Why the Poultry Industry Must Align Collaboratively Or Face Disruption?

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In recent months we have experienced, once again, outbreaks of Avian Influenza (AI) in poultry flocks across Canada. These have become a regular feature of life in our agricultural communities, upsetting not only the economic viability of poultry operations, but posing a potential human health threat in many ways: through potential transmission of infection to humans of course, but more significantly through disruption of rural economies and by posing yet another perceived threat to all Canadians - thereby adding to our national mental health burden and eating away at our individual and collective resilience.

The reality is that Canada has no lack of technology, innovation, creativity, or brain power to solve this issue. Nor does the country lack the financial resources. What we lack is a coherent, collaborative leadership and management framework to do so. We operate in silos, with no coherent vision to drive our collective - or individual - preferred futures and with leadership and management systems to address overarching industry issues grounded in thinking from the 1970s. This must change because our livelihoods, health and country are at stake.

The problem is largely beyond the control of any one person, it is our ***systems*** that are broken, or at the very least, so antiquated as to be almost irrelevant to solving the complex or 'wicked' problems of the twenty-first century. Without solving our leadership and management problems and without learning to do collaborative work, One Health will become yet another broken promise. Fortunately, though, all of this ***can*** change, and it must change. In this paper we review how we can bring about effective change through proper collaborative leadership and management to solve the Avian Influenza and other poultry industry issues.

Keywords: One Health, Avian Influenza, Leadership, Collaboration

The Inhibitory Effect of cLF-chimera, a Recombinant Antimicrobial Peptide, on Avian Influenza Virus Subtype H_9N_2

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Objectives: Avian influenza subtype H9N2 is the most prevalent influenza virus in poultry worldwide. It imposes economic losses on the poultry industry and has zoonotic potential. Currently, there are two main groups of anti-influenza drugs in use; Adamantanes and Neuraminidase inhibitors. In recent years, there has been increasing resistance to existing anti-influenza drugs. Antimicrobial peptides are a group of potential drug candidates with broad-spectrum activity. cLF-chimera is an antimicrobial peptide synthesized from camel milk lactoferrin. This study's objective is to evaluate the inhibitory effects of cLF-chimera on avian influenza, subtype H9N2.

Materials & Methods: For this purpose, one hundred and seventy 11-day-old embryonated eggs were randomly distributed in 17 groups. Different virus and peptide concentrations were injected into the eggs. The eggs were incubated for four days with daily candling for viability assessment. On the 4th day of incubation, each group's alive or dead embryos were sorted and evaluated for gross anomalies. Next, the chick embryos were fixed with 10% neutral buffered formalin for one week for histopathological studies. The MTT assay was also performed for the peptide and virus concentrations.

Results & Conclusion: Overall, the embryo viability results and macroscopic and histopathologic findings showed that the peptide has inhibitory effects against the virus. These findings agree with the MTT assay. Moreover, the peptide has proven effects against pathogenic bacteria that can be advantageous compared with common anti-influenza medications.

Key words: Avian Influenza; Subtype H_9N_2; Antimicrobial Peptide; cLF-chimera; Drug Candidate; Histopathology

Biosecurity Measures and it's Determinants in Commercial Layer Chicken Farms in High Density Provinces of Iran in 2019: A Cross-sectional Study

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Background and Objectives: The commercial layer chicken farming is one of the most important sectors of the poultry industry. In this study, the status of biosecurity of commercial layerchicken farms in 9 Iranian provinces with the highest layer chicken population has been evaluated.

Methods: In this cross-sectional study, 202 commercial layer chicken farms were selected using stratified random sampling from the 9 Iranian provinces in 2019. Then, the required data were collected using a questionnaire by referring to the farms.We used mean, standard variation, sum and adoption rate index to express the result.

Results: The results showed that the adoption level of total biosecurity measures in the commercial layer chicken farms in Iran was 68.18 percent and for conceptual, structural and operational biosecurity measures was 72.11, 75 and 60.82 percent, respectively. Among conceptual biosecurity measures, the lowest adoption level was due to distance to the nearest garden and agriculture land with 29.20%. Among structural biosecurity measures, the lowest adoption level was due to existence of hand disinfection before shed with 28.96%. Among the operational biosecurity measures, the lowest adoption levels belonged to water filtrations and microbial testing of water, respectively with 31.18% and 33.91 %.

