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Isolation and Identification of Respiratory Viruses from Broiler Flocks with Respiratory Complex in west part of Mazandaran Province

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Abstract

For isolation and identification of viruses that cause respiratory complex in broiler flocks, samples were taken from 20 broiler flocks with respiratory disease in the west of Mazandaran province. Samples were taken with history recording such as general status, age of flock, and vaccination program, from the trachea, lungs, kidney, caecal tonsils, and in cases with nervous signs from the head. A 10 percent suspension of samples in BPS solution was prepared and inoculated to 10-day-old fertile SPF eggs via the allantoic route. And then hemagglutination (HA) and hemagglutination inhibition (HI) properties for Newcastle disease (ND) and subtype H9N2 of Avian Influenza (AI) viruses were considered. For identification of infection bronchitis virus, samples were passaged 3 to 4 times and the presence of the virus was confirmed by typical IBV embryo effects such as dwarfing, curling, or death. The viral RNA was extracted from the harvested allantoic fluid by using RNA purification kit (Roche Germany). A fragment of S1 segment was amplified by universal primers of IB virus in RT-PCR reaction and then Nested-PCR with specific primers for differentiation of Mass and 793/B serotype was conducted. Results revealed, that 11 samples were positive for IB virus, 3 samples were positive for ND virus and one sample was positive for AI+IB viruses' simultaneous infection.

Introduction

As a source of protein for the country, the poultry industry supplies a large portion of the country's protein needs, and is a key job creator due to the existence of many industries associated with it[1]. In spite of various factors such as the instability and fluctuations in the price of poultry products, the increase in the price of production inputs and poultry diseases are among the threatening factors of this industry[2]. The broiler industry suffers the greatest economic losses due to respiratory infections[3]. Various viruses play a role in causing respiratory infections, which either alone or simultaneously cause respiratory disease, or together with mycoplasma and bacterial agents such as Escherichia coli and Ornithobacterium rhinotracheale cause respiratory infection, also known as respiratory complexes or multifactorial respiratory diseases[4, 5]. In recent years, the respiratory complex has been one of the most important threats to the poultry industry in broilers. Among the important damages caused by respiratory complexes in broiler chickens are increased mortality, increased treatment costs, decreased food efficiency, and increased slaughterhouse removals. Among the viral infections, the

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role of Newcastle viruses, avian influenza and infectious bronchitis in meat flock is very important. Because they cause disease both independently and simultaneously or together with mycoplasma or coli bacilli infections[6].

Materials and methods:

In order to identify the viruses that cause respiratory infections in poultry in the fall and winter of 2021 and 2022, samples were taken from 20 broiler flocks with respiratory disease. First, details about the overall state of the farm, the condition of the flock, the schedule of vaccinations, and the history of diseases were gathered using a survey. Then, appropriate samples were selected from different tissues such as trachea, lungs, and caecal tonsils, and in case of head swelling and neurological symptoms, the head area was completely isolated. After preparing a 10% suspension of the samples in a phosphate buffered saline solution with neutral pH, antibiotics including penicillin 10000 IU/ml, streptomycin 0.2mg/ml and amphotericin B 0.02mg/ml were added to prevent secondary contamination. The obtained suspension liquid was centrifuged for 20 minutes at 1000 to 1500 rpm and the supernatant was taken in sterile conditions and stored in appropriate vials for storage and inoculation. Then 200 µl suspension of samples in BPS solution was prepared and inoculated to 10-day-old fertile SPF eggs(Specified Pathogen-Free) via the allantoic route. The eggs were placed in an incubator with a temperature of 37 degrees to provide conditions for embryo development. The inoculated SPF eggs were exposed to light on a daily basis. Eggs whose embryos died 48 hours after inoculation were removed, and weak and live embryos were transferred to the refrigerator 48–72 hours later and used to gather allantoic fluid. Embryo losses during the first 24 hours were also removed. A centrifuge operating at 1500 rpm was used to filter the collected allantoic fluid. Then, allantoic fluid was examined for hemagglutination (HA) and hemagglutination inhibition (HI) properties for ND and AI viruses. Also, one milliliter of allantoic fluid was collected from the inoculated eggs to identify the infectious bronchitis virus and injected again into the fertile SPF eggs, and the passage was repeated at least 3 to 4 times to observe symptoms such as shrinking and twisting of the embryo. The collected allantoic fluid was also used to perform RT-PCR and Nested-PCR tests, with general and specific primers of infectious bronchitis virus designed by Adzhar et al[7] as described in Table 1.

