Susceptibility of Egyptian native breed chickens (Gmisa) to experimental infection with highly pathogenic avian influenza virus (H5N1)

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Abstract: Highly Pathogenic Avian Influenza (HPAI) H5N1 virus is an ongoing public health and socio-economic challenge, particularly in Egypt. H5N1 is now endemic in poultry in many countries, and represents a major pandemic threat and have caused multiple human infections. The main objective of this work to highlights on the susceptibility of Egyptian Gimazachickens to experimental infection with H5N1 avian influenza virus and to get a comprehensive view of Gimaza adaptation to H5N1 virus. Nucleotide and amino acid alignment of HA gene after serial passage in Gimaza chickens revealed no changes in cleavage site and receptor binding site. Only few amino acid substitutions in some antigenic sites were recorded. Gimaza breed appeared to be more sensitive to H5N1 Giza strain as evaluated by the MDT and virus shedding.

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1. Introduction

Avian influenza (AI) is a greatly transmissible respiratory disease affecting poultry caused by influenza A virus that belonged to family Orthomyxoviridae, and represents a major threat to the poultry industry worldwide **(Moemen and Gamal, 2016**). Influenza A viruses are negative-sense, single-stranded, segmented RNA viruses. Several subtypes of AIA virus are existed according to haemagglutinin neuraminidase spikes **(CDC, 2013).** There are 18 different known H antigens (H1 to H18) and 11 different known N antigens (N1 to N11) **(Tong et al., 2012).**

Indigenous native poultry in many countries of the world were contributed to the cultural and social life of rural people **(Sonaiya, 1990).** In developing countries in Africa and Asia, indigenous birds constitute up to 80 percent of the standing poultry population (**FAO. 2010).** In Egypt, the endogenous chickens have an important role in keeping household food security, incomes as well as gender equity **(Kitalyi, 1998).** In this regard, Egyptian native breeds have been recently successively raised in semi-intensive systems in rural areas for meat and egg production with more output per bird **(Hossary and Galal 1994).**

Native chicken breeds in Egypt is generally known to have good disease resistance. Initial studies on infection with coccidiosis have shown that Fayoumi birds survive considerably better than White Leghorn, Rhode Island Red or Mandarah breed **(Hamet and Mérat, 1982).** Conversely, the Fayoumi has proven to be more susceptible to IBDV **(Anjum et al., 1993).** Avian influenza is an infectious disease of birds, causing a severe form of illness associated with high mortalities and threats the poultry production and public health **(WHO 1980).**

Despite the endemic situation of AI H5N1 in Egypt with high mortalities in broiler chicken **(FAO 2010),** the susceptibility of native breed chickens to infections with such virus are still a limited issue. So far, searching for sustainable poultry breed with relative better resistance to infectious disease is important. The main objective of this work to highlights on the natural resistance of Egyptian Gimaza chicken to experimental infection with H5N1 avian influenza virus and to get a comprehensive view of Gimaza adaptation to H5N1 virus. Moreover to characterize the seriallypassaged H5N1 avian influenza virus (five passage) by real time PCR and histopathology. Full length HA gene were sequenced and analyzed to predict the genetic difference observed in each virus passage.

2. Material and methods

Gimaza native breed chickens

Two hundred and ten, one day old chickens of Gimaza native breed were used for experimental infection with HPAI H5N1 virus. Chickens were purchased from Al-Sobaiha farm**, a**nimal production

research institute Animal Health Research Institute, Alexandria. The chicks submitted at one day old and were housed in isolators (10 bird/isolator) in Animal Health Research Institute, Cairo, Egypt for experimental infection. The ventilation in isolators was set to negative pressure with HEPA filtered air. The chicks were fed with ad Libitum feed and water. All birds were screened for AI H5N1 antibodies daily on day one till 30 day by using HI and ELISA test to prove the freedom of chicks from AI H5N1 maternal antibodies tell reach MDA zero.

High pathogenic AI H5N1 virus

Influenza A/chicken/Giza/3/2015was isolated in 2015 from broiler chicken in Giza governorate. Giza strain was identified and characterized as HPAI H5N1 classic genotypebelonging to clade; 2.2.1 at Animal Health Research Institute, Cairo, Egypt. AI H5N1 Giza virus was published in the Genbank database with accession number KY951988.

