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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(S1-2):95-106. **OPEN ACCESS**

Characterization of Newcastle Disease Virus (NDV) genotype VII isolated from wild birds and efficacy evaluation of genotype VII vaccines against velogenic NDV challenge

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Wild birds have a critical role in transmission of wide variety of viral diseases to poultry flocks worldwide, and considered as a natural reservoir of Newcastle disease virus (NDV). However, no available data on the prevalence of NDV genotype VII isolated from wild birds especially in Egypt. For this purpose,240 samples were collected from (cattle egrets, sparrows and feral pigeons) in Kafrelsheikh Provence during the period (Septemper2016 to Aprii2018) For identification the most circulating NDV strain responsible for devastating out breaks with in poultry flocks. Oropharyngeal swabs were taken from diseased birds and slide agglutination test was done. The diseased birds suffered from ruffled feathers and whitish diarrhea with enteritis, congested pectoral muscles, lungs, liver and spleen with cloudiness of the air sacs. Amplification of (400bp) of (F) protein by RT-PCR in 2 out of28 samples from cattle egrets (7.1%), 1 out of 10 samples from sparrows (1%) and 1 out of 10 samples from feral pigeons (10%) . Phylogenetic analysis depending on F gene partial sequence clustered these isolates in NDV genotype VII with identity (94.9%) with NDV-chicken china-SOWF-07-2011.The protection efficiency of commercially produced genotype VIINDV inactivated and live vaccines also evaluated when compared with existing programs of vaccination against challenge. The results revealed that the combination of recombinant and live virus of genotype VII vaccines give protection rate (99.6%) against challenge with isolated velogenic NDV in commercial broilers in comparison to inactivated genotype VII alone.

Keywords: Wild birds, NDV genotype VII, RT-PCR, phylogenetic alignment, vaccination and challenge.

INTRODUCTION

Wild birds play a critical role in transmit ion of Newcastle disease virus to domestic poultry farms (Ma-Kay1976.,El-Dahaby 1979) . Newcastle disease virus remains the major cause of huge economic losses and the most harmful pathogen for poultry breeding, NDV Classified as class "A "by OlE was a sever contagious disease affecting variety of domestic and wild birds all over the world (El-Ebiary1979). Newcastle disease caused by NDV genus Avulavirus family paramyxoviridae (Hassan et al1980) which is negative sense single stranded RNA virus (Alexander and Senne 2008). NDV has a great host range about 27-50 birds orders could be affected by NDV (Mayo 2002) depending on some biological parameters,

such as the mean death time (MDT) in ECE ,the (IVPI) in six-week-old chickens and the intracerebral pathogenicity index (ICPI) in one-day-old chickens., strains of NDV are classified into (velogenic), (mesogenic) and (lentogenic) (Dortmans et al 2011). The glycoprotein FO is cleaved into two parts F1 and F2 by host cell furin-like proteases, that is vital for viral virulence. Lentogenic viruses has monobasic motifs at F protein (112G-R/K-Q-G-R;L117) are cleaved by trypsin-like proteases in intestinal tract and respiratory, while virulent virus has multi basic motifs (112R/G/K-R- Q/K-K/R-R;F117) are cleaved by furin-like proteases in whole body. So, the stains virulence can be expected depending on the F protein cleavage site (Samal et al 2011).Wild birds such as Cattle egrets are susceptible to infection with WNDV and play an important epidemiological role in transmission of WNDV among poultry flocks and may act as potential carriers (Metwally 2004). In United States, very virulent strain of NDV isolated from wild birds had caused sever outbreaks in chickens, which lead to drastic economic losses.

The source of these infection was Psittacian origin at 1970 s in south California and during the period from 1990 to1992 another outbreak had been occurred by Cormorants, which was neurotropic virulentvirus (Yussoff and Tan 2001). Therefore, control spreading of Newcastle disease depends mainly on contact with wild birds. This study aimed to characterize the epidemiological role of Cattle egrets, Sparrows and feral pigeons in transmission of NDV associated with recent outbreaks in poultry flocks at Kafrelsheikh Governorate.

