correlation between the levels of pro-inflammatory cytokine mRNA in the spleen and SAA mRNA expression in the liver. Additionally, the data imply that measuring SAA mRNA could serve as an indirect indicator of stress, inflammation, and overall innate immunity in chickens.

Key words: Pro-inflammatory cytokines, IL-6, IL-1, LITAF, serum amyloid A, chicken.