Virus titration and mean death time

Procedures of virus titration and mean death time were done according to the manual of OIE ***(OIE 2009).*** Virus suspension were diluted in PBS to get a ten-fold dilution series between 10^2 and 10^9. For each dilution, 0.1 mL was inoculated into the allantoic cavity of each of five 10 day old embryonated SPF eggs and incubate at 37°C. Eggs were examined twice daily for 7 days and the times of embryo deaths are recorded. Minimal lethal dose (LD 50) was calculated according the methods of ***Reed and Munch 1938***.

Screening of AI H5N1 maternal antibodies by ELISA

Random blood were collected daily for 30 day from Gimaza chickens before experimental infection to ensure the decaying of maternal AI H5N1antibodies in serum. Serum were examined by ELISA and HI test using a commercial ELISA kits (Biochecksynbiotic kits). Individual and geometric HI titer was calculated as described by **(OIE 2009).** Individual and geometric mean titers of AI H5N1 antibodies were calculated using Biochecksynbiotic kits software (Biochecksynbiotic lab, Inc, Holland).

Experimental design

At 30 days old, Gimaza chickens were classified into 6 groups (1- 6), each group contains 20 chickens in a separate isolators. The first group, were inoculated with 100 ul of 10^6 LD50/ml AIH5N1 seed virus by intra nasal instillation except 2 non inoculated chickens were maintained among to check the lateral transmission of the virus. In the second chicken group, the same protocol as previously described in the first group except the chickens were inoculated with 100 ul of 10^5 LD50/ml AIH5N1 virus (1^{st}passge) isolated from the first group chickens. In the third, fourth and fifth chicken groups, the same protocol as in group 2 except the passage of inoculated virus, as 3^{rd} group were inoculated with 100 ul of 10^4 LD50/ml AIH5N1 virus (2^{st}passge) isolated from the second group chickens and so on till the fifth chicken group. The sixth chicken group are separated in other isolators faraway from the inoculated group and kept as control negative chicken.

Chickens observed daily for body weight and AI clinical sings and mortalities. At 24, 48 and 72 hours, cloacal and tracheal swabs were collected for isolation of shaded virus in ECE and titration. Dead birds were necropsied for AI lesions and collection of trachea and lung for histopathology.

RNA extraction and real time RT-PCR

Virus suspension of AI H5 allantoic fluid should be examined for bacterial sterilitybefore inoculation in experimental chickens. Viral RNA were extracted from 140 ul of homogenates of each pooled samples by using a QIAamp viral RNA kit (QIAGEN, Valencia, USA, cat. No. 52904) according to the manufacturer instructions. A total of 5 ul of extracted viral RNA was amplified using a QuantiTect TM probe RT- PCR Kit (QIAGEN, Valencia, CA) following the manufacturer instructions in lightcycler real time PCR. For partial amplification of M and H genes of avian influenza virus, specific primers and probes manufactured by ***Metabion(Germany)*** were used as previously described ***(Spackman et al. 2002).*** Also, real time PCR were done for detection of other respiratory viruses NDV, AI H7, H9 and IBV by using specific primers and probes (table 2).

Sequencing of PCR product and sequence analysis

Amplified HA gene product of AI H5 virus was purified using a PCR purification kits (QIAGEN, Valencia, CA) and sequenced using internal specific primer with BigDye terminator cycle sequencing kit on an automatic sequencer. The nucleotide sequence was submitted to GenBank database and the accession No KY951988 (A/chicken/Giza/3/2015) was assigned. Sequence of HA gene from each passage of the 4 passage in Gimaza chickens were compared with each other's and with the original HA sequence isolated from commercial broiler chickens. A comparative analysis of deduced amino acids and nucleotide sequences of the HA gene was created using the CLUSTAL W Multiple Sequence Alignment Program, version 1.83 of MegAlign module of Lasergene DNA Star software **(Madison, WI).**