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MATERIALS AND METHODS

Clinical Specimens

A total of 140 wild birds,(40 Cattle-egrets, SO Sparrows and SOFeral Pigeons) were collected from different localities in Kafresheikh Governorate included Balteem, Sakha, Motobas and Elhamol during the period from September 2016 till April 2018.Moreover,100 broiler chickens were collected from 2chicken flocks at the same localities. The examined wild birds suffered from ruffled feathers, anorexia, and whitish diarrhea. The sacrificed birds revealed enteritis with greenish intestinal contents, congested pectoral muscles, lungs, liver and spleen with cloudiness of the air sacs and enlarged cecal tonsils. lung, trachea , spleen and brain tissue were aseptically collected from the farm birds while the wild birds were housed for one week and nasopharengeal swabs collected from the suspected ones , All samples were pooled, and homogenized in phosphate buffered saline, pH 7.2, with antibiotics mixture (2 mg/ml) streptomycin, (2000 U/ml) penicillin (50 mg/ml) gentamycin. After repeated cycles of freezing and thawing, the specimens were clarified by centrifugation at 7000 rpm for 7 min. The clarified supernatant stored at -40 c until used.

Virus isolation

The clarified supernatants were inoculated in SPF -ECE (9-11-days) five for each pooled sample via allantoic cavity (0.2 ml/egg) (Kaleta and Baldouf 1988.,Pollard and Walker 1997). Three blind egg passages were carried out before identification of the isolated virus.Embryonic fluids were harvested after 24 hours post inoculation for assessment of haemagglutination activity.It was carried out according to the standard method (Anon 1971).

Slide agglutination test:

Freshly prepared 10% washed chicken RBCs suspension was mixed with the collected allantoic fluid by ratio 1:1 (50% v/v) on grease free glass slide. The suspension was mixed throughout gentle slid rotation. By diffused light the slides were examined, sample with clumping after 1-2 minutes was considered positive (Anon 1971).

RT-PCR

RNAs were extracted from pooled samples of trachea, lung, spleen and brain obtained from wild birds using Viral RNA Mini Kit (Qiagen, Valencia., USA) based on company instruction. cDNA synthesis from the extracted RNAs using one step RT-PCR Kit (Qiagen, Germany) according to instruction procedures. The cDNAs were used for the amplification of F gene of NDV using gene specific primers (Table 1) the PCR mixture contain of 5 µl of RNA template, 4.5 µl RT-PCR buffer, 1 µl of forward primer and 1 µl of reverse primer, 12.5 µl of dNTPs master mix. The thermocycling was as follow30 min at 50 oc; 95 oc for 15 min(RT reaction); 40 cycles of 95 oc for 30 s, 50 oc for 45s and 72 oc for 2 min; then 72 oc for 10 min (final extension). After amplification, 5 µl of PCR products were analyzed by electrophoresis on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide at 1OOV for 40 min in TBE buffer, against 100 bp Plus DNA Ladder (Fermentas).

Nucleotide Sequence and phylogenetic analysis:

The RT-PCR products of five selected positive isolates were purified from the gel using (QiagenInc. Valencia CA)) as manual instruction. The purified PCR products were sequenced using Seqscape ® software for primary analysis of the raw data. A comparative analysis of sequences was performed using the CLUSTAL V multiple sequence alignment program, version 1.83 of Meg Align module of Lasergene DNA Star software (Madison, Wisconsin, USA). to determine nucleotide and amino acid sequence similarities and relationships. A phylogenetic tree of the nucleotide and amino acid sequences was constructed using MEGA6 software (Tamura et al 2013)

Experimental design and challenge.

In order to evaluate the efficacy of commercially produced vaccines of NDV genotype VII .A total of 240 (day old chicks) were divided into six groups (40 birds each). The chicks housed in disinfected separate units under all biosecurity parameters, Food and water were freely available to the chicks throughout the experiment and they observed for one week as a preliminary period .All chicks were reared under the guidelines for animal experiments at Animal Research Institute, Kafrelsheikh, Egypt with approval by the committee of animal care and welfare. The chicks vaccinated with different vaccination programs (table2). Serum samples were collected from all groups on days 7, 21, and 28 for assessing active immune response using haemagglutination inhibition (HI) test according to (Allen and Gough 1974) .AII birds were challenged intramuscularly (1/M) by 0.25 ml of clarified allantoic fluid from positive isolates containing (210) HA units on day 28th.Experimentally infected chickens were daily observed for recording the clinical signs and mortality rate for one week post infection. Tissues samples (trachea, lung and spleen) were collected aseptically from freshly dead chickens and stored frozen for virus re-isolation.