Conclusion: This study showed the biosecurity situation of commercial layer chicken farms in 9 Iranian provinces with the highest layer chicken population and revealed some weaknesses in the implementation of these measures at conceptual, structural and operational levels.

Keywords: Biosecurity, Chicken, Commercial layer farm, Iran

A molecular survey of *Chlamydia* spp. infection in commercial poultry and detection of *Chlamydia pneumoniae* in a commercial turkey flock in Iran

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Objectives: Chlamydiaceae are a group of gram negative obligated intracellular bacteria that can cause infection in a wide variety of animals and human. Infection by these agents in avian species including poultry, can be an asymptomatic to an acute systemic disease with relatively high mortality or egg production reduction especially in commercial turkey and turkey breeders. Chlamydiacea are considered potentially zoonotic and there are numerous reports of human infection from poultry specially in slaughter houses. In this study we conducted a survey to assess the chlamydia infection rate in commercial poultry flocks in Iran.

Materials & Methods: 24 commercial poultry flocks including broiler, commercial layer, broiler breeder, commercial turkey and turkey breeder were sampled by triple swab method. After DNA extraction these samples were subjected to 23S rRNA real time PCR. Positive samples were also examined by other PCRs including a 16S rRNA PCR suitable for species identification and an*omp*A PCR suitable for genotyping.

Results & Conclusion: Out of these flocks only one commercial turkey flock was found positive in diagnostic real time PCR. Sequencing the 16S rRNA gene of positive samples showed 100% similarity between found isolate and *Chlamydia pneumoniae* isolates from koala and frog. Also, sequencing a part of *omp*A gene showed slight dissimilarity between turkey isolates and other isolates from other animal and human isolates. In phylogenic tree based on partial sequencing of *omp*A gene, turkey isolates located in a different clade. This is the first report of this agent in poultry industry in the world. *Chlamydia pneumoniae* which is a prevalent agent in human population and causes different types of maladies was zoonotically transmitted from wild animals in the past and animal isolates are potentially dangerous to public health.

Keywords: Chlamydia infection, Chlamydia pneumoniae, poultry, turkey, chicken

Pathogenicity of Fowl Adenovirus Serotype 1 in Japanese Quails

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Objectives: This study reports for the first time the pathogenicity of fowl adenovirus serotype 1 isolated from gizzard lesions of broiler chickens in Japanese quails.

Materials and methods: After the primary propagation of previously identifiedFowl Adenovirus Serotype 1 virus in embryonatedeggs, cell culture method was used for the determination of virus inoculation titer. After the titer determination, 150 quails were purchased from a local hatchery and kept in a cage system. On the 10th day of breeding, 20 quails were randomly selected for each group and allocated after weighing to three groups in separated rooms. A day after virus inoculation to the quails via oral and intratracheal routes, 10 non-infected quails and 4 SPF chickens were added to these two groups. After virus inoculation, weight gaining were recorded for 10 days and clinical signs were inspected for 7 days.On days 4, 7, and 11 after virus inoculation, quails were euthanized and after the inspection of gross lesions, sampling was done for histopathology and PCR. In addition, contact birds were also euthanized at 4 and 7 days after contact for gross lesions and microscopic and molecular evaluations.

Results and discussion: Oral and intra-tracheal inoculation of gizzard erosion induced fowl adenovirus serotype 1 in Japanese quails led to anorexia, mild diarrhea, and weight loss in both of the groups.Oral and intra-tracheal inoculations were led to obvious liver gross lesions and some cases of hemorrhage in serosal surface of the heart and lung hyperemia. Gross lesions and histopathology of SPF chicks and contact quails showed that virus could transmitted from inoculated quails to the SPF chicks and non-infected quails with similar gross lesions with virus inoculated groups. Histopathology evaluation of inoculated and contact groups showed dilation of hepatic sinusoids, hyperemia and hemorrhage ofliver without specific histopathologic lesions in gizzard and trachea. PCR tests were positive for Liver sampleson days 4 and 7 after virus inoculation in quails.