Histopathology

Lung and trachea from each chicken group were fixed in 10% formaline and processed according to the standard protocol described by **(Laudert et al., 1993).** The lung and tracheal lesions were scored as 1= normal lung and trachea, 2= scattered or partial damage in lung and tracheal tissues, 3= 50% or less damage in tracheal and lung tissues, 4= 50-75% damage of lung and tracheal tissue and 5=75-100%

damage in tracheal and lung tissues.**(Laudert et al., 1993).**

Table (1): Primers and Probes used in Real-Time RT-PCR

H5		
Probe	**5 FAM TCWACAGTGGCGTTCCCTAGCA – Tamra-3**	***Slomka et al., 2007***
H5- LH1	**5-ACATATGACTACCCACARTATTCA-3**	
H5- RH1	**5-AGACCAGCTAYCATGATTGC-3**	
H7		
Probe	**5-HEX-CCGCTGCTTAGTTTGACTGGGTCAATCT TAMRA-3**	**VLA,2007**
H7-LH6	**5-GGCCAGTATTAGAAACAACACCTATGA-3**	
H7-RH4	**5-GCCCCGAAGCTAAACCAAAGTAT-3**	
H9		
Probe	**5-CY5-AACCAGGCCAGACATTGCGAGTAAGATCC-BHQ2-3**	***(Monne et al, 2008)***
H9-F	**5-GGAAGAATTAATTATTATTGGTCGGTAC-3**	
H9-R	**5-GCCACCTTTTTCAGTCTGACATT-3**	
NDV		
Probe	**M4169 [FAM]TTCTCTAGCAGTGGGACAGCCTGC[TAMRA]**	**Wise et al., 2004**
M+4100	**AGTGATGTGCTCGGACCTTC**	
M-4220	**CCTGAGGAGAGGCATTTGCTA**	
IBV		
Probe	**FAM-TTGGAAGTAGAGTGACGCCCAAACTTCA-TAMRA**	**Meir et al., 2010**
IBV-fr	**ATGCTCAACCTTGTCCCTAGCA**	
IBV-as	**TCAAACTGCGGATCATCACGT**	
Table 2	**Primers of H5 AI used in conventional one step PCR**	
HGGT	**CTC TTC GAG CAA AAG CAG GGG T**	
H5-KH3	**TAC CAA CCG TCT ACC ATK CCY TG**	
H5F4	**AGT AAT GGA AAT TTC ATT GCT CCA GAA**	
Bm-NS 890R(HR)	**ATA TCG TCT CGT ATT AGT AGG AAA CAA GGG TGT TTT**	

3. Results and Discussion

Screening of AI H5N1 maternal antibodies in Gimaza chickens

As the pathogenesis of HPAIV to the host is dependent on various host and viralfactors. To examine the natural resistance and susceptibility of native breed chickens to H5N1 AI virus, chickens should be seronegative to maternal AI antibodies. Egyptian Gimaza native chickens firstly screened for H5N1 maternal antibodies by ELISA and HI test.

Mean H5N1 antibody titer were observed as high as 10 log 2 and 6 log10 in the first week by HI and ELISA respectively, the titer begins to decrease in the 2nd and 3rd weak to reach 6 log 2 and 3 log 2 when examined by HI, moreover ELISA titer reach 5 log 10 and 2 log 10 respectively. All chickens reacted negative by ELISA in the 4 th and 5 th weak of age, however chickens reacted negative by HI in the 5thweak of age respectively. After 5 weak age, Gimaza chickens showed seronegative by ELISA and HI assay as presented in table 2.

Rural chicken population constitutes 80% of the total world chicken population. It have a pool of all possible genes found in domestic poultry. Poor traditional husbandry, veterinary intervention and stressful environment has created diversity in productivity, body size and disease resistance **(Minga et al 1989).** In Egypt, the endogenous chickens have an important role in keeping household food security, incomes and gender equity **(Kitalyi et al., 1998).** In this regard, Egyptian native breeds have been raised in semi-intensive systems in rural areas for meat and egg production **(Hossary and Galal 1994).**

Table (2): Maternal antibody titer of Gimaza chickens to AI H5 virus screened by ELISA and HI assay.

Group	Age/day	No.	Mean titer of maternal antibodies	
			ELISA titer log10	HI titer log2
1	1	7	6	10
2	7	6	5	6
3	14	6	2	3
4	21	8	0	1
5	26	4	0	0

Real time PCR standard curve for Giza AI H5N1 virus

The detection and quantification limits of real time PCR for H5N1 virus were determined using CT values obtained for each reaction containing from 10^2 to 10^7 copies of the standard RNA curve. The values were plotted against the log of the number of template copies and a linear equation was generated (Fig. 1). Using the slope from the linear equation, the overall efficiency of the assay was estimated to be 91.43%. The assay was negative below 100 template copies. Therefore, the limit of detection and quantification were both determined to be 100 template copies.