Histopathology

Selected specimens from trachea of all groups (vaccinated and control) were collected and fixed with neutral buffered formalin (10%), dehydrated, cleared and embedded in paraffin wax. 5µm tissue sections stained with (H&E) stain and microscopically examined by oil emersion lens (Bancroft and Stevens1996).

RESULTS

Clinical & pathological feature:

Clinically examined wild birds (Cattle egrets-Feral pigeons and sparrow) revealed ruffled feathers, anorexia, and whitish diarrhea .Feral pigeon suffered from anorexia, white greenish diarrhea, nervous signs including coordination, tremors, head deviation (torticollis and opisthotonus position). With internal lesions of cloudiness in air sacs.

Virus isolation:

The virus isolation done by inoculation 9-11-day SPF -ECE via allantoic cavity revealed that 2 out of 28 pooled samples from Cattle egrets (7.1%), 1 out of 10 samples from sparrows (10%) and 1 out of 10 samples from feral pigeon(10%) were positive .the affected emberyos were edematous , congested with gelatinous material on the skin and heamorrhages over the head.theemberyos died on 3rd days post infection in the third passage fig (1).

Slide agglutination test:

The result revealed that 2 out of 28 pooled samples from Cattle egrets were positive with an incidence of 7.1%, one out of ten pooled Sparrows sample were positive with an incidence of 10% and one out of ten pooled feral pigeon samples was positive with an incidence of 10% Table(3).

RT-PCR:

Newcastle disease virus molecular identification was made by RT-PCR for the field-collected samples as well as lyophilized vaccine (Iasota strain). Out of forty-eight tested samples, five samples were NDV positive with specific amplification of 400 bp of fusion protein (Fig 2).

Sequence analysis:

In order to with stand the genetic relatedness between the obtained viral isolates, a phylogenetic tree was made. A group of sequences, representing global genotypes and others reported from Egypt, were aligned with the sequences presented in this study using the Clustal W algorithm in Bio Edit.

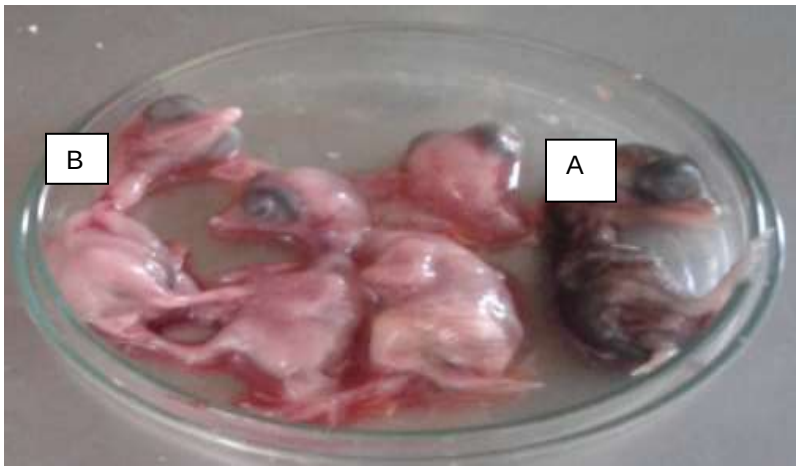


Figure 1: **inoculation of 10 day old SPF-ECE with suspected samples (A)severe congestion, while(B) control negative showed normal embryo.**

