Emerging of Infectious Bronchitis Virus (renal mutant) Evading Chicken Vaccinal Immunity in Ismailia, Egypt

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Abstract: In spite of regular vaccination of chicken flocks in Egypt with Infectious Bronchitis Virus (IBV) vaccine, respiratory and kidney lesions had developed. Six vaccinated chicken flocks (5000/flock) in Ismailia, Egypt showed IB- like symptoms was screened for IBV isolation in SPF embryonated chicken eggs. Four Isolates of IBV were characterized and identified at the pathological, biological, physical and molecular level. IB virus spike (S1) gene was amplified by RT-PCR and sequence analysis of the hypervariable region of S1 gene using specific forward and reverse primers was done. Electrophoretic pattern of amplified S1 gene showed a specific band at 380 bp. IBV Ismailia isolates are clustered in distinct phylogenetic group with 4 recent IBV isolates circulating in Egypt since 2012 (**Egypt/12197B/2012**, Eg/**CU-2/2012**, **Egypt/12120s/2012** and **Egypt/01-13/VIR9715/2012**). IBV Ismailia isolates showed 99.41%, 99.12%, 98.83 % and 98.54 % nucleotide sequence identity and 99.12 %, 100 %, 99.12% and 98.25% amino acid identity to the Egyptian IBV variants, respectively. In a conclusion, IBV Ismailia isolates are closely related to the recent Egyptian IBV isolates circulating in Egypt except minor changes in nucleotides and amino acids sequences. IBV Ismailia isolates are different from the currently IB virus strains used for vaccine production, indicating a constant evolution of IBV in Egypt. This difference necessitates continuous montoring to control the spread of infections and the development and use of vaccine should be based on indigenous viruses.

Introduction

IB is an acute and highly contagious respiratory disease of chickens characterized by high mortalities and severe respiratory distress in young chickens including gasping, coughing, sneezing, tracheal rales, and nasal discharge, moreover, decrease in egg production and loss of internal and shell quality of eggs in layers. It is one of the most important viral disease of chickens concerning the economic impact on

poultry industries (Sultan et al., 2004). IB caused by a virus belong to group 3 coronavirus **(*Cavanagh and nagi, 2003*).** It is an enveloped, non-segmented, positive sense ssRNA virus. IBV genome consists of 27 kb and codes for three structural proteins: the spike (S) glycoprotein, the membrane (M) glycoprotein, and the nucleocapsid (N) phosphoprotein. The S glycoprotein is composed of two glycopolypeptides: S1 and S2 **(*Cavanagh, 1983*).** Neutralizing and serotype-specific antibodies are directed against the S1 glycoprotein.

IBV has the ability to mutate or change its genetic makeup readily by point mutation or deletion or insertion or recombination. As a result, numerous serotypes have been emerged and escaped the vaccinal immunity ***(Meir et al, 2004; Abdel-Moneim et al, 2006).*** Several strains of infectious bronchitis virus have a strong affinity for the kidney causing severe renal damage and associated with high mortalities in chickens ***(Gorgyo et al, 1984).***The affinity for kidney tissue may be resulted from mutation as a result of selection pressure following prolonged widespread use of modified live IB vaccines.

Controlling IB infection is difficult due to many factors e.g wide variations in the serotypes and virulence of strains that have developed from time to time, a highly contagious nature, and the evolution of specific tissue tropism and recombinants due to simultaneous infection of multiple virus types and use of live vaccines ***(Bayry et al, 2005).*** Outbreaks can occur in vaccinated flocks due to the lack of cross-protection against antigenically unrelated serotypes and variant strains of the virus ***(Gelb et al, 1991; Capua et al, 1994; Jia et al, 1995).***

In spite of routine IBV vaccination in Egypt, outbreaks of IB frequently occur in the field due to the presence of different serotypes as well as the emergence of multiple subtypes. Accordingly, genotyping of IBV field strains is very important for screening the emergence of new variants as well as evaluating the existing vaccination programs. In the current study, we present an analysis of the partial S1 gene sequences of IBV isolates from broiler chickens in Ismailia, Egypt to determine the cause of IBV vaccination failure and escaping the virus from vacccinal immunity. To achieve this goal, IBV were isolated and identified at the nucleotide and amino acid levels and compare them with the recent variant circulating in Egypt and other countries in the Middle East.

Material and methods

1- Broiler flocks

Six broiler chicken flocks of Cub breed (5000 bird each), 35-40 day old suffered of significant mortalities and respiratory and renal

symptoms are included in this study. The flocks located in Abou-soier, Ismailia governorate, which represent the eastern part of Egypt. The broiler flocks demonstrating a respiratory disorders, anorexia, and loss of bodyweight, possibly associated with increased mortalities. Mortality rates ranged from 15 to 30 %. Postmortem findings included petecheal hemorrhages in the larynx and trachea, and severe congestion and urates deposition in the kidneys and ureters.

2- Samples

Five bird of each flock were collected for virus isolation and post mortem examination. A part of kidney, lung, trachea, bronchi and liver of each bird are pooled and aseptically transferred in transportation media for virus isolation. Another parts of previous organs are kept in 10% formalin for histopathological examination and the third parts of organs kept frozen for PCR assay.

3- Virus isolation

Virus isolation was carried out according to the method described by ***Momayez et al (2002).*** Briefly, pooled samples were homogenized to give approximately 10% (w/v) suspension in PBS pH 7.2 containing 100IU/ml penicillin, 100µg/ml streptomycin, and 30 IU amphotericin B/ml. The homogenized samples were centrifuged at 1000g for 15min at 4°C and then filtered through a 0.45µm filter membrane. The supernatant was inoculated at 0.2ml via the allantoic cavity of groups of ten 9-11 day-old SPF eggs.

Inoculated eggs were checked twice a day. Those that died within 24h after inoculation were discarded. Mortality between 2 and 7 days post inoculation (PI) were considered to be virus specific. The allantoic fluid was harvested aseptically from embryos that died between 48 and 72h PI, if the fluid showed no Hemaglutination (HA) activity. Dead embryos were examined for the presence of embryo stunting, curling, urates in the mesonephros, or focal necrosis in the liver. On day 3 PI, part of the eggs were removed from the incubator and were placed at 4°C for 24h and the allantoic fluid was collected for the next passage.

4- Viral RNA extraction

Viral RNA was extracted from infected allantoic fluid of the third egg passage and from clinical pooled samples using a DNA/RNA extraction kit (QIAamp minikit, Qiagen, Germany) as recommended by the supplier.