RT-PCR assays can detect influenza viruses directly in clinical samples, and real-time RT-PCR is the diagnostic method of choice in many laboratories **(Suarez et al.2007)** Viral antigens can be detected with ELISAs including rapid tests **(Suarez et al., 2007).**

Agüero et al, (2007) Real-time RT-PCR, usually based around the hydrolysis probe or 'TaqMan' method for generation of the target-specific fluorescence signal, has become the method of choice in many laboratories for at least partial diagnosis directly from clinical specimens.

Modifications to the use of RT-PCR have been applied to reduce the time for both identification of virus subtype and sequencing. used a 'real-time' single-step RT-PCR primer/fluorogenic hydrolysis probe system to allow detection of AI viruses and determination of subtype H5 or H7 **(Spackman et al., 2002).**

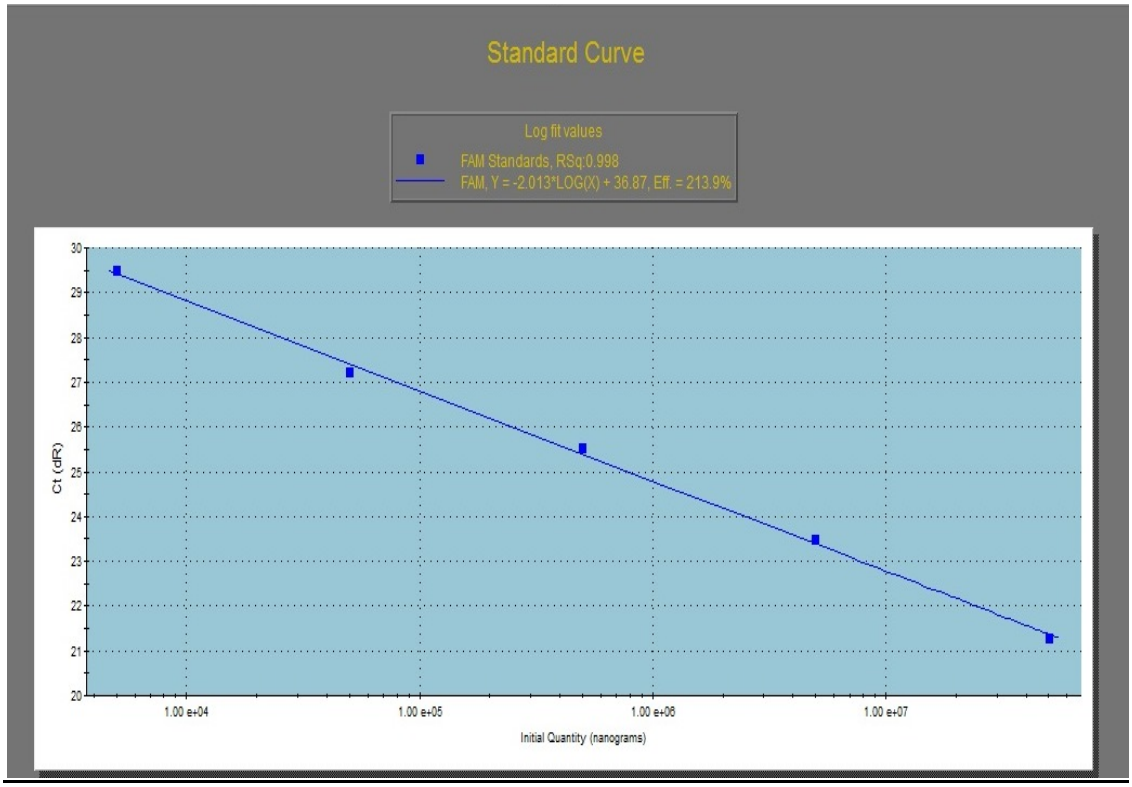

Fig. 1: Real time RT- PCR standard curve for control positive AI H5N1

Real time PCR for AI H5N1 and other chicken respiratory viruses

Real time RT-PCR was performed on positive H5 allantoic fluid. Real time RT-PCR was carried out on extracted and reverse transcribed RNA for detection of NDV, IBV, AI H7 and AI H9 virus to ensure that if the examined samples are contaminated with other respiratory viruses or not. AI H5 virus isolate examined give negative results with NDV, IBV, AI H7, AI H9 and positive result to AI H5 virus, indicating that the virus suspension contain AI H5 and free from other chicken respiratory viruses **(Figure 2)**