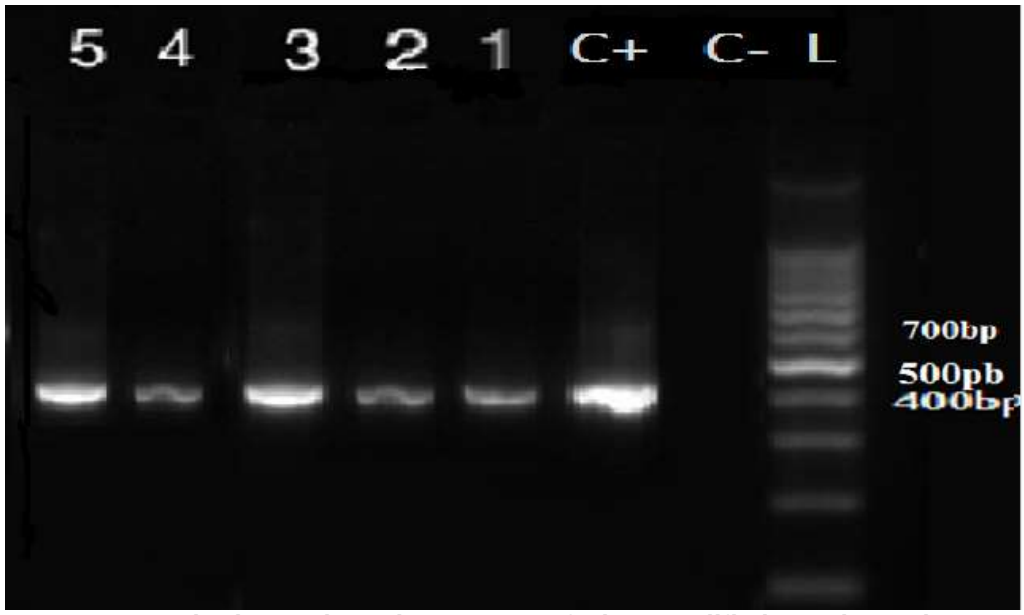
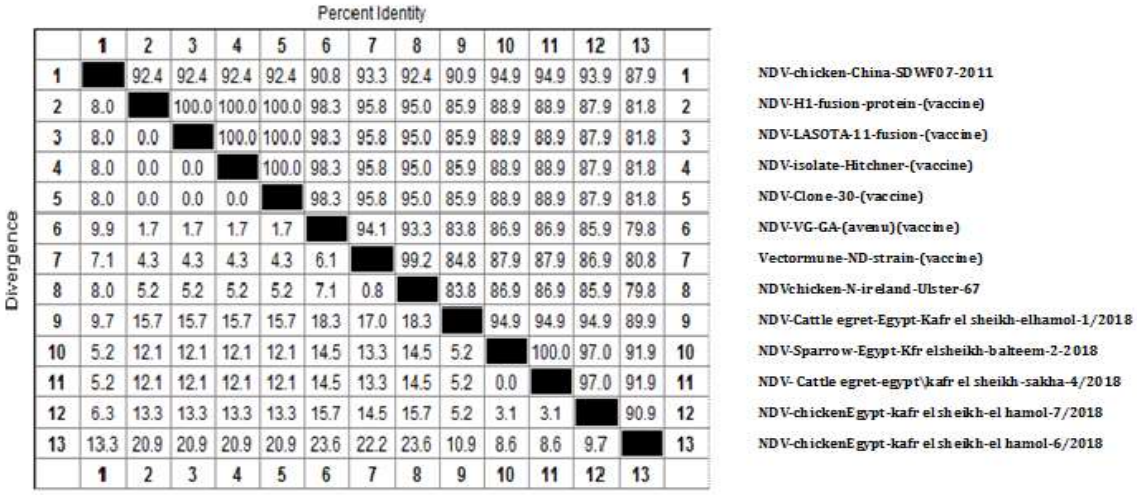


Figure 2: Agarose gel electrophoresis pattern of the amplified products by RT-PCR; for samples.1,2, 3,4 and 5, C- = negative control, C+ :positive control. L :100bp DNA ladder .



	1	2	3	4	5	6	7	8	9	10	11	12	13	
1		92.4	92.4	92.4	92.4	90.8	93.3	92.4	90.9	94.9	94.9	93.9	87.9	1
2	8.0		100.0	100.0	100.0	98.3	95.8	95.0	85.9	88.9	88.9	87.9	81.8	2
3	8.0	0.0		100.0	100.0	98.3	95.8	95.0	85.9	88.9	88.9	87.9	81.8	3
4	8.0	0.0	0.0		100.0	98.3	95.8	95.0	85.9	88.9	88.9	87.9	81.8	4
5	8.0	0.0	0.0	0.0		98.3	95.8	95.0	85.9	88.9	88.9	87.9	81.8	5
6	9.9	1.7	1.7	1.7	1.7		94.1	93.3	83.8	86.9	86.9	85.9	79.8	6
7	7.1	4.3	4.3	4.3	4.3	6.1		99.2	84.8	87.9	87.9	86.9	80.8	7
8	8.0	5.2	5.2	5.2	5.2	7.1	0.8		83.8	86.9	86.9	85.9	79.8	8
9	9.7	15.7	15.7	15.7	15.7	18.3	17.0	18.3		94.9	94.9	94.9	89.9	9
10	5.2	12.1	12.1	12.1	12.1	14.5	13.3	14.5	5.2		100.0	97.0	91.9	10
11	5.2	12.1	12.1	12.1	12.1	14.5	13.3	14.5	5.2	0.0		97.0	91.9	11
12	6.3	13.3	13.3	13.3	13.3	15.7	14.5	15.7	5.2	3.1	3.1		90.9	12
13	13.3	20.9	20.9	20.9	20.9	23.6	22.2	23.6	10.9	8.6	8.6	9.7		13
	1	2	3	4	5	6	7	8	9	10	11	12	13	