Table1: General and specific primers for infectious bronchitis virus to perform RT-PCR and Nested-PCR tests

Type of reaction	Primer name	Sequence	position in the S1 gene	PCR product	Property
RT-PCR	XCE2-	CTCTATAAACACCCTTACA	1168 - 1193	464bp	
RT-PCR	XCE1+	CACTGGTAATTTTTCAGATGG	728-749	464bp	
Nested-PCR	XCE3_	CAGATTGCTTACAACCACC	1093-1111		
Nested-PCR	BCE1+	AGTAGTTTTGTGTATAAACCA	958-978	154bp	793B
Nested-PCR	MCE1+	AATACTACTTTTACGTTACAC	817-837	295bp	Mass

Result:

Identification of Newcastle and influenza viruses

Allantoic fluid was collected 48–72 hours after inoculation using eggs whose embryos had died 48 hours earlier as well as weak and live embryos. The hemagglutination (HA) for ND and AI viruses was checked in the collected allantoic fluid. Four samples out of the 20 that were injected in fertile SPF eggs tested positive for hemagglutination. HA positive samples were mixed with Newcastle and influenza specific antiserum and evaluated for the presence of Newcastle and influenza virus by HI method. Three of the four cases that tested positive for a HA response also reacted to antiserum specific for the Newcastle virus, and one case also reacted to antiserum specific for the influenza virus.

Identification of infectious bronchitis virus by RT-PCR and Nested-PCR tests

Out of the total of 20 samples tested, 11 cases had the infectious bronchitis general primer react positively, according to analysis of the extracted allantoic fluid using the RT-PCR method to identify the infectious bronchitis virus and then the Nested PCR method to identify the mass and 793/B serotypes of the virus. Among them, 6 cases were positive with both types of primers of mass and 793/B serotypes. One case only responded to mass serotype primer, while four cases only reacted to serotype 793/B primer. One of the samples that tested positive for infectious bronchitis also had influenza virus. In total, out of 20 samples taken from broiler flocks with respiratory symptoms, fourteen cases (70%) were positive for respiratory viruses. Out of a total of 14 positive samples, 3 cases (21.43%) were infected with Newcastle virus, 11 cases (78.57) were infected with bronchitis virus, and one case (7.14%) was infected with influenza virus, which was the case of simultaneous infection with bronchitis and influenza virus (Table2)

Table 2: The results of the total respiratory samples taken in 2021 and 2022

Sampling year	The number of samples taken	Number of negative samples	Number of positive samples	IB	ND	AI/IB
2021	11	3	3	7	1	1
2022	9	3	3	4	2	0
Total	20	6	6	10	3	1
	Percent	30	30	78.57	21.43	7.14

Discussion:

The respiratory system of poultry is significantly influenced by viral pathogens, particularly Newcastle viruses, influenza, and infectious bronchitis. Because of their severe contagiousness, wide range of strains, and high mortality rates, these pathogens have a significant negative economic impact on the poultry business. Therefore, studying and determining the role of these viruses in causing respiratory infections has helped a lot to identify viral infections in each region and can be used in planning to prevent these diseases. In the present study, which was carried out with the aforementioned objectives, out of a total of 20 samples taken from meat herds with clinical symptoms related to respiratory tract infection, fourteen cases (70%) were positive for respiratory virus infection, and out of 6 cases (30 percent) also no respiratory virus was isolated, which is either related to the conditions of sampling, storage and sending of the sample, or that the samples were basically not infected with the virus and their respiratory symptoms were probably caused by bacterial factors.

The most viral infection in this study was related to infectious bronchitis virus, which includes both 793/B and Mass serotypes. Infectious bronchitis is one of the acute and highly contagious diseases of poultry caused by a virus from the coronavirus family, which causes great economic losses in broilers, reduced egg and egg quality due to respiratory tract lesions[8]. Coronaviruses are classified into three groups. Infectious bronchitis virus is in the third group of coronaviruses. Infectious bronchitis was first observed in 1930 in the North Dakota region of the United States. Since the beginning of the 50s in America, in addition to the Massachusetts strain, which is the main serotype of the virus and has global distribution, other serotypes were also identified. Since the 40s, the Massachusetts strain was isolated in Europe. One of the most significant strains of the virus, aside from American strains, has been found in North Africa, Asia, Australia, and Europe. [9-13].