Evaluation of clinical signs and mortalities post challenge

Gimaza chickens challenged with the H5N1 seed virus (10^6) chickens showed ruffled feather, depression (Fig.3), anorexia, watery greenish diarrhea, neurologic signs and died in less than 3 days. However, contact chickens, showed the same clinical findings indicating the lateral transmission of the virus was evident. Chickens showed subcutaneous

hemorrhages in thigh and foot (Fig. 3). Cyanosis and swelling in the comb and wattles and leg swelling is prominent. Some birds showing neurologic signs included tremors, uncontrollable shaking, and marked loss of balance, tilted head and seizures. Chickens in contact to challenged groups showed a high mortalities and lower MDT.

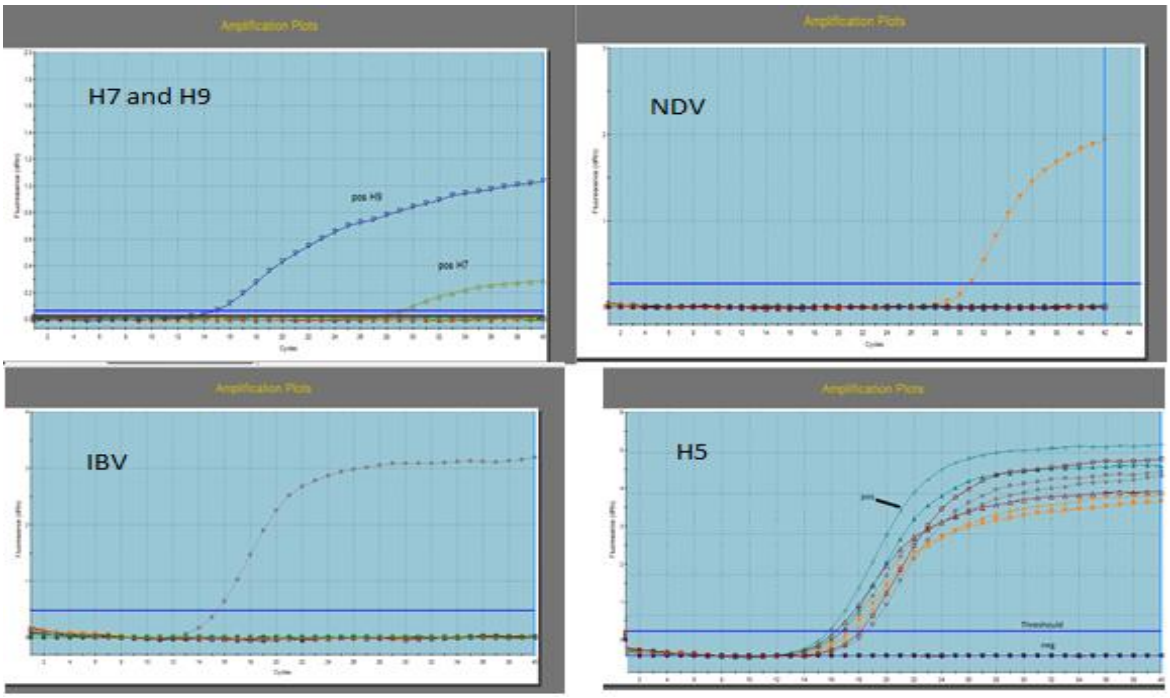

Fig 2: Real time PCR for detection of NDV, IBV, AI H7, H9and AI H5

Gimaza chickens infected with 100 ul of 10^6 seed virus showed clinical findings similar to AI H5N1 infection in commercial broilers**.** Differences in sensitivity among breeds of chickens have been reported for several diseases, such as Marek's disease **(Jiao et al. 2008)**. Variable responses of different chicken lines to HPAIV infection (**Sironi et al., 2011).** In addition, some Thai indigenous chickens displayed resistant traits during the H5N1 outbreak in 2003–2004 **(Kalaya et al., 2006).**

