Epidemiological Distribution of respiratory viral pathogens in marketable vaccinated broiler chickens in six governorates in the Nile Delta, Egypt, January to October 2022

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Research Article

Keywords: Chicken, Avian influenza virus, subtype H5 and H9, Newcastle disease, Infectious bronchitis, RT-PCR, Egypt

Posted Date: May 22nd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2944417/v1

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Abstract

Background and Aim:

Respiratory viral infections have a considerable detrimental impact on animal welfare as well as significant financial ramifications in the poultry industry. Avian influenza virus (AIV) subtypes H5 and H9, Newcastle disease (ND), and infectious bronchitis (IB) are the most economically significant illnesses impacting the poultry sector worldwide, including Egypt. From January to October 2022, this study examined the presence of respiratory viral infections (AIV-H5, AIV-H9, ND, and IB) in 359 flocks of broiler chickens (33–38 days) in six Egyptian governorates (Beheira, Gharbia, Giza, Monufiya, and Qalyoubia).

Results

Out of 359 flocks examined, 293 tested positive, whereas 66 tested fully negative for the four viruses tested with the highest positive results in Beheira. Out of 293 positive flocks, 211 were positive to a single virus with Beheira having the highest rate, followed by Qalyoubia, Giza, and Monufiya. NDV was found to be the highest across all governorates, followed by IBV, AIV-H9, and AIV-H5. Double infection was detected in 73 flocks with either H9 or ND or both H9 and IB would co-infect one another. The most common viral co-infection was H9 + IB, ND + IB, and ND + H9. Giza had the greatest prevalence of co-infection with ND + H9, H9 + IB, and ND + IB in the governorates, followed by Monufiya and El Buhyera. Only 6 out of 359 flocks were tribally infected with ND + H9 + IB in three governorates: Giza, Monufiya, and Beheira. According to the number of flocks and the month of the year, July had the fewest tested flocks (23) and both September and October had the most (48 flocks). The positive flocks were highest in October and lowest in January.

Conclusion

The results revealed that IBV and H9 as a single or a mixed infection had a great role in the respiratory infection in broiler. The used vaccine (regardless their origin and type) is not able to protect broiler chickens from developing infection and shedding of virus to the poultry environment. Therefore, poultry vaccines need regular evaluation, renovation in face infective field virus mutants and also, poultry farms must be adopting more biosecurity measures.

Background

In poultry, respiratory co-infections are more common due to the presence of several causal agents. Respiratory viral infections in the poultry industry have a significant negative influence on animal welfare in addition to significant financial implications. As respiratory disease in chickens gets clinically worse, determining a clear diagnosis and finding an appropriate therapy becomes difficult. Accordingly, both precipitating causes and predisposing variables should be addressed in respiratory complex infection control efforts (1, 2).

Pathogens that cause respiratory illnesses in poultry work independently or in conjunction with one another. Because of their multifaceted character, respiratory illnesses pose a significant challenge in Egypt's poultry sector. Several poultry respiratory infections cause clinical indications that are similar and can be misinterpreted. Several clinical indications have become more common in Egyptian commercial chicken flocks in recent years. These infections are significant and have a substantial economic impact since they can cause disease individually or in conjunction with one another (3). The primary respiratory causes of high death rates in broiler chicken flocks include infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and avian influenza (AI), which can be highly pathogenic as H5 or low pathogenic as H9 (4).

Infectious bronchitis (IB), a highly contagious disease that causes a large financial loss to the commercial chicken flocks, is one of the main contributors to mixed infections. IBV belongs to the family *Coronaviridae* and many distinct IBV variants circulate worldwide (5). The virus causes usually mild respiratory disease and egg production drops and can, depending on the viral variant, induce renal damage (6). The IBV virus can infect chickens. Economic losses in broilers are caused by a decline in weight gain, reduced feed efficiency, and an increase in carcass condemnations, especially when infectious bronchitis is worsened by secondary diseases such as bacterial infections (7). The disease frequently causes respiratory signs including gasping, coughing, sneezing,
tracheal rales, and nasal discharge (8). In addition, some strains have been associated with kidney lesions (9). The severity of the symptoms in chickens is related to their age and immune status. Other signs of IB including wet droppings are due to increased water consumption. The type of virus strain infecting a flock determines the pathogenesis of the disease and the degree and establishment of lesions in different organs. The upper respiratory tract is the primary site of infection, but the virus can also replicate in the reproductive, renal, and digestive systems (10). To monitor the existing different IBV in a geographical region, PCR on the reversely transcribed RNA is a potent technique for the detection of IBV. Comparing with classical detection methods, PCR-based techniques are both sensitive and fast (11).

