

## Avian Influenza (H9N2) Outbreak In Iraq

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### Summary

An outbreak of avian influenza (AI) (H9N2) occurred in broiler, layers and breeder chicken farms in Iraq during 2004-2007. Mortality between 30% and 70% in broilers and 5% and 10% in layers and breeders were commonly observed on the affected farms. Mixed infections of the influenza virus with other respiratory pathogens particularly Newcastle Disease (ND) virus and Infectious Bronchitis (IB) virus were thought to be responsible for such high mortality. ELISA test was used to detect ND, AI and IB antibodies. Clinical signs included , decrease feed consumption, swelling of the face and typical severe respiratory signs. Gross lesions included extensive hyperemia of the respiratory system, cheesy exudates in the tracheal bifurcation extending into the secondary bronchi, hyperemia and hemorrhagic ulcers in the proventriculus and intestine.

### أنفلونزا الطيور (H9N2) في العراق

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### الخلاصة

انتشر مرض انفلونزا الطيور ( H9N2 ) في حقول الدجاج اللحم والبياض وامهات افراخ اللحم والبياض في العراق في السنوات 2004-2007. بلغت الهلاكات 30 - 70% في الدجاج اللحم و5% و 10% في الدجاج البياض وامهات افراخ اللحم والبياض في الحقول المصابة. تعزى الهلاكات الى الاصابة بفيروس انفلونزا الطيور اضافة الى فيروسات الامراض التنفسية الاخرى وخاصة فيروس مرض نيوكاسل وفيروس مرض التهاب القصبات المعدي. استعمل اختبار الاليزا لمعرفة الاجسام المضادة لمرض نيوكاسل ومرض انفلونزا الطيور ومرض التهاب القصبات المعدي. اشتملت العلامات السريرية على قلة استهلاك العلف وتورم الوجه والعلامات التنفسية النمطية. التغيرات المرضية كانت احتقان شديد

للجهاز التنفسي و وجود مواد متجينة في منطقة تفرع الرغامى وفي القصيبات الثانوية وكذلك وجود احتقان وقرح نزفية في المعدة الغدية والامعاء.

### **Introduction**

Avian Influenza (AI) is caused by viruses of the influenza A genus of the family Orthomyxoviridae (1). At present sixteen hemagglutinin subtypes (H 1-16) and nine neuraminidase subtype (N 1-9) of influenza A viruses have been recognized. High pathogenicity (HP) AI virus isolates are usually of H5 or H7 subtypes, however, most H5 or H7 subtype viruses are not HP based on experimental inoculation of chickens (2). While HPAI occurs relatively rarely, ND is enzootic in some areas of the world and a constant threat to most birds reared domestically (3). Newcastle Disease is regarded throughout the world as one of the two most important diseases of the poultry and other birds, the other disease being highly pathogenic avian influenza (4). The virulent forms of Newcastle disease virus a devastating disease of poultry, hardly a single commercial flock of poultry is reared that, if not infected, is not influenced in some way for measures aimed at controlling ND and spread of the virus (5). Infectious Bronchitis (IB) virus caused late-onset respiratory disease and increased airsacculitis condemnation in affected flocks despite the use of an established infectious bronchitis virus vaccination (6).

The purpose of this study is to report AI incidence and the real cause of the high mortality occur during and after infection depending mostly on the level of Newcastle Disease (ND), Avian Influenza (AI), and Infectious Bronchitis (IB) antibodies using Elisa technique.

### **Materials and Methods**

Broiler farms that showed mortality between 30% and 70% and breeder and layer farms that showed mortality between 5% and 10% were closely monitored for clinical signs, gross pathological lesions and food consumption for the period between the 1<sup>st</sup> of September, 2004 to the 1<sup>st</sup> of September, 2007. Not less than 18 blood samples were collected from each poultry house of each farm. Very few farms were vaccinated against AIV, whereas, all farms were vaccinated against ND. Just broilers were not vaccinated against IB. ELISA technique was proceeded to detect ND, AI, and IB antibodies during and after infection mostly using (Synbiotic company) kits.

**Results**

All farms investigated have showed decrease in feed consumption specially during infection. Some broilers and layers and breeders showed swollen head. During postmortem examination, broilers showed severe congested trachea with caseated materials extended to the bifurcation of the trachea and lower air passages. Airsacs were cloudy or thickened with cheesy materials. Also, ulcers were noticed in proventriculus and intestine. Breeders and layers showed less symptoms and pathological changes characterized by congested trachea and less severe airsacculitis, with congested viscera. The following data show the levels of ND, AI and IB antibodies during and after the outbreaks during three years: Each number represents the mean of ten values (each number represents 180 blood samples tested).

Table (1): ELISA results of ND, AIV and IB antibody titres from 1<sup>st</sup> September to the end of 2004

ND	MEAN	Geometric Mean	% CV
	137227	12128.9	34.815
	11106.9	7543.9	51.651
	9074.2	7413.8	44.862
	10503.7	8900.2	31.219
	8631.8	7890	28.443
	13453.22	13563.444	24.717
	6643.66	5109.77	52.684
AIV	682.3	29	101.496
	898.4	115.2	127.616
	1128.285	94.857	135.441
IB	41341	35193.75	54.5275