In our study, variations in genetic suscebtability and/or resistance of Gimaza chickens to HPAI virus were studied. Gimaza breed appeared to be more sensitive to H5N1 Giza strain as evaluated by the MDT and virus shedding. The same results obtained by **Hassan et al 2004** who stated that there is variations in genetic susceptibility and/or resistance of Egyptian native breed chickens to Newcastle and IBD**.** These results also supported by the initial studies on Fayomi birds infected. Fayoumi birds survive considerably better than White Leghorn, Rhode Island Red or Mandarah breed **(Hamet and Mérat, 1982).** Fayoumichickens has proven to be more susceptible to IBDV **(Anjum et al., 1993).**

Fig. 3: Gimaza chicken breed infected with 100ul of 10^6 EID_{50} AI H5N1 Giza strain showed subcutaneous hemorrhage in leg, thigh, ruffled feather, lethargy, restlessness and extended leg (Nervous signs).

Table 5: Summary of mortalities and mean titer of shedded H5N1 virus in tracheal and cloacal swabs by inoculation in ECE.

Groups	Inoculum titre	passage	Mortality %	Mean titer of shedded virus log 10 EID50/ml		
				24 hrs	48hrs	72hrs
1	10^6	Seed	100	3.5	5.6	7.5
2	10^5	1^{st}	61.1	2.1	3.4	4.5
3	10^4	2^{nd}	22.2	00	00	3.1
4	10^3	3^{rd}	00	00	00	2.5
5	10^2	4^{th}	00	00	00	00
6	PBS	0	00	00	00	00

Table 6: Mean titer of 5 passage of AI H5N1 virus shedded from infected Gimaza chickens as detected by real time RT-PCR

Hrs post challenge	H5N1 titer expressed as EID50/ml equivalent to real time PCR ct			
	10^6seed virus	1^{st} passage 10^5	2nd passage 10^4	3rd passage 10^3
24 hour post challenge	1.489 x 10^4 3.481 x 10^3 1.406 x 10^4 3.06 x 10^4 3.707 x 10^5 1.286 x 10^7 9.86 x 10^3 6.204×10^1 7.428 x 10^2	- - - - - - - - -	- - - - - - - -	- - - - - - - - -
48 hour post challenge	1.286 x 10^7 1.501 x 10^9 1.655 x 10^7 3.521 x 10^7 2.777 x 10^6 9.609 x 10^4 4.991 x 10^5 3.204 x 10^4 1.151 x 10^6 1.286 x 10^7	- 6.15 x 10^4 - - 9.527 x 10^3 - 7.428 x 10^2 1.608 x 10^5 - -	- - - - - - - - -	- - - - - - - - -
72 hour post challenge	1.511 x 10^9 - - - - - - - -	- - 3.767 x 10^5 2.534 x 10^6 5.909 x 10^6 - 3.696 x 10^6 - 3.204 x 10^4 9.609 x 10^4	1.251 x 10^2 3.153 x 10^1 - - 3.213 x 10^1 8.524 x 10^3 - - 3.153 x 10^1 -	2.683 x 10^2 1.261 x 10^2 - - - - - - - -
96 hour post challenge				1.261 x 10^2 2.116 x 10^1 4.555 x 10^1 4.204 x 10^1 2.683 x 10^2

Estimation of pathological scores in chicken tissues post challenge

Pathological changes in lung and trachea were examined and the severity of lesions was evaluated and scoredaszero score (no lesion),+ (mild lesion), ++(moderate lesion or multifocal lesion), +++(sever and wide spread lesions). Severity of the pathological score in lung and trachea were increased with increasing of virus concentration inoculated (fig 2 and table 7). The pathological lesion in the trachea

showing moderate epithelial erosion to severe sloughing, edema and hemorrhage in submucosa. The lesion in the lung were expressed as profuse cellular exudate filling the alveolar capillaries and parabronchial lumen with severe congestion of blood capillaries.