Figure 3: Amino acids identity and divergence percent of the isolated viruses in comparison to vaccines and reference strains available in the gene bank.

Studying the percent of divergence and homology between the five selected NDV isolates named(Cattle Egert/ Egypt/ KFS- el hamol-1/2018, NDV -Cattle egert- Egypt -KFS- Sakha-4-2018, NDV -chicken- Egypt -KFS- elhamol-7-2018, NDV-sparrows- Egypt-KFS- Balteem 2-2018 and NDV- chicken -Egypt - KFS-elhamol6-2018)and vaccinal strains showed (90.9-94.9%) identity with NDV chicken/China/SOWF0712011, and (85.9%)homology to NDV-isolate Hitchner fusion genes, NDV-Lasota-11-fusion and NDV-Clone 30, respectively, and (91 %) to vectormune NDV as shown in (Fig3). Phylogenetic alignment revealed that (NDV- Cattle Egert -Egypt- KFS-elhamoi1- 2018) and (NDV-sparrows-Egypt-KFS-Balteem-2-2018) were clustered with NDV-Chicken-Egypt-EI-Arish16-2016,NDVchicken-egypt-ismailia-32-2016.NDVchiken-egypt-dkahlia 30-2016.NDV-egypt ismailia-29-2016.NDV-Chicken-Egypt-Dakahlia 27 2016, NDVchicken-egypt-elarish-15-2016,NDV-Chicken-Egypt-Qaliobia11 2016, NDV-Chicken-Egypt-Sharkia-10-2016,NDV-Chicken-egypt-sharkia7-2016.(NDV-Cattle-Egert-Egypt-kfs-Eihamol-1-2018) was clustered with (NDV-chicken-egypt-sakha-4-2018) NDV-CK/CH/JL/1/03,ndv-FU4-egypt-NLQP-2014, NDV-fu13-egypt-NLQP 2014,NDV-fu8-egyptNLQP-2014and NDV-yz-22-07-Os and all of them belonged to NDV Genotype VII (fig 4).

Haemagglutination inhibition test

The mean HI titers estimated for the broilers before and after-vaccination with the live and recombinant genotype VII inactivated vaccine as shown in (Table 4). At the day (7 -21 and28) post-vaccination, chickens in all groups exhibited NDV HI titers, which elevated throughout the vaccination course. For all groups, the antibody titers produced at the day 7 ranging from 1.9 log2 to 2.3 log2. However, sera collected from the chicks of group 2 had high HI titers (2.31og2 to 6.3 log2) when tested with recombinant genotype VII NDV antigens (A-V11 strain YEBIO) .In addition, the HI titer of the group 4 recorded the highest level of immunity ranging from 2 log2 to 7 log2 and decreased to 5.6 log2 in the day 28 PV when tested against ND 6/10 live vaccine in combination with A-V11 strain YEBIO. Chicks of the group 1and 3 recorded the lowest HI titer ranging from1.9 log2 to 2.6 log2 all over the course of vaccination when tested with either inactivated vaccine (kbnp-c4152R21 strain)or combination of live and inactivated(kbnp-c4152R21 strain).

Table 1: Oligonucleotide primer sequence used for amplification of NDV fusion (F) gene

Name	Primers	Reference
NDV-F330	AGG AAG GAG ACA AAA ACG TTT TAT AGG	(13)
NDV-R700	TCA GCT GAG TTA ATG CAG GGG AGG	*(13)*

Table 2: Vaccination design and challenge:

Group	Vaccine	Route of administration	Age
G1	(Inactivated vaccine 1) (Kbnp-c4152 R2L NDVVII strain	intramuscular route	7 days
G2	(inactivated vaccine 2) Recombinant NDVA-VII strain	intramuscular route	7 days
G3	(inactivated vaccine 1) +(live vaccine1)	intramuscular route drinking water	7 days
G4	(inactivated vaccine 2+ (live vaccine 4)(ND6/10)	intramuscular route , drinking Water	7 days
	Challenge all birds with cattle – egret Kafrelsheikh-2018	intraocular,	28 days
G5(Control +ve)	Non vaccinated challenged with cattle – egret Kafrelsheikh -2018	intramuscular route	28 days
G6(Control-ve)	saline	drinking water	7 days

Table 3: Results of slide agglutination test:

spp.	Total No. of birds	Pooled sample	+ve result of pooled samples to slide agglutination test		-ve result of pooled samples to slide agglutination test	
		No.	No.	percent	No.	percent
Cattle egret	140	28	2	7.1%	26	92.9 %
Sparrows	50	10	1	10 %	9	90%
Feral pigeon	50	10	1	10 %	9	90%
Broiler G1	50	10	1	10%	9	90%
Broiler G2	50	10	1	10%	9	90%

Table 4: Result of heamagglutinaton inhibition test

Group Age	*1*inactivated vaccine 1*	*2**inactivated vaccine 2)*	*3***live vaccne1+inactivated vaccine 1*	*4****live vaccine3+Inactivated vaccine 2*	*5(Control +ve)*
7	$2^{1.9}$	$2^{2.3}$	$2^{1.6}$	2^{2}	$2^{1.9}$
21	$2^{2.3}$	$2^{6.3}$	2^{3}	2^{7}	$2^{2.3}$
28	2^{1}	2^{5}	$2^{2.6}$	$2^{5.6}$	2^{1}

1*Dolgoban inactivated vaccine, 2**YEBIO inactivated vaccine, 3***dolgoban live +inactivated vaccine, 4****ND 6/10 live vaccine+YEBIO inactivated vaccine.

Table 5 : mortality results of challenged birds:

AgeGroup	28	29	30	31	32	33	Total NO.	Total dead	Mortality%
1	**0**	**1**	**4**	**9**	**10**	**8**	**40**	**32**	**80 %**
2	**0**	**0**	**1**	**1**	**0**	**0**	**40**	**2**	**5 %**
3	**0**	**0**	**0**	**0**	**0**	**1**	**40**	**1**	**0.4 %**
4	**0**	**0**	**0**	**0**	**0**	**1**	**40**	**1**	**0.4 %**
5	**0**	**2**	**6**	**10**	**22**	**-**	**40**	**40**	**100 %**
6	**0**	**0**	**0**	**0**	**0**	**0**	**40**	**0**	**0 %**

Figure 4: Neighbor joining phylogenic analysis based on nucleotide sequence clusteration of F-gene of NDV Genotype VII subtype d.

Clinical and post mortem lesions in experimentally infected chicks

Ciinical examination revealed ruffled feathers decrease feed intake, greenish diarrhea, respiratory manifestation and swollen head appeared on 29-31 day in the first and fifth groups. While groups 3 and 4 showed greenish diarrhea .PM lesions were sever heamorhagictrachitis, sever lung congestion and enlarged cecal tonsils with greenish proven tricular contents in the first and fifth groups. While groups 3 and 4 showed mild heamorrhagictrachitis and enlarged cecal tonsils. Mortality reached 100% in the non-vaccinated NDV-challenged broilers began on day 1 p.c .Neither clinical signs of disease nor mortality were observed for broilers from group 6. In contrast, about 80% (32/40) of birds in group 1 were dead with reduced necrotic foci in liver, ureter impacted with urates, parasitic manifestation along the gastrointestinal tract.

Histopathology

Trachea of experimental challenged birds of group 5(control +ve) showed sever degree of trachitis with congestion, necrosis and desquamation of the covering mucosa. Submucosal layer showed marked leukocyticin flitraton manly heterophils (fig 5) . Control -ve group 6 showed normal epithelium lining the tracheal rings represented with pseudo stratified ciliated epithelium with goblet cells(fig6).Trachea of experimentally challenged group 1 recorded degenerative changes within the epithelial lining associated with hyperplastic and disquamative changes (fig 7).Trachea of experimental challenged birds group 2 showed marked decrease in the infiltration and necrotic lesions, the epithelium lining revealed mild degree of hyperplasia and mild submucosalleukocytic infiltration (fig 8).Trachea of experimental challenged birds ofgroup 3 showed decrease in the inflammatory lesions within the tracheal tissue and mild to moderate degree of heterophile infiltration with marked hyperplasia of both epithelium and goblet cells (fig9).Trachea of experimental challenged birds group 4 showed decrease in the inflammatory lesions within tracheal tissue and mild to moderate degree of heterophilic infiltration and marked hyperplasia of both epithelium and goblet cells with mild to moderate trachitis(fig 10)