Newcastle disease is one of the most severe viral diseases of poultry and has a significant economic impact on the poultry industry, with velogenic strains of the virus causing 100% bird mortality in infected flocks (12). The ND virus is considered endemic in many countries, including Egypt (13). Exotic Newcastle Disease Virus (ENDV) is an extremely virulent strain that causes serious losses in pet and game birds in the USA. These birds are also thought to be an excellent reservoir for the virus' transmission between domestic commercial flocks, which results in substantial losses in the poultry industry (13). According to the strain infecting the flock and the clinical signs it produces, NDV infection is divided into different pathotypes. There are 4 different strains: Lentogenic strains, which cause mild respiratory symptoms and are used as secondary live vaccines; mesogenic strains, which are fatal only to young chicks; viscerotrophic velogenic strains, which are fatal to all ages of chicken and almost always manifest as enteric signs, and neurotropic velogenic strains, which are characterized by nervous signs (14, 15). Research in last years proved the mutation and genetic diversity of ND viruses become more apparent (16–19). The wide genetic distance between the currently circulating NDV isolates and the used vaccines to combat the disease is an area of discussion in many countries (19, 20).

Avian influenza (AI) is a poultry disease that has caused significant economic losses in addition to its zoonotic potential. Orthomyxoviridae type A influenza viruses are the ones responsible for the disease. According to the severity of the disease in vulnerable birds, avian influenza is further divided into highly pathogenic AI (HPAI) and low pathogenic AI (LPAI) virus strains. The H5 and H7 subtypes have been mostly responsible for HPAI outbreaks in hens and turkeys; however, some H5 and H7 subtypes have been identified as HPAI, and numerous strains of these subtypes have been demonstrated to be LPAI (21). A further risk factor for the poultry industry has been identified by the silent spread of LP H9N2 that has been observed in the Middle East and the Far East for several years. Although being classified as low pathogenic avian influenza (LPAI) viruses, H9N2 viruses were highly lethal (22). The most recent emergence of H9N2 virus in Egypt occurred in May 2011 from a clinically healthy commercial bobwhite quail flock, and the virus co-circulated with HPAIV subtype H5N1, with the potential for the silent spread of H9N2 viruses to impair the usual spread of HP H5N1 (23, 24).

Avian influenza virus (AIV) and avian infectious bronchitis virus (IBV) are two main viruses that infect chicken airways. AIV is a member of the Orthomyxoviridae family and, like IBV, belongs to the group of low pathogenic avian influenza viruses (LPAIV), which cause mild respiratory symptoms, egg production loss, and growth reduction (25, 26). Although dual infections with two viruses or even many infectious agents have been known to happen, dual infections with a virus and a bacterium are more frequently recorded in chicken. After infection with IBV, AIV, NDV, or avian metapneumoviruses, there is increased susceptibility to subsequent respiratory bacterial infections (25, 27, 28). In addition, Immunosuppressive viruses such as the chicken anemia virus, can significantly increase the risk of developing secondary infections (29). When two pathogens coexist, the ensuing disease is typically more severe than when the two pathogens are present separately (30, 31). Despite the numerous proposed underlying mechanisms of virus-virus interactions, disease mechanisms underlying infection with two viruses have only sporadically and rarely been studied within the topic of chicken respiratory disease. Mechanisms underlying virus-induced susceptibility for bacterial disease have been investigated quite extensively in several species.

In Egypt, commercial chicken flocks continuously had outbreaks of respiratory affections with variable mortality rates and diverse clinical presentations (32) According to recent studies, the most prevalent condition in Egyptian poultry was mixed infection, particularly with IB and AIV-H9N2 viruses (28). According to recent in vivo study, a dual infection with the first IBV and the second AIV does appear to cause a more severe clinical illness that is accompanied by an increased inflammatory response, but comparisons are still challenging because no sequential IBV infections or receptor binding were studied (33). A mechanistic study (34) showed that loss of cilia and goblet cells in infectious bronchitis virus-induced tracheitis is associated with varying epithelial susceptibility to secondary viral infections. The degree of binding reduction varied depending on the virus and was more common between the two IBV variants than between IBV and AIV. Superinfection reduced the viral titers of the first injected virus both in vivo, in ovo, and in vitro (cell cultures); however, there was no effect on the replication of the second virus, regardless of which of the two viruses was initially
and which was secondarily inoculated. Instead of a virus-specific mechanism primarily based on competition for the same susceptible cells, as concluded in one of the previous studies, these observations might indicate an underlying epithelial antiviral mechanism-based surface receptor molecule reduction that varies for different viruses. This mechanism could potentially be regulated by an interaction between different cytokine and interferon types or result from specifically induced host sialidase activity (35).