5-RT-PCR

RT- PCR was used to amplify the S1 gene of IBV using Qiagen one step test Kit according to manufacturer's instructions. The mix per one reaction are; 25 µl of 2x RT-PCR buffer, 1 µl of each Forward and Reverse primers, 1 µl of RT-enzyme, 1 µl of MgSO4, 11 µl of RNase-free water and 10µl of Template RNA in 50 µl Total volume using thermal cycler

ABI2720 (ABI-USA). Thermal profile; 96 C for 1 min followed by 25 cycles of 96 C for 10sec; 50 C for 5 sec and 60 C for 2 min then followed by rapid ramp to 4C.

The sequence and length of the primers used for amplification of S1 gene of IBV are designed according to Adzhar et al., (1997). Sequence of foreward and reverse primers are **IBV-S1-F** (5'-CACTGGTAATTTTTCAGATGG -3') and **IBV-S1-R** (5'-CAGATTGCTTACAACCACC-3')

6-Agarose Gel Electrophoresis

RT-PCR products were loaded in agarose gel and covered with electrolyte solution in 1X TBE and allowing running the PCR product in the gel at 95 V for 40 minute, to determine the base pairs of the PCR product (380 bp) which could be visualized compared to 100 bp ladder (Qiagen, Germany) using Gel documentation system (Biometra, Germany).

7-Phylogenetic analysis

The RT-PCR product were separated and purified using purification kits (Fermentas Inc.,Ontario, Canada) according to the manufacturers protocol. Purified RT-PCR products were sequenced in forward and reverse directions. Sequencing reaction was performed with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) as described by the manufacturer in an ABI Prism310 Genetic Analyzer (Applied Biosystems, USA).

Sequence of S1 gene of Ismailia IBV isolates were compared with the IBV sequences in GenBank database using a BLAST search via the National Center of Biotechnology Information (USA), and sequence similarities were included in alignment and phylogenetic construction. The phylogenetic tree was constructed by Unweighted Pair Group Method using Arithmetic averages (UPGMA) together with a bootstrap analysis. The analysis was carried out in Mega 6 program.

The S1 gene sequences of other previously IBV retrieved in the GenBank in this study are: **Eg/12197B/2012**(Acc no.KC533683), **Eg/CU-2/2012** (KC985213), **Eg/12120s/2012** (KC533684), **Egypt/01-13/VIR9715/2012** (KC527831), **RF/06/2008** (HQ840489), **RF/07/2008** (HQ840490), **UK/142/86** (X58066), **UK/123/82** (X58067), **IS/589/98** (AY789963), **IS/572/98** (AY789961), **IS/1366-Sp1** (Acc no.EU350550), **IS/236-S1** (Acc no.AY135205), **IS-1494** (Acc no.HM131453)**, H120** (Acc no.JN600610), **Ma5** (Acc no.AY561713), **Connecticut** (Acc no.AF094818), **4/91** (Acc no.AF093794) and **D41** (Acc no.AF036937).

8- Histopathology

The collected samples from kidneys, trachea, lung and liver were fixed in 10% neutral buffered formalin for 24 hours. The samples

were then washed, dehydrated in ethyl alcohol, cleared in xylene and embedded in paraffin wax, as routinely allowed. Histological sections at 4 -5 microns from embedded samples were done and stained with the routine stain, hematoxylin and eosin (Luna, 1988).

Results and discussions

1- Gross necropsy findings

All birds examined for post mortem findings showed similar pictures. The most frequent finding was mucosal thickening with serous or catarrhal exudates in the nasal passage, trachea, bronchi and air sacs. Respiratory tract was partially or completely blocked with dry, caseous yellow casts results in high pitched cheeping and may be the main cause of death. Pneumonia, conjunctivitis and swollen sinuses were also seen.

The kidneys were dark, congested, swollen and showed petecheal hemorrhages and urates deposition (Fig. 1). Ureters were hyperemic, congested and showed urates deposition. Liver, bursa of fabricious and spleen were congested, swollen and showed pinpoint hemorrhages in subserosal surfaces.

Respiratory system is the main site of IBV multiplication, following which viremia occurs and the virus become widely distributed in the body particularly kidneys and reproductive system (Darbyshire, 1981). In kidneys, the virus probably occurs in epithelial cells lining the ducts and tubules produce marked swelling and hemorrhages in kidney tissues in an acute form (Duff et al., 1971) and possibly also in a chronic form (Alexander et al., 1978).

2- Virus isolation

After the third passage of six IBV isolates in specific-pathogen-free embryonated eggs, lesions were observed in the form of mortality of embryos, stunting, curling (Fig.2), and uric acid deposition in the kidneys and ureter. The results obtained in (Fig.2) were in accordance with that obtained by Susan and El-Hady (2010) and DeWit et al. (2011). The allantoic fluid of inoculated eggs were found to be negative for Newcastle disease virus and avian influenza virus by Hemaglutination assay.

The isolate recovered from kidney of the IB vaccinated flock was shown to produce dwarfing of the infected embryos and showed similar characteristics of coronavirus IBV. The clinical manifestations, gross and microscopic lesions of the infected chickens were the same as those infected with nephropathogenic IBVs (***Calnek, 1997; Zhou and He 2000***).

3-Histopathological finding:

Histopathology can be useful in studying of IBV virulence and pathogenesis, and help in diagnosis of acute and non-complicated IB. The respiratory tract showed severe acute hemorrhagic tracheitis and

pneumonia. The changes in trachea included: edema, desquamation of epithelium, mononuclear cell infiltration of the submucosa and severe congestion and hemorrhages of the trachea (Fig. 3). The lung suffered also from interstitial pneumonia (Fig. 4). The reaction of trachea and lung are extensively studied and well described by ***Purcell and McFerran (1972).***

The prominent histopathological change in the liver was focal infiltrations of the hepatic tissues with mononuclear inflammatory cells and some heterophils. In the kidneys, severe congestion, hemorrhages and necrosis of the tubular epithelium were seen. Focal infiltrations of the interstitial tissue with lymphocytes was seen between the urinary tubules. Signs of regeneration of the tubular epithelium could be seen. Hyperplasia of the glomerular epithelium was also an evident finding (Fig. 5, 6 and 7).