In numerous investigations conducted in Iran, the infectious bronchitis virus was isolated from herds that had respiratory infections. Serotype identification attempts have been extensive. In the study the presence of this strain in Iran's poultry farms was proven by conducting a VN neutralization test using the specific antiserum of strain 793/B[14]. During a study, 12 isolates of infectious bronchitis virus were isolated after inoculation in SPF embryonated eggs, and in the Nested-PCR test using specific primers, it was determined that 5 isolates belong to serotype 793/B, 5 isolates belong to serotype mass and Two samples contained a mixture of the above two serotypes[15]. Hosseini and his colleagues with Using RT-PCR method, they confirmed the presence of mass and

793/B serotypes of infectious bronchitis virus in respiratory infections[16]. According to the results of various studies and the present investigation, the role of infectious bronchitis virus serotypes in causing respiratory infections in broiler poultry is very important. After infectious bronchitis, the most cases were related to Newcastle disease, and out of the total of 20 investigated herds, 3 cases were positive for Newcastle virus infection. Newcastle disease is a highly contagious and deadly viral disease of domestic and wild birds, especially chickens and turkeys. An RNA virus belongs to the Paramyxoviridae family and the Oylavirus species. The pathogenicity of the Newcastle disease virus varies greatly in terms of type and intensity. Although, there are different isolates and strains of the virus, they all belong to the same serotype and are considered serotype 1 of paramyxovirus PMV-1[16]. In Iran, the first outbreak of Newcastle disease was reported from Tabriz city in 1951 AD and the disease was diagnosed by isolating the virus by the experts of Razi Institute[16].

Since that time, Newcastle disease has spread throughout the nation and now annually affects various areas of Iran. Depending on its severity and pathogenicity, it can result in fatalities as well as financial losses for the poultry industry. The significance of respiratory complex epidemics in recent years has increased awareness of the function of the Newcastle virus. Newcastle virus infection was found to be 2% in the research to examine the factors causing concurrent respiratory infection[17]. In the study conducted by Roussan in Jordan on 115 beef herds with respiratory symptoms, 13% were infected with Newcastle virus[18]. In this study, one case (5%) was also positive for H9N2 influenza virus. Influenza is one of the most important viral diseases of birds, especially industrial poultry, which is caused by RNA virus from the orthomyxovirus family. The virus has several main and sub-proteins, which are divided into 3 types C, B, and A based on the main proteins, which type B and C are found only in humans and type A in humans, other mammals and birds[19]. Avian influenza viruses are classified into two main groups, which include highly pathogenic influenza viruses (HPAI) and low pathogenic influenza viruses (nHPAI) or low pathogenicity viruses[20]. Since 1998, a widespread epidemic of bird flu was identified in Iran and the H9N2 serotype was isolated[21]. Several reports of disease occurrence with significant mortality have been made, despite the fact that this serotype belongs to the influenza viruses with minimal morbidity. The occurrence of respiratory complex and an increase in disease severity are caused by the co-infection of influenza with other infectious respiratory agents, particularly the infectious bronchitis virus and bacteria like Mycoplasma gallisepticum and Escherichia coli.

As a result, it has become crucial to understand the virology of respiratory infections in chickens and how the influenza virus contributes to the development of respiratory complex. Gholami and his colleagues reported that out of ten suspected respiratory complex herds that they studied in Isfahan province in 2018, three herds were infected with influenza and one herd was infected with influenza and infectious bronchitis[22]. Pourbakhsh et al conducted virology tests on flocks suspected of having influenza from industrial poultry farms in Tehran province. From a total of 85 suspected herds, 43 herds (6.50%) of type A influenza under the H9N2 type were isolated[23]. The importance of the role of respiratory viruses in the occurrence of respiratory complications and the occurrence of economic losses in the poultry industry is emphasized once more at the conclusion of this study, which also reveals that infectious bronchitis virus has a relatively high prevalence among poultry respiratory viruses during the time period under study. Escherichia coli, which is frequently found in chicken farms, and other bacterial infections have a high probability of co-occurring, complicating the disease and raising the mortality cost. In the meantime, an outbreak of Newcastle virus can be very dangerous for the poultry industry in the area due to the presence of highly virulent pathotypes. Both the presence of Newcastle virus and bird flu has also been verified. It is advised to find suitable strategies for prevention and countermeasures taking into account the above viruses because the concurrent contamination of respiratory viruses and the development of a respiratory complex increases the severity of the disease and results in an increase in mortality due to illness and damages. The most crucial of these are hygiene, proper sterilization and cleaning of halls during preparation and hatching, and selecting the right type and method of immunization for each of the viruses provided in the Disease prevention program.