With reference to AI, ND and IB virus co-infections in commercial broiler chicken flocks, the primary goal of this article is to highlight the current field difficulties facing the poultry industry. It will assess how prevalent single and combined viral infections are in the field and how they affect the severity of respiratory illnesses and the high fatality rates that follow. This may assist us in creating a rough epidemiological map that shows the circulating viruses in the field and aids the government in implementing scientific remedies among flocks of poultry.

**Results**

Three hundred fifty-nine flocks of broiler chickens with respiratory issues from five different governorates were evaluated (Table-2, Figure-1). Depending on the virus strain causing the epidemic, the flock’s vaccination regimen, and whether the sickness was brought on by a single infection or numerous infections, different clinical symptoms and post-mortem lesions were observed in the examined respiratory disease outbreaks. Gasping, rales, and nasal secretions were the primary clinical signs of respiratory distress. There have been reports of head tilting in some flocks as a result of nervous manifestations. During post-mortem examination, the main pathological findings included tracheitis, tracheal caseation at the tracheal bifurcation, and engorged viscera. Other findings included pneumonia, enlarged kidneys, and thickened, cloudy air sacs. Lesions had also been seen in the central nervous system, the gastrointestinal tract, and tissues including the kidney, in addition to the respiratory system.

Swab samples collected from the tested 359 flocks indicated that 293 (81.6%) were positive for one of the pathogenic respiratory virus infections, while only 66 samples (18.4%) were completely negative for the 4 tested viruses (Table-2, Figure-2). The highest positive results were recorded at 84.1% in Beheira, followed by 79.8%, 78.6%, 78.3%, and 72.2% in Monufiya, Gharbia, Giza, and Qalyoubia, respectively.

Tested flocks showed that 211 (58.7%) were positive to single virus with the highest rate in Beheira (64.7%) followed by Qalyoubia (61.1%), Giza (50.0%) and the lowest was in Monufiya (48.31%) (Table-3, Figure-3). NDV was the highest detectable virus in all governorates (11.1%- 22.4%) with total rate of 20.6%. IBV was the 2nd most prevalent (10.1–26.4%) with a total rate of 20.1%. AI-H9 was the 3rd in range of 8.7–22.2%) with a total rate of 11.4%. The AI-H5 was the least detectable pathogen in our tested samples with a range of 3.57–11.11% with total rate of 5.85% (Table-3, Figure-4).

Double infection was reported in 73 flocks with an average rate of 20.33%. The highest rate was in co-infection between H9 + IB (7.52%) followed by ND + IB (6.41%) and ND + H9 (3.90%), while H5 was sharing ND, H9 and IB in 1.67%, 1.39% and 1.11%, respectively. Regarding the distribution of co-infection with ND + H9, H9 + IB and ND + IB in the governorates the highest was in Giza, followed by Monufiya and Beheira. Both H9 and IB were the mostly co-infecting each other and either H9 or ND (Fig. 4). The obtained results showed that that only total 6 out of 359 flocks (1.7%) had tribal infection with ND + H9 + IB in 3 governorates Giza (3.57%), Monufiya (3.37%) and Beheira(0.9%) (Figure-5, Table-4). The number of Flocks in relation to year month, lowest number was in Julie (23 flocks) and the highest was in both September and October (48 flocks). The positive flocks were the highest rate in October (89.58%), followed by 86.96% in June, 84.62% in Julie and the lowest (75.61%) was in January (Table-5, Fig. 6).

**Discussion**

The fast expansion of Egypt’s poultry farming industry, as well as the global transportation and commerce of poultry, have led to the emergence and spread of numerous viral diseases (36). In this study, the prevalence of respiratory viral pathogens (AIV) subtypes H5 and H9, IBV and NDV, was investigated in broiler chickens that’s the most economically important diseases of the poultry industry in Egypt.