Conclusion: The results of this study could emphasize the importance of game birds in the incidence and distribution of fowl adenoviruses.

Key words:fowl adenovirus serotype 1, pathogenesis, Japanese quail, contact transmission

Characterization, whole-genome sequencing and phylogenetic analysis of three H3N2 avian influenza viruses isolated from domestic ducks at live poultry markets of Iran, 2017: first report

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Avian influenza type A viruses (AIV) can infect a broad range of hosts including human and birds, making them an important viral pathogen with zoonotic potential. Ducks are a known reservoir for many avian viruses including the AIV. To sequence the entire genome of duck-derived H3N2 and ran comprehensive phylogenetic analysis on them to study their origin. In this study, 962 cloacal swabs were collected from domestic ducks at several live poultry markets (LPMs) of Gilan, Mazandaran and Golestan provinces of Iran in the year 2017. Preliminary assays such as Hemagglutination Inhibition assay(HI), Neuraminidase Inhibition assay(NI) and RT-qPCR suggested that 0.5% of the birds were infected by H3 low pathogenic influenza viruses (LPAI). Three isolates were selected for whole genome sequencing. The cleavage site of the HA genes showed a PEKQTR/GLF motif, an indicator of LPAI. Furthermore, BLAST and phylogenetic analyses of the HA gene showed high homology to the Eurasian lineage of H3N8 AIV (95.5-97.1% to several European and East Asian isolates). However, the NA genes showed high homology (at most 96.5-96.9%) to those belonging to AIV N2 subtype. Furthermore, internal genes showed high homology (96-98%) to a variety of duck-origin subtypes and glycoprotein combinations, which were different for each segment. This showed a complex reassortment between different subtypes. This report is the first whole genome sequencing and complete characterization of H3N2 AIV from Iran. Such surveillance should continue to study the evolution and possible emergence of viruses with pandemic potential .

Keywords: Avian influenza virus, domestic ducks, Iran, H3 subtype, live poultry market.

The arrival of highly pathogenic avian influenza viruses H5N8 in Iran through two windows, 2016

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Objectives: The highly pathogenic avian influenza(HPAI) H5N1 virus has received considerable attention during the past two decades due to its zoonotic and mutative features. The purpose ofthis study was the phylogenetic analysis andmolecular characterization of hemagglutinin (HA) and neuraminidase (NA) genes of the H5N8 strain were identified in Malard county of Tehran province and Meighan wetland of Arak city, Markazi province were investigated

Materials & Methods:Prepared trahea and lung tissue samples were inoculated in to the embryonic eggs of 14-10 days and after extraction of allantoic fluid and RNA extraction and RT-PCR and sequencing of genes, phylogenetic trees were produced by distance –baseeed Neighbor Joining method in software MEGA7, the validity of the tree is evaluated by bootstrap test with 500 replicates and molecular properties including Cleavage site, Glycosylation site(NetNGlyc 1.0 Server – prediction), Antigenic site, Receptor Binding site of HA genes and gene-related mutations were investigated.

Results &Conclusion : Based on the analysis of the amino acid sequence of the HA genes, the cleavage site of the genes include the PLREKRRKR / GLF polybasic amino acid motif, which is a characteristic of highly pathogenic influenza viruses. The HA genes had T156A, S123P, S133A mutations associated with the increased mammalian sialic acid-binding, and the NA gene of two viruses had H253Y mutations associated with the resistance to antiviral drugs. Phylogenetic analysis of the HA genes indicated the classification of these viruses in the 2.3.4.4 b subclade. Although the Gs / Iran-meighan / 180/2016 virus was also an H5N8 2.3.4.4 b virus, its cluster was separated from the Ck / Iran-malard / 162/2016 virus. This means that the entry of these viruses in to the country happened through more than one window. Furthermore, it seems that the introduction of these strains in Iran probably occurred through the West Asia-East African flyway by wild migratory aquatic birds.