CV= Coefficient of Variations

Table (2): ELISA results of ND, AIV and IB antibody titres of 2005

	MEAN	Geometric Mean	% CV
ND	14045.4	12727.6	28.933
	1077.2	8945.6	46.13
	7069.9	5611.8	38.111
	6731.9	5271.9	47.425
	10070.8	8909.4	38.386
	7599.8	6310.6	36.416
	11998.1	11447.3	26.371
	11019.4	10372.8	31.355
	8974	8538.6	21.166
	9805.8	9106.6	33.649
	9162.4	8020.3	50.109
	5544.4	4759.9	46.797
	8962.2	7862.5	44.851
	11301.9	9968.5	29.3
AIV	2737	1742.1	36.56
	865.2	397.6	54.869
	542.4	207	59.234
	520.4	360.2	73.002
	1542.1	555.2	59.042
	799.2	448	45.94
	647.5	350.7	97.08
	2249.2	1939.8	28.79
IB	7806.1	5525.8	50.45
	10553.8	8255.1	47.064
	6915.7	4555.6	52.001
	3214.3	2183.9	48.714
	11617.2	9943.4	35.88
	14790	12747.6	29.597
	14783.1	12437.4	53.179
	9229.3	7296	54.539
	18001.8	15108	33.481
	9320.8	7143.3	52.619
	12995	10334.66	38.74

CV= Coefficient of Variations

Table (3): ELISA results of ND, AIV and IB antibody titres of 2006

	MEAN	Geometric Mean	% CV
ND	18213.1	15076.6	41.148
	28167.5	27343.8	17.832
	29934.3	34171.9	15.902
	11921.6	24710.2	30.372
	22243.1	22004.7	28.168
	7065.6	6366.3	37.652
	16769.5	15294.7	28.272
AIV	1390.1	1136.8	56.868
	9078.7	4010.8	25.252
	1746.2	1613.4	29.854
IB	15748.5	13708.7	45.365
	19402.5	1745.15	29.489
	22119.8	18254.7	27.022
	21370.1	19580.7	22.948
	30811.6	22529.2	47.361
	19186.7	16703.3	27.752
	14751.4	12609.8	30.683
	10073.9	7040.8	45.607

CV= Coefficient of Variations

Table (4): ELISA results of ND, AIV and IB antibody titres o the from the 1<sup>st</sup> of of January to the 1<sup>st</sup> of September, 2007

	MEAN	Geometric Mean	% CV
ND	13005.1	11425.7	38.708
	21081.9	20138	19.826
AIV	1534.3	1287	49.992
	2993.666	2890.333	43.148
IB	15120.9	14943.9	54.793
	15280.7	12590	41.725

CV= Coefficient of Variations

### **Discussion**

Two poultry diseases are considered to be sufficiently serious to be included in List A of the Office International des Epizootics (OIE), namely: highly pathogenic avian influenza (HPAI) and Newcastle disease (ND) (7). Different vaccines have been imported and applied in different programs in Iraq , among them ND, AI and IB vaccines.

Inactivated influenza virus vaccine have been successfully used in various outbreaks (8). ELISA assays have been developed to detect antibodies to avian influenza viruses (9). Two influenza A viruses and one NDV were isolated from Anseriformes, with isolation rates of 5.0% and 2.5% , respectively, for influenza A and NDV (10).The use of vaccination as a tool to eradicate AI requires the enforcement of a coordinated set of control and monitoring measures. In fact, only certain attempts at eradicating AI with the support of vaccination have been successful, and the outcome of the vaccination campaign has been shown to depend greatly on effective application of the field strategy that complemented the vaccination program (11).

Results of ELISA test showed that most of high titres were of ND and IB and mostly ND whereas AI antibodies titres were low indicating that AI role was only immunosuppressive and the main mortality was due to ND or IB infection, except of some results showed high titre of AI revealed that the real cause was AI infection. The positive infection has been confirmed by Dr. Sabbar when he sent samples of lung and trachea from infected broiler farm with high AI antibody titre, tested in this study, to Veterinary Laboratories Agency-Weybridge, England and the result appeared positive and identified the strain as H9N2 (AMR.ANT/IRAQ/2005) (12). The positive infection of broiler farms with AI virus in Iraq was previously reported (13). High coefficient numbers indicate high variation in mean and geometric mean antibodies titres results which refer to either inefficient vaccination or a beginning of infection. Very high titres means a positive infection, while moderate titres means a good result of vaccination. Although number of samples tested in 2004 were less than other years, the number of negative samples for AIV were 5, whereas, number of negative samples in 2005 were 5 also, and 3 negative samples in 2006, and 2 negative samples in 2007. Most of samples sent to the laboratory were tested for ND, AIV and IB, but the in common was ND. ELIZA kits used (Synbiotic company) detect H9- AIV.

The frequent emergence of new variants of IBV is one of the major obstacles to the effective control of the infection caused by this pathogen (14). Relevant changes in IBV antigenicity and virulence have been reported in several countries (15). Because of the different strains of IB vaccines imported and

related to the reasons mentioned above led to unusual respiratory difficulties in poultry farms which caused high mortality. Besides the antigenic variation, other factors can interfere on efficacy of a vaccine program, among them, the viral interference. (16),

Viral interference can also occur between different viruses, as between infectious bronchitis virus (IBV) and Newcastle disease virus (NDV) (17) or avian pneumovirus (18).

To overcome spread of respiratory diseases, vaccination comes in the first step, but it does not always work for many reasons. The High Pathogenic Avian Influenza (HPAI) outbreak was eventually overcome by enforcing biosecurity measures, controlling poultry movements, using inactivated vaccines, and introducing a comprehensive AI surveillance network. However, similar measures undertaken to control H9N2 outbreaks have not been successful in the affected areas, with continuing increased mortality and heavy production losses in broilers and layers, respectively (19).

Because of all these reasons we concluded that the real cause of the high mortalities in poultry farms in Iraq was mainly due to ND and to some extent IB infection, while AI infection was the real cause in some farms only, but its impact was an immunosuppressive predisposing situation to other infection with ND and IB diseases.

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