The clinical sign findings of this study were consistent with those of (37, 38) who reported that the primary clinical signs of respiratory disease included nasal and ocular discharge, dyspnea, gasping or open mouth breathing, and various mortalities in a flock. Occasionally, nervous signs were seen.
Pneumonia, enlarged kidneys, and cloudy, thickened air sacs were also noted. Other than the respiratory system, lesions had been observed on the gastrointestinal tract, the central nervous system, and tissues like the kidney. The reported results are in accordance with previously published studies in Egyptian flocks (32, 37, 38).

According to data from the current study, of the 359 flocks tested, 293 (81.6%) tested positive for common respiratory virus infection (H5N1, H9N2, NDV, and IBV), whereas just 66 (18.4%) tested negative. Behira had the largest percentage (84.08%), followed by Monufiya, Gharbia, Giza, and Qalyoubia with 79.8%, 78.8%, 78.6%, and 72.2%, respectively (Table 2, Fig.2). Mixed infection with IB and AIV-H9 represented 66.3% (57 flocks) while single viral infection of the tested viruses was found in 33.7% (29 flocks) (32).

In the present study multiple respiratory viruses as single or mixed infections were detected. NDV was detected as the highest percent (11.1%-22.39%) with a total rate of 20.61%. IBV comes in 2nd (10.1-26.4%) with a total rate of 20.1%. H9N2 was the 3rd in the range (8.70-22.22%) with a rate of 11.42%. The H5N1 shows the lower range of 3.57-11.11% with a total rate of 5.85% (Table 3, Fig.3). The most common cause of respiratory affection in Egypt and that mixed infection with IB and AIV-H9 viruses was the most common situation in the examined flocks (32).

The avian influenza virus (HP, LP), IBV, and NDV were the main detected viruses alone or as coinfected in broiler chickens (32). In recent years, the avian influenza situation in Egypt has been more complicated due to the detection and circulation of many serotypes, including HPAI (H5N1), HPAI (H5N8), and LPAI (H9N2) (39, 40).

Yehia, et al. (2021) reported that The AIV subtypes and IBV were recorded in 48 out of 53 farms. Single infection represented 90.6% (37.7% I.B, 30.2% H5N8, 9.4% I.B and 5.7% NDV) (table 4). Furthermore, co-presence of HPAI (H5N8) and IBV, unique detection of these co-infected flocks, and LPAI (H9N2) and IBV, were detected in 3.8% as previously recorded by (38). Another study reported that out of 39 farms investigated, 35, 27, 12, 9 and 9 samples were positive for HPAIV H5N8, H9N2, IBV, HPAIV H5N1 and NDV, respectively. Various combinations of these viruses were detected in the majority of farms, Single infections, in contrast, were evident in only 7 of 39 (17.9%) of the holdings. Co-circulation of HPAIV H5N8 and H9N2 was the most commonly detected mixed infection (11/39 farms, 31.8%) (28).

In the present study the circulation of H5 & H9 subtypes of AIV (5/359) 1.4% could be a risk for emergence of new AI viruses (table 4, Fig3 and 4). That complies with results (41). In forty-two layer and broiler chicken farms (21/each) suspected to be infected with AIV of different breeds and ages during 2012 - 2014 were examined at Sharkia Governorate. The occurrence of AI in chickens using HI assay was 40.47%. Subtyping for H9 and H5 subtypes has been done by specific antisera. The H9 was detected in 30.95% and H5 was 9.52% (42-44).

Under field conditions coinfection of AI H9 was observed to increase severity of other pathogens (25), including IB (4, 30, 33, 45) or H5 (41, 46, 47) or ND (48, 49), also increase the severity of E.coli (50) as well as secondary bacterial infection (51, 52).

In order to develop effective management measures in the future, other respiratory pathogens bacterial and viral require further study and characterization. The Egyptian poultry sector will be improved by providing farmers with biosecurity measures training and follow up.

**Conclusion**

It could be concluded from the presented study that common respiratory virus infection (H5N1, NDV, H9N2 and IBV) are circulating along Delta region Governarate in broiler chickens from January to October 2022. The molecular epidemiology of prevalent respiratory viral diseases in Egypt is crucial for assessing the situation and developing efficient control measures. Beside periodic evaluation of the respiratory viral diseases vaccines efficacy and vaccination strategies being applied.

**Material and methods**

**Study period and location**

This study was conducted from January 1st to October 31st, 2022. Samples were collected from five different Egyptian governorates in the Nile delta in which poultry farms are concentrated. These governorates are Beheira (201), Gharbia (23), Giza (28), Monufiya
(89) and Qalyoubia (18) in the period from January to end of October 2022. Samples were submitted to National Research center, Faculty of Veterinary Medicine, Cairo university, and Egyptian Lab for Poultry Health for further analysis.