IBV strains isolated from Ismailia are nephropathogenic and replicate in respiratory tissues and kidney, but the lesions are more evident in the kidney. Several reports confirmed the tropism of IBV to Kidney tissues ***(Purcell et a., 1976; Albassam et al, 1986; Ignjatovic et al, 2002; Lee et al, 2004; Benyeda et al, 2009, 2010; Boroomand et al, 2012).*** The tubular epithelial cells of the kidney are the target cells of IBV (Owen *et al*., 1991; Janse et al.,

4-RT-PCR

Four IBV isolates out of six isolates were positive in RT-PCR. Amplification of the S1 gene from the infected allanotic fluids and pooled clinical samples resulted in a 380 bp amplicon as shown in Fig. 2 (didn't not appear with the uninfected allantoic fluid as a negative control). The positive results were seen with 10^{-2} to 10^{-5} dilutions of allantoic fluid. Further dilutions could not yield any PCR products. No amplifications were seen when using primers for NDV, AI and IBD viruses.

A primer pair forward and reverse was used in this study to detect IBV by S1 gene amplification in clinical samples and in allantoic fluid of infected eggs. It was showed that the primer can amplify 380 bp of S1 gene (Fig. 8). These results shown to be the same as obtained by ***Peyman et al (2013) and Susan El-Hady (2010).*** The primers was designed specific to a conserved region of S1 gene to ensure a wide detection range. PCR amplification of S1 gene: was applied for the first 1230 base of S1 gene which contains the 3 hyper variable regions (HVRs) of S gene ***(Dolz et al, 2006; Cavanagh et al, 2005).***

5-Phyogenetic analysis of S1 gene of IBV Ismailia strain

342 nucleotide sites in hyper variable region (HVR) of the S1 gene of IBV, Ismailia strain was characterized for the whole dataset and compared with the IBV sequences in GenBank database using BLAST search via the

National Center of Biotechnology Information (USA). BLAST analysis revealed that, **IBV-isolate-Ismailia-Egy/2014** is shared significant similarity at the nucleotide level with other IBV Egyptian isolates emerged in 2012 as **Egypt/12197B/2012**, Eg/**CU-2/2012**, **Egypt/12120s/2012** and **Egypt/01-13/VIR9715/2012** with identity percentage of 99.41%, 99.12%, 98.83% and 98.54% respectively.

This isolate also found to be different from other Egyptian IBV vaccinal strains as **H120**, **Ma5**, **Connecticut** and **D41**with a lower percent of identity 38.60%, 72.22%, 47.36% and 71.93%respectively as showed in figure (9) and table (1). IBV-Ismailia-isolate had less similarities when compared with IBV Israeli strains (**IS/1494, IS/589/98**, **IS/572/98**, **IS/1236-Sp1** and **IS/236-S1**) with an identity percent 88.60%, 88.60%, 86.84% 76.90% and 72.51% respectively (Fig.12 and table 1).

Deduced amino acid sequence of S1 of IBV Ismailia isolate demonstrated high similarity and little variations when compared with other recent IBV Egyptian isolates emerged in 2012 as **Eg/12197B/2012**, **Eg/CU-2/2012**, **Eg/12120s/2012** and **Egypt/01-13/VIR9715/2012**. Amino acid sequences of IBV Ismalia isolate was found to be closely related to Eg/CU-2/2012 with identity percentage of 100%. Only one amino acid substitution at site 75 from A to S (A75S) and at site 56 from N to H was found with **Eg/12197B/2012** and **Eg/12120s/2012** respectively. Two amino acid substitution were found in **Egypt/01-13/VIR9715/2012** at site 67 and 44 (S67I and S44V). Low similarity and wide variations between Ismailia isolate and other IBV vaccinal strains as **H120, Ma5, Connecticut** and **D41** with variations in 94, 38, 92, 26 and 38 amino acids.

Deduced amino acid sequence of S1gene of IBV Ismailia isolate in comparison with **IS/1494**, **IS/589/98, IS/572/98, IS/1236-Sp1** and **IS/236-S1** revealed wide divergence in 15 ,13, 13, 28 and 38 amino acids.

The low nucleotide and amino acid similarities between IBV vaccine strains and Ismailia isolate may account for the occurrence of the IBV outbreak in vaccinated flocks. Production of a new vaccine based on the Egyptian genotype is necessary to protect the poultry industry in Egypt.

Phylogenetic analysis of recent Egyptian IBV isolates revealed that the sequences of S1 gene of blasted IBV strains formed two main distinct groups (Fig. 11). The first group subdivided into three subgroups: group A, including recent Egyptian IBV (**IBV isolate Ismailia, Eg/12197B/2012**, **Eg/CU-2/2012, Eg/12120s/2012 and Egypt/01-13/VIR9715/2012)**. The subgroup B including: European

IBV sequences **(UK/123/82, RF/07/2008, RF/06/2008 and UK/142/86)**, the subgroup C including: IBV Israeli sequences **(IS/589/98, IS/572/98 and IS/1494/06).** The second group including: IBV vaccinal strain sequences and Israeli sequence **(IS/236, Ma5 and D41)**.

It is known that theS1 subunit was involved with infectivity of IBV and carries serotype-specific sequences (*Cavanagh and Davis, 1986; Cavanagh 1995*) and antigenic epitopes inducing virus neutralizing antibody. The different serotypes, subtypes or variants of IBV was thought to be generated by nucleotide point mutations, insertions, deletions or RNA recombinations of S1 genes (*Cavanagh 1983; Cavanagh and Davis, 1986; Jia et al., 1995*), which were responsible for outbreaks of IB in the vaccinated chicken flocks. In addition to serotype changes, the genetic variation may result in changes of the tissue tropism and pathogenicity of the virus, which lead to the generation of new IBV pathotypes.

The genetic diversity of IBV strains arises primarily by mutations, which are induced both by the high error rate and limited proofreading capability of the viral RNAdependent RNA-polymerase and by recombination (Jackwood et al., 2012). The S1 protein commonly differs by 20 to 25 % of the amino acids among different IBV serotypes (Keeler et al., 1998 and Kingham et al., 2000); however, some IBV serotypes show as little as 2 % differences in S1 (Cavanagh et al., 1992). The antigenic sites in the amino acid sequence of the spike sequence were determined (Kant et al., 1992).Three HVRs are located within residues of 38-67, 91-141 and 274-387(Cavanagh et al., 1988, Koch et al., 1990 and Moore et al., 1997).

Multiple sequence alignment and phylogenetic tree analysis separate IBV isolates into different groups which are genotypically related to each other. Most of the serotypes are different from each other in the first HVR of S1 spike glycoprotein. This region seems to be conserved in each geographically isolated virus, which can be used for genotyping of the virus. It is most likely that this region is a serotype specific determinants of IBV and contains antigenic epitope, which is serologically important for serotyping of IBV (Abdel Moniem et al., 2012).