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References

1. Dolganyuk, V., et al., *Food Proteins: Potential Resources.* Sustainability, 2023. **15**(7): p. 5863.
2. Miani, A.M., et al., *Toward sustainable rural livelihoods approach: Application of grounded theory in Ghazni province, Afghanistan.* Applied Geography, 2023. **154**: p. 102915.
3. Hafez, H.M., et al., *Influence of COVID-19 on the poultry production and environment.* Environmental Science and Pollution Research, 2021. **28**: p. 44833-44844.
4. Abdelaziz, A.M., et al., *Molecular survey and interaction of common respiratory pathogens in chicken flocks (field perspective).* Veterinary world, 2019. **12**(12): p. 1975.
5. Yehia, N., et al., *Common viral and bacterial avian respiratory infections: an updated review.* Poultry Science, 2023: p. 102553.
6. Gray, P., et al., *Antimicrobial prescribing guidelines for poultry.* Australian Veterinary Journal, 2021. **99**(6): p. 181.
7. Adzhar, A., et al., *Molecular analysis of the 793/B serotype of infectious bronchitis virus in Great Britain.* Avian pathology, 1997. **26**(3): p. 625-640.
8. Nakanishi, M. and J. Soma, *Efficacy of commercial live vaccines against QX-like infectious bronchitis virus in Japan.* Poultry Science, 2023. **102**(5): p. 102612.
9. Kalokhoran, A.Y., et al., *Co-circulation of three clusters of 793/B-like avian infectious bronchitis virus genotypes in Iranian chicken flocks.* Archives of virology, 2017. **162**: p. 3183-3189.
10. Demeke, H.D., et al., *Pathological and Molecular Characterization on Clinically Sick Chickens with Infectious Bronchitis Virus in Small Scale and Commercial Poultry Farms in Bishoftu Town, Ethiopia.* 2023.
11. Karimoddinia, H. and S.R. Larijania, *Evaluation of the effect of using Citrus aurantium extract with Acepromazine and Ketamine during induction of anesthesia on the heart rate of male dogs during sterilization surgery and comparing it with the treatment used with acepromazine and ketamine and control treatment.* Evaluation, 2021.
12. *Investigation of infection of pregnant dogs in the central parts of Mazandaran province with Brucella Canis.* 2021.
13. Rezaei Larijani, S., S.M. Hosseini, and B. Ekrami, *Study of different doses of zinc oxide nanoparticles by intraperitoneal and gavage methods on testicular tissue in Wistar rats: An experimental study.* Int J Reprod Biomed, 2023. **21**(6): p. 499-508.
14. Masoudi, S., L. Pishraft-Sabet, and S. Shahsavandi, *Immunogenicity and efficacy of live infectious bronchitis 793/B. 08IR vaccine in SPF chickens.* Archives of Razi Institute, 2020. **75**(1): p. 23.
15. Najafi, H., et al., *Molecular characterization of infectious bronchitis viruses isolated from broiler chicken farms in Iran, 2014-2015.* Archives of virology, 2016. **161**: p. 53-62.
16. GHALYANCHILANGEROUDI, A., et al., *New insights on epidemiology of infectious bronchitis virus in Iran by comparing two genotyping methods.* Turkish Journal of Veterinary & Animal Sciences, 2023. **47**(1): p. 91-99.
17. Jaganathan, S., et al., *Observation of risk factors, clinical manifestations and genetic characterization of recent Newcastle Disease Virus outbreak in West Malaysia.* BMC veterinary research, 2015. **11**(1): p. 1-9.
18. Roussan, D., R. Haddad, and G. Khawaldeh, *Molecular survey of avian respiratory pathogens in commercial broiler chicken flocks with respiratory diseases in Jordan.* Poultry science, 2008. **87**(3): p. 444-448.
19. Parija, S.C., *Orthomyxoviruses*, in *Textbook of Microbiology and Immunology*. 2023, Springer. p. 797-809.
20. Baggio, G., F. Filippini, and I. Righetto, *Comparative Surface Electrostatics and Normal Mode Analysis of High and Low Pathogenic H7N7 Avian Influenza Viruses.* Viruses, 2023. **15**(2): p. 305.
21. Dong, J., et al., *Status and challenges for vaccination against avian H9N2 influenza virus in China.* Life, 2022. **12**(9): p. 1326.
22. Gholami Ahahngaran, M., et al., *Molecular identification and typing of infectious bronchitis virus in respiratory syndrome cases of broiler chickens in Isfahan province.* Journal of Iranian Veterinary Science, 2008. **3**: p. 469-76.
23. Tavakkoli, H., K. Asasi, and A. Mohammadi, *Effectiveness of two H9N2 low pathogenic avian influenza conventional inactivated oil emulsion vaccines on H9N2 viral replication and shedding in broiler chickens.* Iranian Journal of Veterinary Research, 2011. **12**(3): p. 214-221.