Keywords: Highly pathogenic, Avian influenza virus ,Iran ,H5N8, Phylogenetic analysis

Evaluation of different ND-AI Inactivated Vaccines Regimes in Commercial Broilers

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Objectives: Newcastle disease (ND) and avian influenza subtype H9N2 (H9N2 AI) are two of the most important diseases of poultry, causing severe economic losses in the global poultry industry. Vaccination is an effective way to prevent and control the spread of ND virus (NDV) and H9N2 AI virus (AIV), but the antigenic differences between the current circulating strains and the vaccine strains might account for recent ND and H9N2 AI outbreaks in vaccinated poultry flocks.

Materials & Methods: The present study was carried out to evaluate the Newcastle Disease (ND) and Avian Influenza (AI) antibody levels after same vaccination programs.This trial was carried out in broiler farm belongs to Dehkade Co. with 27 houses by Capacity of 300,000 chicks. All groups received same vaccination protocols during the trial as bellow:Day old: Ibird+Vitabron spray and Transmune injection,Day 8: Lasota DW, Day 15: Vitapest DW, Day 25: hitchner B1 DW, Day 32: Cloned Lasota DW. In order to compare the antibody response obtained using various types of killed ND-AI vaccines, the flocks were classified into 9 groups.Four groups received different brands of ND-AI inactivated vaccines in day old (A1, A2, A3 and A4), and 4 groups received the above-mentioned brands of ND-AI inactivated vaccinesaround day 7-9 of age (B1, B2, B3 and B4), and one group received no ND-AI inactivated vaccine as control group (C).on days, 1, 7, 14, 21, 28, 35, 42 blood samples were collected (15 samples from each group) andHI test was used to evaluate the titer against the AI and ND.

Results & Conclusion: Vaccination against AI and ND using inactivated vaccines is necessary. The results of this study showed that regardless the vaccination time (day old vaccination vs 7–9-day old vaccination), the quality of vaccine brand to achieve higher antibody titers is crucial (in this case A1, A2, B1and B2) showed better results both AI and ND antibody titers and also CV. At the same time the quality of vaccination performance at day old shows optimal results based on lower CV of antibody titers. This study indicated that inactivated ND and AI vaccines influence antibody titers increase above 28 days of age. Over all results determine that choosing vaccine brand and performance quality is key to achieve the best results.

Keywords:Vaccination Programs, ND-AI Inactivated Vaccines, HI test,CommercialBroiler, Antibody

Astroviruses' Infection in Turkeys; New Findings

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Objectives: Astroviruses are non-enveloped, single-stranded, positive-sense RNA viruses that can infect a wide range of hosts including avian species and are often linked to gastrointestinal sickness. Among the most important of these diseases in avian species, there is Poults Enteritis and Mortality Syndrome (PEMS). The symptoms include: diarrhea, poor weight gain, and in some cases, high mortality. The aim of this study was to investigate the infection of industrial and backyard turkey flocks with turkey astrovirus using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method.

Materials and Methods:There are different methods for determining an astrovirus infection. Electron microscopy, cell culture, immunoassays, polymerase chain reaction, and a variety of other molecular techniques are currently being used in diagnostic and surveillance researches. In order to investigate astrovirus infection, fecal sampling was performed from 15 turkey flocks in Tehran, Gilan, Mazandaran and Golestan provinces. At least 288 fecal samples were taken based on the average prediction of 25% prevalence, 5% accuracy and 15% confidence interval. RT-PCR test was performed to determine the presence of astrovirus in the collected samples using type 1 and 2 turkey astrovirus primers. Statistical analysis of the samples was performed using SPSS software and Chi-square and Fisher's exact tests and p value ≤ 0.05 was considered significant.