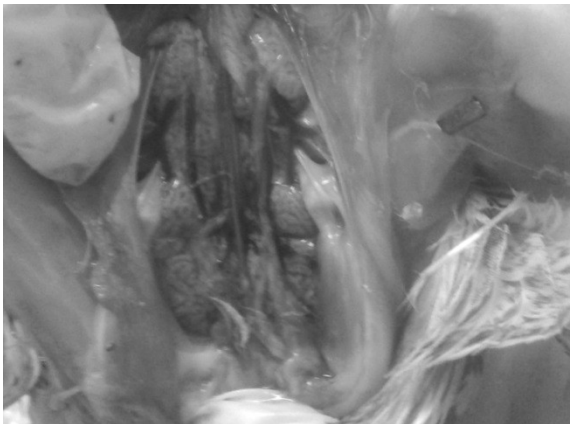


Fig 1: *Kidneys and ureters of chicken showed swelling, congestion and petechial hemorrhages in the surface.*

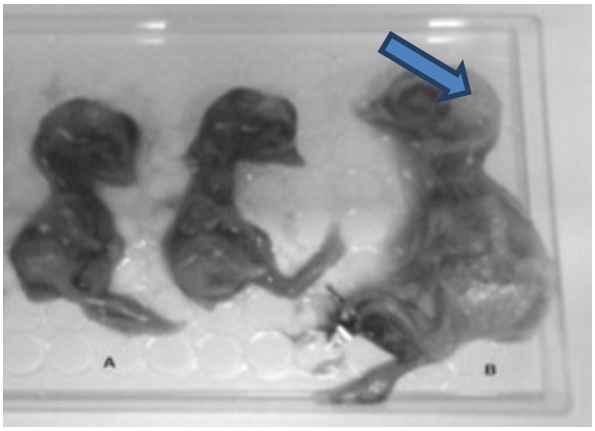


Fig 2: *Stunting and curling of chicken embryos inoculated with IBV (arrow: control)*

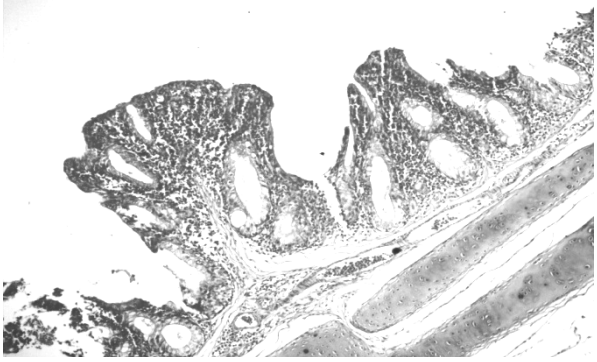


Fig 3: *Tracheal mucosa and submucosa showing severe congestion with hemorrhages, necrosis and loss of surface epithelium and leukocytic infiltrations in the lamina propria. H&E. X200*

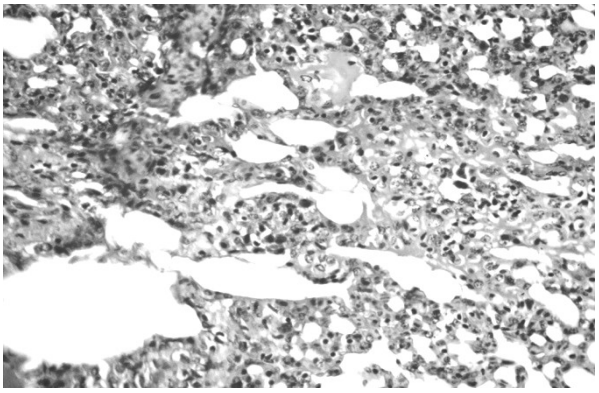


Fig 4: *Lung tissue showing severe congestion and heavy infiltration of the interalveolar septa with heterophils and lymphocytes. The alveolar lumina either collapsed or contain inflammatory exudate. H&E. X200*

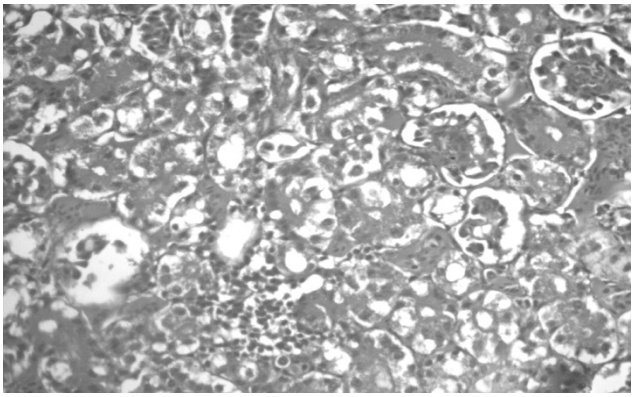


Fig 5: *Histopathological picture of the kidney showing focal infiltration of the interstitial tissue with inflammatory cells, and degeneration of the tubular epithelium. H&E. X200.*

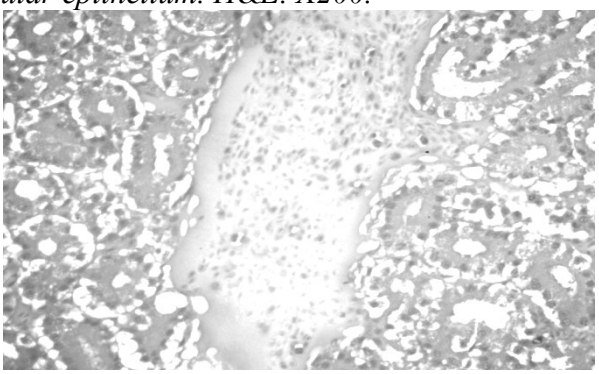


Fig 6: *Histopathological picture of the kidney showing severe congestion of the intertubular blood vessels. H&E. X200.*

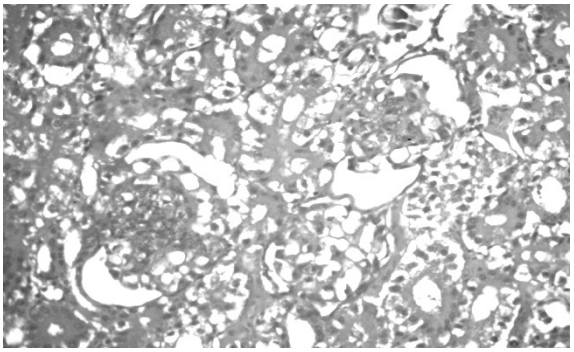


Fig 7: *Histopathological picture of the kidney showing hypercellularity of the glomeruli and degenerative changes of the renal tubules. H&E. X200.*