**Chicken flocks**

The flocks were raised on floor open farms with capacities ranging from 3000 to 50000 birds. Commercial ready-made pelleted rations with the standard requirements of each breed are used. Birds were vaccinated against H5 (inactivated or vector vaccine), H9 (inactivated), ND (live, inactivated, or vector (Vaxxtek), and IBV (live against classical or variant strains).

**Sampling**

At flock the marketing time (33–38 days of age), combined oropharyngeal, cloacal swabs and tissue samples were collected from clinically diseased or freshly dead birds (3–5 samples per flock) suffering from respiratory disease problems. The samples were transported to our laboratory, where the liver, lung, spleen, and trachea were pooled as one sample, tagged, and stored at -20 °C in a sterile plastic bag. Tissue samples were ground in a 1:5 (w/v) dilution of phosphate-buffered saline pH 7.0 to 7.4 with gentamycin (50 g/mL) and mycostatin (1,000 units/mL), centrifuged, and tissue supernatant recovered. Swabs from each flock were combined and suspended in 2 ml of phosphate buffer saline (PBS) pH 7.0-7.4, cleared by centrifugation at 1,740 g for 10 minutes, and then processed for RNA extraction. (53, 54).

**Real time polymerase chine reaction**

**Extraction of viral RNA**

The RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, Calif., USA, Cat. no. 52904) according to the manufacturer's recommendation. Different causative agents of these diseases were investigated using Real–Time Reverse Transcriptase PCR (qRT-PCR), QuantiTect probe RT-PCR kit (Qiagen, Inc. Valencia CA, Cat no 204443).

**Primer and probe used for RT-PCR**

Primers and probe used for RT-PCR were designed as shown in Table-1. In brief, primers for AI-H5, AI-H9, ND, and IBV were designed according to previously published literature (55–58).

**Abbreviations**

AI
Avian Influenza
IBV
Infectious bronchitis virus
NDV
New Castle Disease Virus
Real–Time Reverse Transcriptase PCR
qRT-PCR

**Declarations**

**Ethical approval and consent to participate**

Ethical approval was not necessary for this study. The study involves swab and tissue samples bought from Egyptian poultry farms by poultry veterinarians; thus, no ethical declaration or further permits are necessary.

**Consent for publication**
Not applicable.

Availability of data and materials

All collected data were tabulated and photographed in the manuscript. All data included in this article are original.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was completed solely through the author's efforts, with no funding provided.

Authors' contributions

Ahmed Elshemy, Mohamed Elaish, Heba Hassan collected samples, conducted laboratory investigation. Ahmed El-shemy, Mohamed Amer, and Mohamed Elaish supervised the work, wrote, and revised the original draft. The authors read and approved the final manuscript.

Acknowledgments:

All authors acknowledge the veterinary division of NRC, clinical pathology department and departments of poultry diseases faculty of Vet. Med., Cairo university and NRC.

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References


41. Gado HAA, Ead AAM, Ghanem IA, Moursi MK. Occurrence of Avian Influenza Virus (H9 subtype) in Broiler and Layer Chickens at Sharkia Governorate. Zagazig Veterinary Journal. 2017;45(Supplementary 1):79–90.


51. ENGELICH G, WHITE M, HARTSHORN KL. Role of the respiratory burst in co-operative reduction in neutrophil survival by influenza A virus and Escherichia coli. 2002;51(6):484–90.


Tables

Table-1: RT-PCR primers against tested viruses.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Type</th>
<th>Primer Sequence (5′-3′)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N1</td>
<td>F</td>
<td>5-ACATATGACTAC CCACARTATTCA G-3</td>
<td>(55)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5-AGACCGAGCT AYC ATGATTGC-3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5-FAM-TCWACA GTGCAGGT TCCCTAGCA-TAMRA-3</td>
<td></td>
</tr>
<tr>
<td>H9</td>
<td>F</td>
<td>5-GGA AGA ATT AAT TAT TAT TGG TCG GTA C-3</td>
<td>(56)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5-GCC ACC TTT TTC AGT CTG ACA TT-3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5-FAM- AAC CAG GCC AGA CAT TGC GAG TAA GATCC –Tamra-3</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>F</td>
<td>5-GCT TTT GAGCCT AGC GTT-3</td>
<td>(59)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5-GCC ATG TTG TCA CTG TCT ATT G-3</td>
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<td>Probe</td>
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<tr>
<td>ND</td>
<td>F</td>
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<td></td>
<td>P</td>
<td>5-[FAM]AAGCGTTTCTGTCTCCTCTCCTCCA[TAMRA]-3</td>
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</table>

