## **CONTENTS**

|      |                        | Page    |
|------|------------------------|---------|
| I    | INTRODUCTION           | 1-3     |
| II   | REVIEW OF LITERATURE   | 4-48    |
| III  | MATERIAL AND METHODS   | 49-67   |
| IV   | RESULTS                | 68-115  |
| V    | DISCUSSION             | 116-124 |
| VI   | SUMMARY AND CONCLUSION | 125-127 |
| VII  | REFERENCES             | 128-170 |
| VIII | ARABIC SUMMARY         | 1       |

## LIST OF CHARTS TITAL

**PAGE** 

| Chart 1 | Plan for detection of Ab against AI.  | 66  |
|---------|---|-----|
| Chart 2 | Plan for viral detection in different bird species.                             | 67  |
| Chart 3 | The positive serological results of broiler breeder flocks during period A.     | 79  |
| Chart 4 | The positive serological results of broiler flocks during period A.             | 80  |
| Chart 5 | Incidence of AI among different governorates during period C in backyard birds. | 103 |
| Chart 6 | Incidence of AI among different governorates during period C in backyard birds. | 104 |

## LIST OF APPREVIATION

AI AVIAN INFLUENZA

Ab ANTIBODY

AC-ELISA AG CAPTURE ELISA

AGPT AGAR GELL PERCIPITATION TEST

AIV AVIAN INFLUENZA VIRUS

AIVS AVIAN INFLUENZA VIRUSES

CEF CHICKEN EMBRYO FIBROPLAST

DIVA DIFFERENTIATING INFECTED FROM

**VACCINATED AVIAN INFLUENZA** 

DNA DEOXI RIBONUCLEIC ACID

ECE EMBRYONATED CHICKEN EGG

EID50 EMBRYO INFECTIVE DOSE 50

ELISA ENZYME LINKED IMMUNE SORBANT ASSAY

HA HEAMAGGLUTINATION

HP HIGHLY PATHOGENIC

HPAI HIGHLY PATHOGENIC AVIAN INFLUENZA

HPNAI HIGHLY PATHOGENIC NOTIFIABL AVIAN

**INFLUENZA** 

LBM LIVE BIRD MARKETS

LPAI LOW PATHOGENIC AVIAN INFLUENZA

LPM LIVE POULTRY MARKET

LPNAI LOW PATHOGENIC NOTIFIABL AVIAN

**INFLUENZA** 

M MATRIX PROTEIN

MP MILD PATHOGENIC

MPAI MILD PATHOGENIC AVIAN INFLUENZA

mrna Massanger Rna

NA NEURAMINADASE

NASBA NUCLEIC ACID SEQUENCE- BASED

**AMPLIFICATION** 

NP NUCLEO PROTEIN

NS NON STRUCTURAL PROTEIN

OIE OFFICE DES EPIZOTIC INTERNATIONAL

(WORLD ANIMAL HEALTH ORGANIZATION)

PCR POLYMERASE CHAIN REACTION

RRT-PCR REAL TIME- RT- PCR

RT-PCR REVERSE TRANSCRIPTASE- PCR

RNA RIBONUCLEIC ACID

SNA SPECIFIC ANTIBODY NEGATIVE

SPF SPECIFIC PATHOGEN FREE

USAHA UNITED STATE OF AMRICA ANIMAL HEALTH

WHO WORLD HEALTH ORGANIZATION

## **VI. SUMMARY**

Avian influenza become a global disaster threatens poultry industry as well as international trade during the last 10 years, especially after the high economic losses and the repeated human cases which reported all over the world, so that the economic, public health and the zoonotic importance of AI were increased, with maximizing the global fear of occurrence a new human pandemic of avian influenza that become the main target of the scientists and public all over the world. So we began to study the prevalence of AI in Egypt before the outbreak by about three years.

The surveillance during the period of 1/6/2003 - 10/2/2006 was carried out on both clinically diseased and apparently health commercially and backyard flocks.