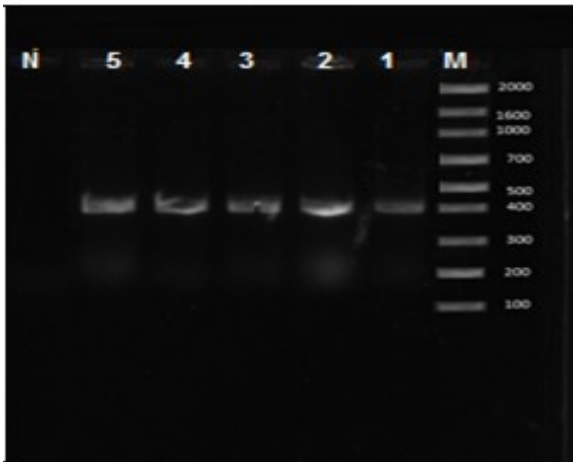
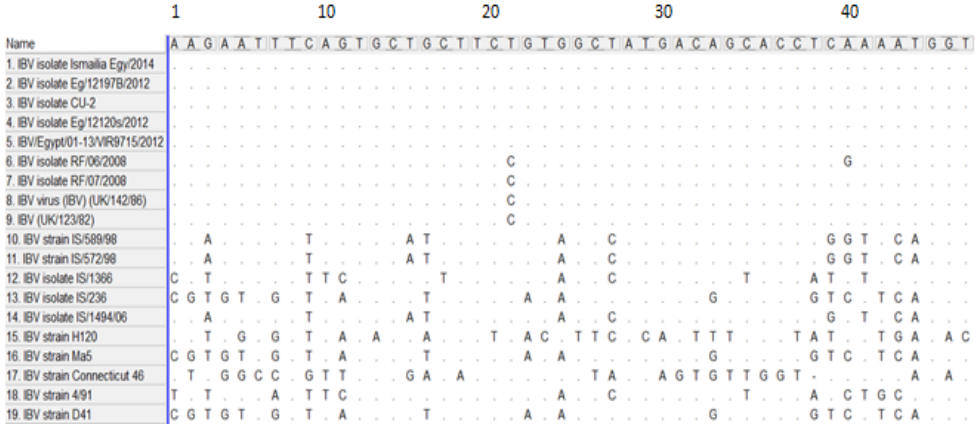
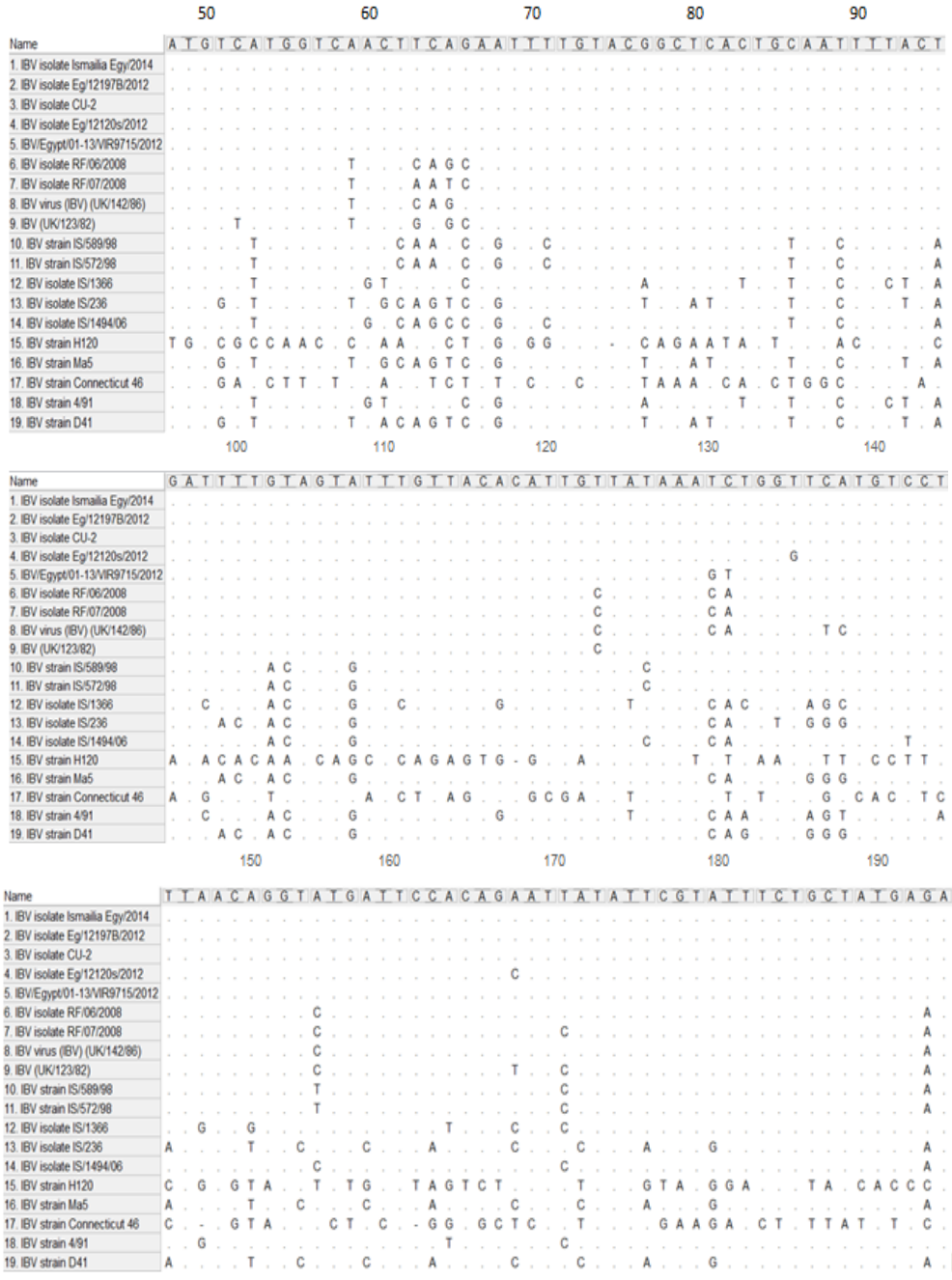
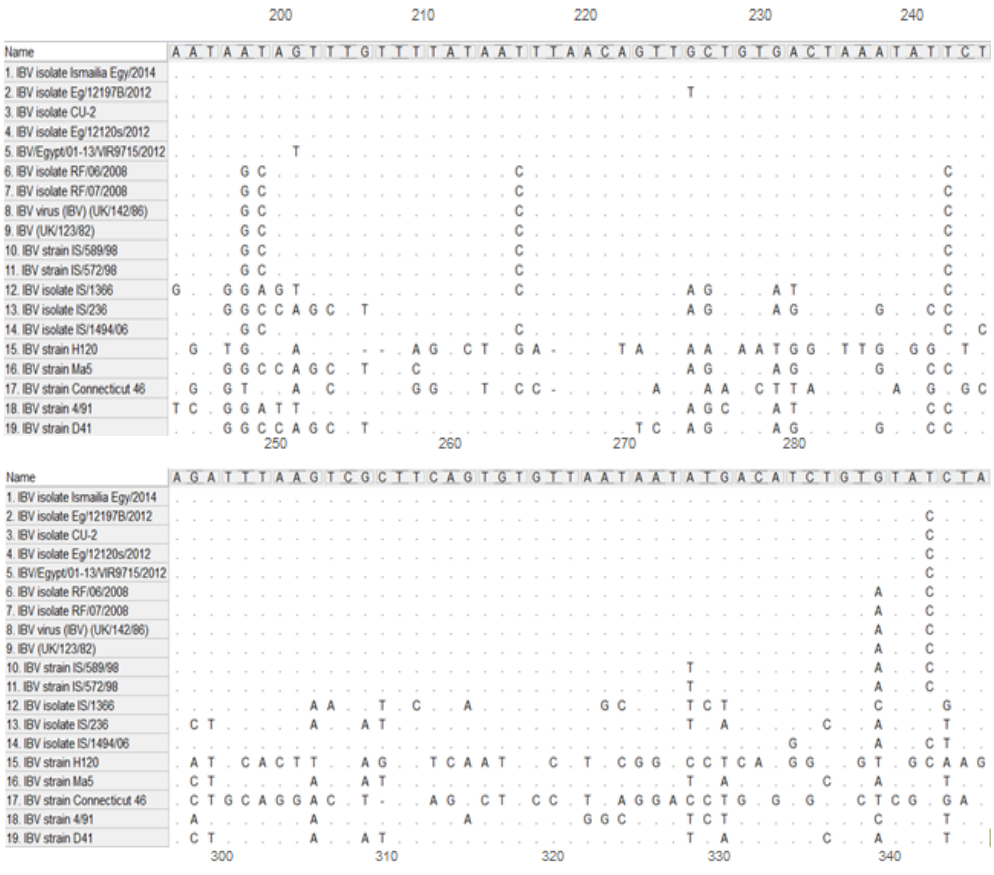