Differences in the pathogenicity of genetically closely related H5N1 highly pathogenic avian influenza viruses (HPAIVs) were evaluated in White Leghorn chickens. Among these breeds, Chee, Dang, and LHK showed significantly longer survival times than White Leghorns. Virus shedding from dead Thai indigenous chickens was significantly lower than that from White Leghorns **(Matsuu et al., 2016).** Another study suggested that El-Salam chicken strain can be selected for its better growth performance traits compared with others while, Dokki-4 strain can be selected for its higher immune response against ND and AI viral vaccines **(Taha et al., 2012).**

Table7: pathological scores of H5N1AI in lung and trachea of Gmiza chicken correlate to inoculum titer

Organ/lesion	**Pathological score**	
Organ/lesion	**10^5**	**10^6**
1) Trachea		
Subepithelial congestion	++	+++
Subepithelial edema	++	+++
Deciliated epithelium	+	+++
Epithelial erosion	+	+++
Epithelial necrosis and sloughed	+	+++
2) Lungs		
Air capillaries congestion	++	+++
Alveolar exudates	++	+++
Inflammatory cells aggregation	+	+++
Parabronchial exudates	-	++

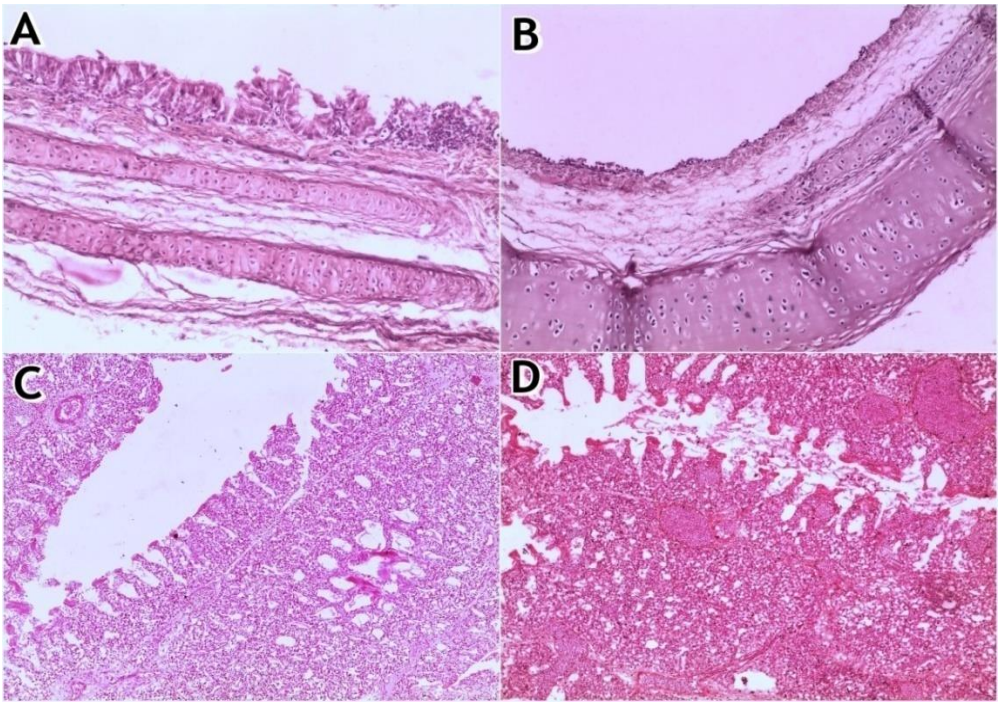

Figure 4: Results of Histopathology

A) Trachea of 10^5 showing moderate epithelial erosion, edema and hemorrhage in submucosa (x200)
B) Trachea10^6: showing severe sloughing of epithelia lining, severe edema in submucosa (x200).
C) Lung$10^{6:}$ showing cellular exudate filling the alveolar capillaries(x100).
D) Lung10^5 showing profuse exudates filling parabronchial lumen with severe congestion of blood capillaries(x100)
H & E staining.

Sequence analysis of HA gene for H5N1 virus after passage in native breed chickens

Nucleotide and amino acid analysis of HA gene after serial passage in Gmiza chickens revealed no changes in cleavage site and receptor binding site. Only few amino acid substitution in some antigenic sites, as in 2nd passage at site 17 in which E instead of D. In 3rd passage at site 17,82,228,372,483 and 493in which E to D, M to I, Ito R, Sto N, Fto I and Cto G were substituted respectively and in 4thpassage at site 87 in which I replaced with H and at site 329in which A is replaced with S (table 8). The pathogenicity of AI H5N1 were conducted in the MDCK cell line, to achieve that goal, ten serial passages of the viruses with and without supplemental trypsin were done. The titers ranged from undetectable by HA testing to as high as $10^{6.8}$ $TCID_{50}$/ml on passages five and seven. Also, was noted lack of differences in titer between passage one and passage ten and hadn't any effect on nucleotide sequence of HA gene **(Kira et al., 2009; Nour, 2010).**

Table 8: Amino acid mutation in AGS, RBS, cleavage, Glycosylation sites of H5N1 HA gene in comparison to original 2006.