We examined 12772 serum samples and 8561 swabs (cloacal and tracheal) from 496 flocks; 34, 206, 167, 18, 8, 12, 4 of grandparent, broiler breeder, broiler, turkey, ostrich, pigeon and quail flocks respectively for presence of AI in 19 provinces. The results of this surveillance during this period revealed freedom of the commercial poultry and backyard flocks from AI infection, as following:

Results of ELISA for detection of AI antibodies were more sensitive than AGPT while the AGPT was more specific than ELISA; all the examined serum samples with ELISA were negative for AI except in 11 broiler breeder and 2 broiler flocks. The positive ratio ranged from 10-40%. The results of investigation by using AGPT were negative for all examined flocks for AI antibodies, In addition 80 serum samples of quail and ostrich were also negative.

Results of comparative study between ELISA test and AGPT, recorded a great difference in the results obtained between the two

different tests. Where the ELISA test recorded positive ratio 10-40% and the AGPT results were negative for AI antibodies in all cases as well as AI viruses isolation from flocks with positive AI Ab sera by ELISA were negative.

Trials for Virus isolation were conducted making two serial passages (3 SPF ECE /passage intra allantoic route) according to the OIE and WHO manuals in SPF embryonated 9-11 days eggs, all of these trials were negative for virus isolation from both clinically diseased and health flocks.

Recovery of other viruses were recorded as following

- No virus isolation in the grand parent and quail flocks.
- (17) NDV isolates, (3) IBD, (2) APV, (4) ILT and (1) PMV3 isolate from broiler breeders flocks, (21) NDV isolates, (5) IBD, (1) IB, (2) ILT,
- (1) Reo and (1) PMV3 isolate from broiler flocks, (2) NDV and (1) PMV3 isolate from turkey and (1) PMV 3 isolate from pigeon flocks.

Recovery of bacterial isolation was recorded as following:

- (2) E.coli, (2) MG, and (2) Salmonella isolates from grand parent flocks.
- (26) E.coli, (13) MG, (14) Pasturella and (6) Salmonella isolates from broiler breeders flocks, (26) E.coli, (5) MG, (6) Salmonella and (7) Pasturella isolates from broilers flocks, (4) E.coli, (1) MG, and (4) Pasturella isolates from turkey and (1) E.coli, (2) Pasturella and (1) Salmonella isolate from ostrich flocks.

We recorded the first outbreak of H5N1 in poultry in Egypt for the first time at 10/2/2006 in the rooftop and LBM in Giza, Cairo and Al Menia. The affected cases suffered from congestion and swelling of comb, wattle, head and face with respiratory, nervous and GIT disturbances with history of high mortality reached 100% in most cases. The post mortem lesions were generalized haemorrhages and congestion

all over the body muscles and organs with ulceration of the GIT and pancreatitis.

Results of the HI using monoclonal and polyclonal antibodies, virus isolation, AC-ELISA and RT-PCR were used in the diagnosis of the disease, after the confirmation of the first reported cases as H5N1 AI virus we have stopped the process of virus isolation and we have used the other rapid diagnostic techniques as RT-PCR and other rapid Ag detection tests after their validation according to CLQP SOPs.

Traditional diagnostic techniques must go parallel with the molecular ones. Molecular techniques are safer, sensitive, time saving and reliable where the isolation methods as well as pathogenicity tests need BSL3 or BSL2+ lab where the PCR techniques not required this facility.

Lack of public awareness, insufficient restriction of movement, randomised poultry production and lack of infra –poultry structure lead to highly and rapidly spread avian influenza all over Egypt within few days.

Examination of 266 backyard cases during the period of 10/2/2006 to 30/4/2006 revealed 131 positive cases with ratio 46.1%. This ratio differed from month to another where 65 (40.4%), 50 (60.9%) and 16 (39%) cases were recorded during 16/2/2006 to first of Mars, Mars and April was positive respectively. This may indicate the wide spread of this virus in the backyard system.

Backyard considered an important reservoir of the virus where it can emerge; remerge the infection of commercial poultry flocks from time to another even act as a source for the new reassortement strains. So especial attention should be awarded in control of the virus toward the backyard system.