Fig 8: *RT-PCR results for IBV–S1 gene: Lane 1: Postive control, Lanes 2-5: Positive IB samples, Lane N: Negative control.*




50 60 70 80 90
Name
1. IBV isolate Ismailia Egy/2014
2. IBV isolate Eg/12197B/2012
3. IBV isolate CU-2
4. IBV isolate Eg/12120s/2012
5. IBV/Egypt/01-13/VIR9715/2012
6. IBV isolate RF/06/2008
7. IBV isolate RF/07/2008
8. IBV virus (IBV) (UK/142/86)
9. IBV (UK/123/82)
10. IBV strain IS/589/98
11. IBV strain IS/572/98
12. IBV isolate IS/1366
13. IBV isolate IS/236
14. IBV isolate IS/1494/06
15. IBV strain H120
16. IBV strain Ma5
17. IBV strain Connecticut 46
18. IBV strain 4/91
19. IBV strain D41
100 110 120 130 140
150 160 170 180 190


200
210
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240
Name
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2. IBV isolate Eg/12197B/2012
3. IBV isolate CU-2
4. IBV isolate Eg/12120s/2012
5. IBV/Egypt/01-13/VIR9715/2012
6. IBV isolate RF/06/2008
7. IBV isolate RF/07/2008
8. IBV virus (IBV) (UK/142/86)
9. IBV (UK/123/82)
10. IBV strain IS/589/98
11. IBV strain IS/572/98
12. IBV isolate IS/1366
13. IBV isolate IS/236
14. IBV isolate IS/1494/06
15. IBV strain H120
16. IBV strain Ma5
17. IBV strain Connecticut 46
18. IBV strain 4/91
19. IBV strain D41
250
260
270
280
Name
1. IBV isolate Ismailia Egy/2014
2. IBV isolate Eg/12197B/2012
3. IBV isolate CU-2
4. IBV isolate Eg/12120s/2012
5. IBV/Egypt/01-13/VIR9715/2012
6. IBV isolate RF/06/2008
7. IBV isolate RF/07/2008
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9. IBV (UK/123/82)
10. IBV strain IS/589/98
11. IBV strain IS/572/98
12. IBV isolate IS/1366
13. IBV isolate IS/236
14. IBV isolate IS/1494/06
15. IBV strain H120
16. IBV strain Ma5
17. IBV strain Connecticut 46
18. IBV strain 4/91
19. IBV strain D41
300
310
320
330
340
Name
1. IBV isolate Ism
2. IBV isolate Eg/
3. IBV isolate CU-
4. IBV isolate Eg/
5. IBV/Egypt/01-1
6. IBV isolate RF/
7. IBV isolate RF/
8. IBV virus (IBV)
9. IBV (UK/123/82
10. IBV strain IS/5
11. IBV strain IS/5
12. IBV isolate IS/
13. IBV isolate IS/
14. IBV isolate IS/
15. IBV strain H12
16. IBV strain Ma
17. IBV strain Cor
18. IBV strain 4/9
19. IBV strain D4