Results and Conclusion:Two of the 15 surveyed flocks were infected with type 1 astrovirus and one flock was infected with both types of astrovirus 1 and 2 and a total of 3 flocks were infected. Among the 15 flocks studied, 8 were commercial flocks and 7 were backyard flocks, and positive cases were obtained from 2 commercial flocks and 1 backyard flock. There was no statistically significant difference between positive cases with province, age, breeding type and density using Chi-square and Fisher's exact tests ($p> 0.05$). According to the obtained results, an overview of the presence of astrovirus in the turkey flocks of Iran was obtained. Undoubtedly, definite conclusions about the occurrence of astroviral infections in turkey flocks require more detailed and extensive virological and epidemiological studies and tests**.**

Keywords: Astrovirus, Diagnostic Methods,Poults Enteritis and Mortality Syndrome, RT-PCR, Turkey Poults

Oral immunization of broiler chickens with arecombinant *Lactobacillus casei*expressing NetB protein from*C. Perfringensprovides* significant protection against necrotic enteritis

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Objectives: Necrotic enteritis is a devastating enteric disease of poultry caused by *Clostridium perfringens*. NetB toxin from*Clostridium perfringens* is considered the causative agent of necrotic enteritis. After the ban on antimicrobial agents from chicken's diet by the European Union, the alternative approaches such as vaccination were suggested to control necrotic enteritis especially in broiler chickens. Currently, there is no available commercial vaccine to control necrotic enteritis in birds.In this study,we immunized the broiler chickens orally with a probiotic bacterium, a recombinant *Lactobacillus casei* strain expressing NetB protein of *C. perfringens* to evaluate the efficacy of the vaccine against the necrotic enteritis challenge.

Materials & Methods: The netb gene from *C. perfringens*(accession No. in GenBank: KY559052.1) was modified and cloned into pT1NX expression vector, and the resultant plasmid was electroporated into the competent Lactobacillus cells. The recombinant *L. casei* vaccine strainexpressed the NetB proteins on the cell surface, and the expression was confirmed by the immunofluorescent assay and western blotting.One-day-old broiler chickens were immunized orally with the vaccine strain on days 3, 13, and 21 for three consecutive days,and then challenged with the virulent *C. perfringens* on day 30 for four consecutive days. Sera were collected from all birds after each immunization, and the anti-NetB antibody responses were determined using an ELISA assay. The body weights of birds were individually measured at 5 day intervals from day 24 to 34. The day after the challenge experiment, birds were euthanized and necropsied, and the small intestine were examined.

Results & Conclusion: The birds immunized with the recombinant *L. casei* vaccine strain expressing the NetB proteins were significantly protected against the experimental induction of necrotic enteritis, and also elicitedrobust serum anti-NetB antibody responses to NetB protein. Moreover, the immunized birds showed higher body weight gains during the challenge experiment compared with control birds.This study showed that the strain, as a probiotic vector vaccine, could be a promising vaccine candidate to protect broiler chickens against necrotic enteritis.

Keywords:*Clostridium perfringens*; *Lactobacillus casei*;Necrotic enteritis;NetB toxin; Probiotic; Vector vaccine

Evaluating the Economic impact of HPAI in backyard and rural farms and live bird markets in Iran during the 2016-2017 Outbreaks

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Introduction: HighlyPathogenic Avian Influenza (HPAI) has been one of the most critical problematic epidemiological issues in the poultry industry during the last decades. In 2016, Iran was faced with H5N8 outbreak that affected poultry industry and backyard and rural birds.Although the disease has received much attention in industry, its effect on backyard and rural birds has been neglected.Evaluating the economic impacts of avian influenza in the backyard and rural birds and Live bird markets in the can highlight the importance of paying attention to the disease in these birds.

Materials and Methods: The economic loss was calculated based on the evaluation of two different items for both direct and indirect effects of economic loss:1) economic loss quantity for people who are involved with the business of backyard and rural birds during the unemployment caused by the outbreak and its consequences, and 2) the amount of increase in import and decrease in export. After that, the amount of economic loss was calculated based on collected data.

Results: During the 2016-2017 HPAI outbreak in Iran, the economic loss of the government was around 2,7 M$, the economic loss of the villagers was around 10,5 M$, and the economic loss of the live bird markets was negligible.