Table-2: Number and percentage of RT-PCR positive and negative broiler flocks in relation to governorate

Table-3: Number and percentage of RT-PCR positive broiler flocks for single infection in relation to governorate.
Table 4: Number and percentage of RT-PCR positive broiler flocks for double and triple infection in relation to governorate.

<table>
<thead>
<tr>
<th>Governorate</th>
<th>No of tested flocks</th>
<th>Positive flocks</th>
<th>Negative flocks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Beheira</td>
<td>201</td>
<td>169</td>
<td>84.1</td>
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<tr>
<td>Gharbia</td>
<td>23</td>
<td>18</td>
<td>78.3</td>
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<td>Giza</td>
<td>28</td>
<td>22</td>
<td>78.6</td>
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<tr>
<td>Monufiya</td>
<td>89</td>
<td>71</td>
<td>79.8</td>
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<tr>
<td>Qalyoubia</td>
<td>18</td>
<td>14</td>
<td>77.8</td>
</tr>
<tr>
<td>Total</td>
<td>359</td>
<td>293</td>
<td>81.6</td>
</tr>
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</table>

Table 5: Number and percentage of RT-PCR positive and negative broiler flocks in relation to governorate.

<table>
<thead>
<tr>
<th>Governorate</th>
<th>No of tested flocks</th>
<th>H5</th>
<th>H9</th>
<th>ND</th>
<th>IB</th>
<th>Total single infection</th>
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<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
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<tr>
<td>Beheira</td>
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<td>8.7</td>
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<td>Giza</td>
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<td>5.6</td>
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<td>Qalyoubia</td>
<td>18</td>
<td>2</td>
<td>11.1</td>
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<td>22.2</td>
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<td>Total</td>
<td>359</td>
<td>21</td>
<td>5.9</td>
<td>41</td>
<td>11.4</td>
<td>74</td>
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Table 4: Number and percentage of RT-PCR positive broiler flocks for double and triple infection in relation to governorate.

Table 5: Number and percentage of RT-PCR positive and negative broiler flocks in relation to sampling month.
<table>
<thead>
<tr>
<th>Month</th>
<th>No. of tested flocks</th>
<th>Positive</th>
<th>Negative</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>No</td>
<td>%</td>
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<tr>
<td>January</td>
<td>41</td>
<td>31</td>
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<td>68.8</td>
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<tr>
<td>May</td>
<td>29</td>
<td>24</td>
<td>82.8</td>
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<tr>
<td>June</td>
<td>23</td>
<td>20</td>
<td>87.0</td>
</tr>
<tr>
<td>Julie</td>
<td>39</td>
<td>33</td>
<td>84.6</td>
</tr>
<tr>
<td>August</td>
<td>41</td>
<td>34</td>
<td>82.9</td>
</tr>
<tr>
<td>September</td>
<td>48</td>
<td>40</td>
<td>83.3</td>
</tr>
<tr>
<td>October</td>
<td>48</td>
<td>43</td>
<td>89.6</td>
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<tr>
<td>Total</td>
<td>359</td>
<td>293</td>
<td>81.6</td>
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Figures
Figure 1

Geographic distribution and cumulative number of poultry sampled in different governorates of Delta region in Egypt during 2022. Pie charts depict the number of samples collected from each governorate.
Figure 2

Overall frequency of positive and negative samples tested across five different Egyptian governorates during 2022.
Summary of different infections (single, combined, and negative) of H5, H9, Newcastle disease virus (NDV) and Infectious bronchitis virus in each governorate.

**Figure 3**

Summary of different infections (single, combined, and negative) of H5, H9, Newcastle disease virus (NDV) and Infectious bronchitis virus in each governorate.
Figure 4

Single infections pie charts of H5, H9, Newcastle disease virus (NDV) and Infectious bronchitis virus in each governorate.
Figure 5

Overall frequency of double and triple respiratory infection detected in the affected flocks. The frequencies were calculated relative to the total number of isolates ($n = 359$)
Figure 6

Seasonal distribution of positive and negative samples in the affected flocks.