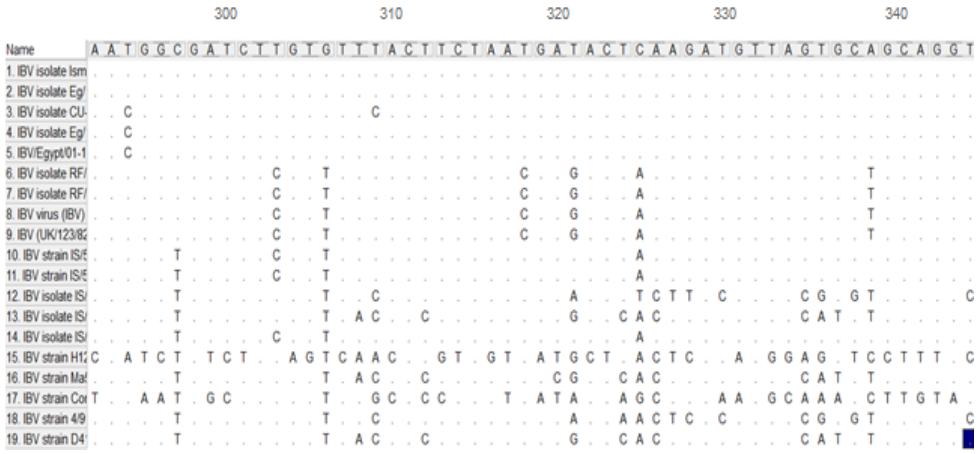
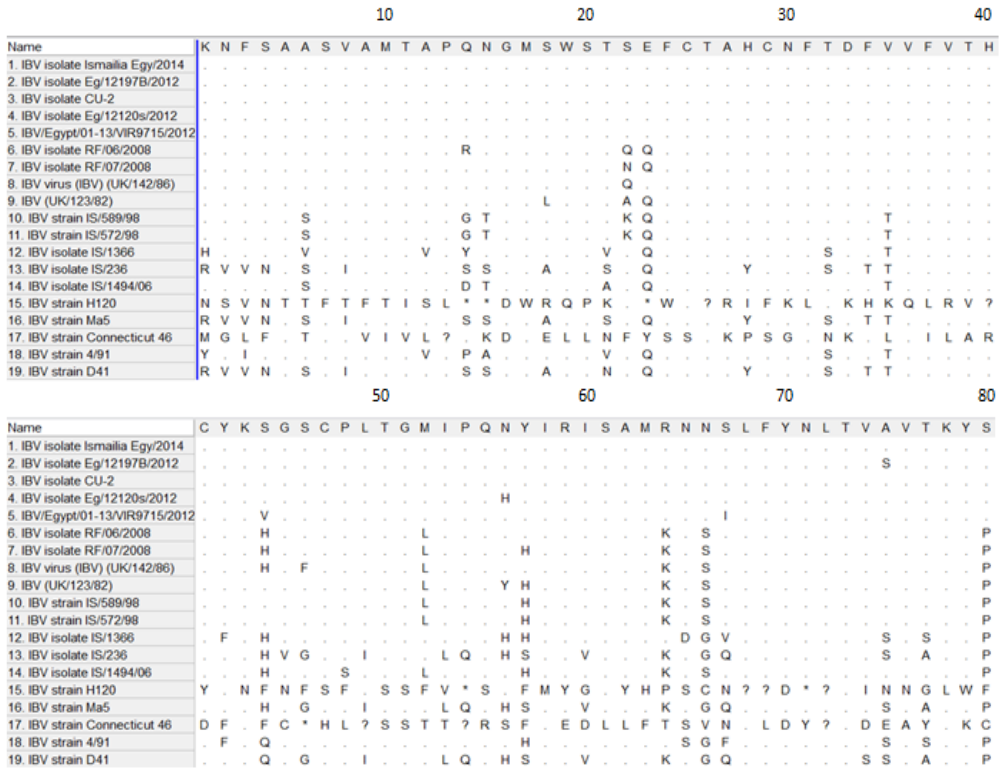


Fig 9: *Nucleotide sequences of different Egyptian IBV variant strains compared with Egyptian and Israeli variants. The horizontal line represents the sequence limits of HVR-3. Dots indicate identical sequences.*



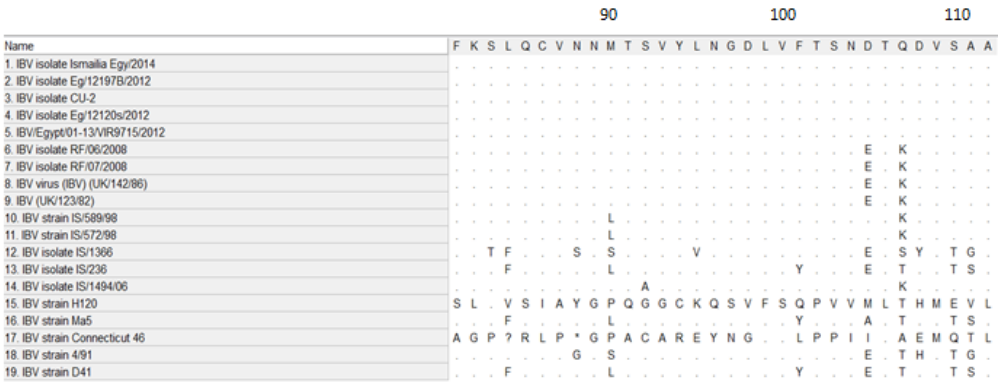


Fig 10: *Deduced amino acid of different Egyptian IBV variant strains compared with other Egyptian and Israeli variants. Dots indicate identical sequences.*

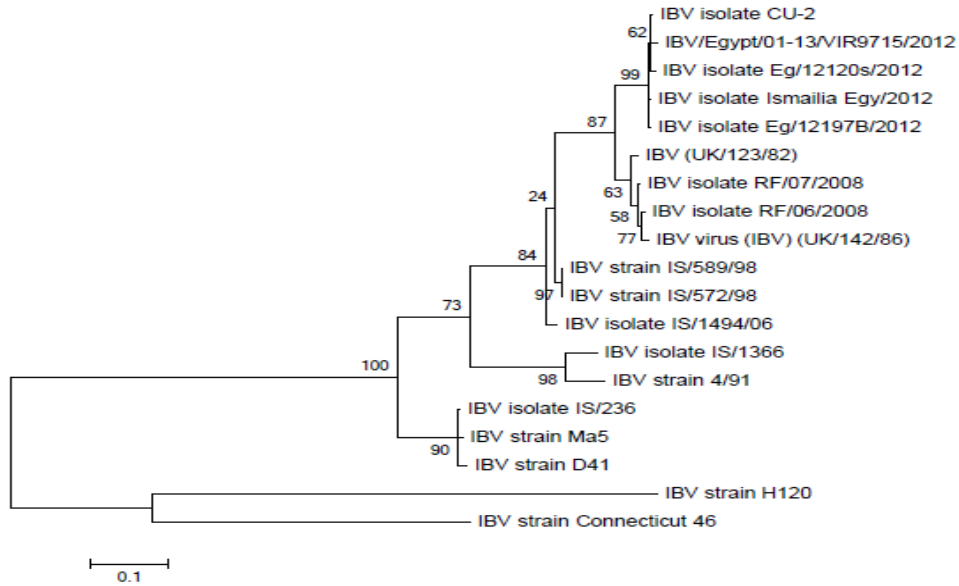


Fig 11: *Phylogenetic tree based on a partial sequence of the S1 gene, showing the relationship between different IBV isolates. The robustness of individual nodes of the tree was assessed using 1000 replications of bootstrap re-sampling of the originally aligned nucleotide sequences.*