Keywords: HPAI, Economic loss, Rural and Backyard Birds, Iran

Comparison of some quantitative and qualitative indicatorsin different commercial sources of Dicalciumphosphate used in the poultry feed industry

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Objective: Effect of chemical parameterswher affects quality in commercial dicalcium phosphatesources

Materials & Methods:For this purpose, completely random samples were prepared from 7 commercial sources producing dicalcium phosphate during the years 1395-1401 and the parameters like phosphorus, calcium, lead, arsenic, cadmium, mercury, fluorine and solubility in the acid of these samples was measured in the identical conditions during the continuous experiments on 500 samples. One-way analysis of variance used to compare the results.

Results & Conclusion: Dicalcium phosphate is one of the important sources of phosphorus and calcium in poultry feed which plays an important role in meeting the requirements of measured parameters and preventing the occurrence of diseases caused by their deficiency in birds.The use of low-quality dicalcium phosphates in poultry nutrition, apart from failure to provide the appropriate level of nutritional parameters, due to the presence of impurities, causes the possibility of bone abnormalities as well as defects in the function of kidneys and liver of birds. Therefore, it is very important to pay attention in choosing the source of dicalcium phosphate in terms of economic efficiency in feeding livestock and poultry.In this study, the chemical parameters affecting the quality of commercial sources of dicalcium phosphate inside the country evaluated.

The results show that there is no significant difference among the parameters measured in these sources, including solubility in acid and heavy metals (lead, cadmium, mercury and arsenic) ($P>0.05$).A significant difference was observed between different commercial brands in terms of phosphorus, calcium and fluorineof dicalcium phosphates ($P<0.05$).

The experiments revealed that correct selection of the dicalcium phosphate source in terms of different parameters and adjusting the feed formulation accordingly, in terms of both achieving proper performance and preventing skeletal abnormalitiesas well asoccurrence of obvious and hidden damages caused by this quality difference in livestock and poultry products, is necessary.

Key words: phosphorus, Dicalcium phosphate, heavy metals, broiler, performance.

Compliance of Principles of Biosecurityin the Prevention of Highly Pathogenic Avian Influenza (HPAI) in the Bird Garden

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Abstract:Highly Pathogenic Avian Influenza (HPAI) in the bird garden, like what happened in Qom province in Iranin early 2021, is caused by non-observance of different levels of biosecurity, including locational, structural, procedural. Construction of a bird garden in the location of the migration corridor of migratory bird is one of the most risk factor in the bird garden, which can be reduced to incidence of disease in other bird gardens by asking the employer from the department of environment protection.Among the structural factors that were effective in incidence of HPAI, the lack of construction of an enclosed area for garden bird, the lack of installation of a water disinfectant in the circulation of the complex from the shed for keeping tropical bird to the open space and waterfowlpool.The procedural level of biosecurity contained several deficienciesfailure to observe the quarantine period at the time of arrival of birdof other units or donated bird, not HPAI vaccination, not identifying the bird, absence of a recording book of events, failure to separate the feed and water used by bird garden from migratory bird, releasing reservoir (goose and duck) and the moresusceptible (turkey)birdto HPAI. In the bird garden of Qom province, turkey, guinea fowl and partridge had the most casualties in the collection that were suspect to HPAI.The suspect center ofHPAIwas quarantined and in order to differential diagnosis with other diseases such as highly pathogenic newcastle disease, molecular tests were performed on the tissue samples of the brain, trachea, lung, spleen and cecal tonsils, and the results of the HPAI tissue samples were positive (H5N8). At this section, preventing of the distribution after the first incidence is determinativeto protect other poultry units. Therefore, the emergency extermination of the bird, disinfection of the stands, exit along with the cleaning of the personnelsworking in the garden and the veterinary technical and administrative staff were carried out with great care. Finally, the veterinary personnel working in the department refused to attend other poultry units for several days. Sampling equipment and sanitary technical equipment were all fumigated with permanganate formalin. The center was free of avian for a long time (three months). Therefore, due to the implementation of the above biological security principlesafter confirming the disease, it was completely prevented from distributionof disease from the bird garden to other poultry units.

Key words: Highly pathogenic avian influenza, Bird garden, Qom, Iran.