Passages	Con.	Amino acid difference in comparison to original seed	Amino acid mutation affect AGS (antigenic site), RBS (receptor binding site), cleavage site, Glycosylation sites in comparison to 2006 isolate			
			AG sites	RB sites	Cleavage site	Glycosylation sites
Seed	10 log 6		S136D, I167T, D170N, R178K,	Δ143	PQGEKRRKKRGLF	26NNS, 27NNT, 39NPT, 181NNT, 209NPT, 309NSS, 500NGT
Passage 1st	10 log 5	D17E				
Passage 2nd	10 log 4	D17E, M82I, I228R, S372N, F483I, C493G				
Passage 3rd	10 log 3	L87H, A329S				

A Alanine, R Arginine, N Asparagine, D Aspartic acid, C Cysteine, E Glutamic acid, Q Glutamine, G Glycine, H Histidine, I Isoleucine, L Leucine, K Lysine, M Methionine, F Phenylalanine, P Proline, S Serine, T Threonine, W Tryptophan, Y Tyrosine, V Valine.

4. Discussion

Rural chicken population constitutes 80% of the total world chicken population. It has a pool of all possible genes found in domestic poultry. In spite of poor traditional husbandry, veterinary intervention and stressful environment, they have been subjected to natural selection imposed by endemic diseases, climate, nutrition and other stresses. This has created diversity in productivity, body size and disease resistance. Many reports indicate that rural chickens are resistant to many endemic diseases and stressful environment and survive better than commercial chickens under rural conditions.

In Egypt, the endogenous chickens have an important role in keeping household food security, incomes and gender equity **(Kitalyi et al., 1998).** In this regard, Egyptian native breeds have been raised in semi-intensive systems in rural areas for meat and egg production **(Hossary and Galal 1994).** Infectious diseases of chickens constitutes a big challenger for production and public health authorities. These diseases, either they do not get treatment or they are not appropriately treated which leads to the development of drug resistance **(Mola et al., 2003; Bekele and Ashenafi, 2010).**

As the pathogenesis of HPAIV is is dependent on various host and viral factors, and to examine the natural resistance and susceptibility of native breed chickens to H5N1 AI virus, Egyptian Gimaza native chickens were experimentally inoculated with an H5N1 virus, and their sensitivity to infection were examined. Firstly, Gimaza chickens were screened for H5N1 maternal antibodies in 1day old and 4 successive weeks by ELISA and HI test (Table --). After 26 days old all Gimaza chickens proved seronegative for H5N1 AI virus.

Gimaza chickens infected with 100 ul of 10^6 seed virus showed clinical signs similar to AI H5N1 infection in commercial broilers like ruffled feathers, depression, anorexia, greenish water diarrhea and neurological signs and died within 3 days **(Fig. 3).** Differences in sensitivity among breedsof chickens have been reported for several diseases, such as Marek's disease **(Jiao et al. 2008)**. Variable responses of different chicken lines to HPAIV infection (**Sironi et al., 2011).** In addition, some Thai indigenous chickens displayed resistant traits during the H5N1 outbreak in 2003–2004 **(Kalaya et al., 2006).** In our study, we demonstrated that Gimaza breed appeared to be more sensitive to H5N1 Giza strain as evaluated by the MDT and virus shedding.

Native chicken breeds is generally known to have good disease resistance. Initial studies on infection with coccidios is have shown that Fayoumi birds survive considerably better than White Leghorn, Rhode Island Red or Mandarah breed **(Hamet and Mérat, 1982).** Conversely, the Fayoumi has proven to be more susceptible to IBDV **(Anjum et al., 1993).** Variations in genetic susceptibility and/or resistance of Egyptian native breed chickens to a specific viral diseases e.g Newcastle and IBD **(Hassan et al. 2004)** is well studied. Little literatures is available for genetic resistance of Egyptian native breed chickens to avian influenza H5N1 virus.

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