Table 1: *Nucleotides and amino acids identity and divergence of IBV_isolate_Ismailia_Egy/2012 compared with theIBV sequences in GenBank database:*

		IBV_isolate_Ismailia_Egy/2012			
		Nucleotides (342)		Amino acids (114)	
		Identity No. (%)	Divergence No. (%)	Identity No. (%)	Divergence No. (%)
1-	**Eg/12197B/2012**	340 (99.41%)	2 (0.59%)	113 (99.12%)	1 (0.88%)
2-	**Eg/CU-2/2012**	339 (99.12%)	3 (0.88%)	114 (100)	0 (0%)
3-	**Eg/12120s/2012**	338 (98.83%)	4 (1.17%)	113 (99.12%)	1 (0.88%)
4-	**Egypt/01-13/VIR9715/2012**	337 (98.54%)	5 (1.46%)	112 (98.25%)	2 (1.75%)
5-	**RF/06/2008**	318 (92.98%)	24 (7.02%)	104(91.23%)	10 (8.77%)
6-	**RF/07/2008**	318 (92.98%)	24 (7.02%)	104 (91.23%)	10 (8.77%)
7-	**UK/142/86**	318 (92.98%)	24(7.02%)	105(92.10%)	9 (7.90%)
8-	**UK/123/82**	319 (93.27%)	23 (6.73%)	103 (90.35%)	11 (9.65%)
9-	**IS/589/98**	303 (88.60%)	39 (11.40%)	101 (88.60%)	13 11.40%)
10-	**IS/572/98**	303 (88.60%)	39 (11.40%)	101 (88.60%)	13 11.40%)
11-	**IS/1366-Sp1**	263 (76.90%)	79 (23.10%)	86 (75.44%)	28 (24.56%)
12-	**IS/236-S1**	248 (72.51%)	94 (27.49%)	76 (66.67%)	38 (33.33%)
13-	**IS-1494**	297 (86.84%)	45 (13.16%)	99 (86.84%)	15 (13.16%)
14-	**4/91**	260 (76.02%)	82 (23.89%)	88 (77.20%)	26 (22.80%)
15-	**H120**	132 (38.60%)	210(61.40%)	20 (17.54%)	94 (82.45%)
16-	**Ma5**	247 (72.22%)	95 (27.78%)	76 (66.67%)	38 (33.33%)
17-	**Connecticut**	162 (47.37%)	180(52.63%)	22 (19.30%)	92 (80.70%)
18-	**D41**	246 (71.93%)	96 (28.07%)	76 (66.67%)	38 (33.33%)

Conclusion: IBV Ismailia isolates are closely related to the recent Egyptian IBV isolates circulating in Egypt except minor changes in nucleotides and amino acids. IBV Ismailia isolates are different from the currently IB virus strains used for vaccine production, indicating a constant evolution of IBV in Egypt. This difference necessitates continuous monitoring to control the spread of infections and the development and use of vaccine should be based on indigenous viruses.

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الملخص العربي

ظهور طفره كلويه من فيروس الالتهاب الشعبي المعدي قادره على الهروب من المناعه في الدجاج المحصن باللقاح الواقي فى الاسماعليه مصر

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على الرغم من التحصين الدوري و المنتظم لقطعان الدواجن فى مصر باللقاح الواقى لفيروس الإلتهاب الشعبى المعدى إلا أنه قد يظهر فى بعض القطعان المحصنه أعراض تنفسيه كلويه مشابهه لأعراض مرض الإلتهاب الشعبى المعدى. تم فحص عدد ٦ قطعان من الدواجن المصابه فى محافظه الإسماعيليه يحتوي كل قطيع على ٥٠٠٠ كتكوت وذلك لعزل فيروس الإلتهاب الشعبى المعدى على بيض الدجاج المخصب الخالى من المسببات المرضيه وقد تم عزل عدد ٤ معزولات فيروسيه وتم التعرف عليهم وتوصيفهم بواسطه الطرق البيولوجيه والجزيئيه المختلفه ودراسة الاثر المرضى على الأنسجة لها فى الطيور المصابة بالفحص الهستوباثولوجى، وقد تم تكبير الجين الخاص بالزوائد الفيروسيه على غلاف الفيروس المعزول بواسطه إختبار تفاعل البلمره المتسلسل المسبوق بالنسخ العكسى وتحليل تتابع القواعد النيتروجينيه فى المنطقه الأكثر تنوعاً فى هذا الجين بإستخدام بوادئ خاصه بهذا الجين وقد أثبتت النتائج أن هذا الجين قد أعطى خطوط بيضاء مضيئه عند ٣٨٠ قاعده نيتروجينيه مزدوجه عند سريانه على الجيل بجهاز الفصل الكهربائى.

وعند تأسيس شجره العائله الوراثيه لمعزوله فيروس الإلتهاب الشعبي المعدي الإسماعيليه وجد أنه قد صنف وراثياً ضمن مجموعه فيروسات تحتوي علي أربعه فيروسات مصريه أخرى متوطنه فى مصر منذ عام ٢٠١٢ وهم مصر١٢١٩٧-٢٠١٢ و مصر سى يو٢-٢٠١٢ و مصر ١٢١٢٠-٢٠١٢ ومصر٠١-١٣ فى اى ار ٢٠١٢ . وقد لوحظ أن معزوله الإسماعيليه تتشابه تماما مع العزلات المصريه الأربعه على مستوي القواعد النيتروجينيه بنسبة 99.41% & 99.12% & 98.54% 98.83% وتتشابه أيضاً على مستوى الأحماض الأمينيه بنسبه 99.12% &100% & 98.25% 99.12% & تباعاً .

الخلاصه: نستنتج أن فيروس الإلتهاب الشعبى المعدى المعزول من الإسماعيليه متطابق الى حد كبير مع المعزولات المصريه الأربعه المتوطنه فى مصر منذ ٢٠١٢ بإستثناء بعض الإختلافات فى بعض القواعد والأحماض الأمينيه ومختلفه تماماً عن فيروسات الإلتهاب الشعبى المعدى المستخدمه فى تحضير اللقاح الواقى فى مصر وتشير أيضاً النتائج إلى أن فيروس الإلتهاب الشعبى المعدى فى تطور مستمر ويتطلب فحصه وراثياً حتى يتم التمكن من السيطره على المرض وعدم إنتشار العدوى به وكذلك تصنيع وتطوير لقاحات جديده من المعزولات المحليه للفيروس .