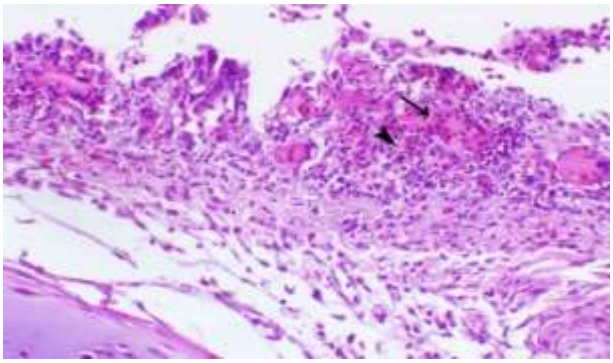


Figure 5: trachea of diseased bird showed severs degree of trachitis associated with congestion.

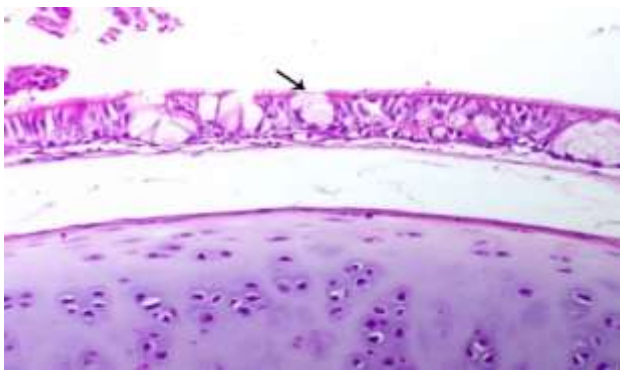


Figure 6.Trachea of control-ve bird showed normal pseudo -stratified epithelium with goblet cells.

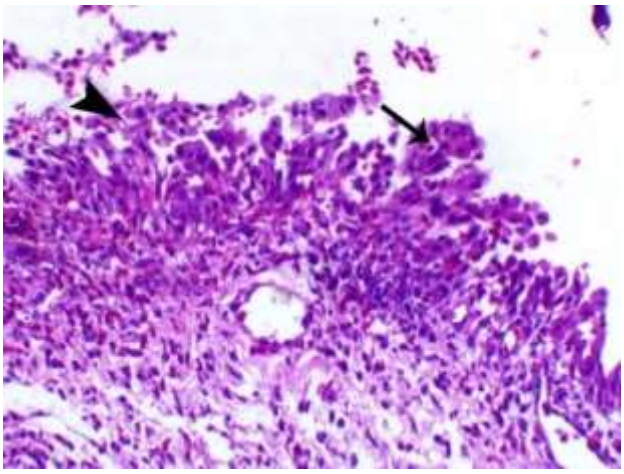


Figure 7: Trachea of experimental challenged bird with Dolgoban® inactivated vaccine showed degenerative changes.

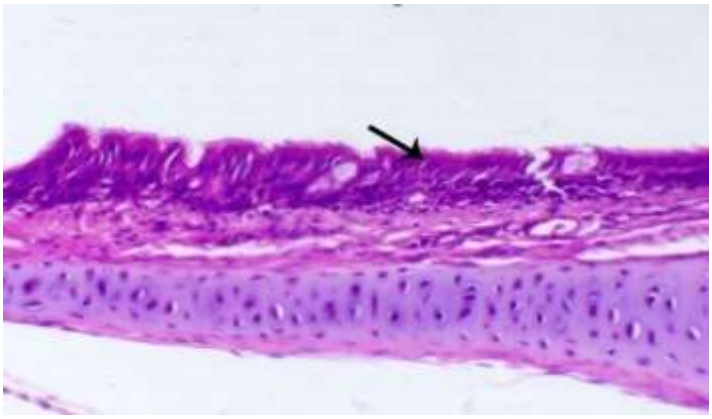


Figure 8: Trachea of experimental challenged birds with Yebio® inactivated vaccine showed mild degree of trachitis.

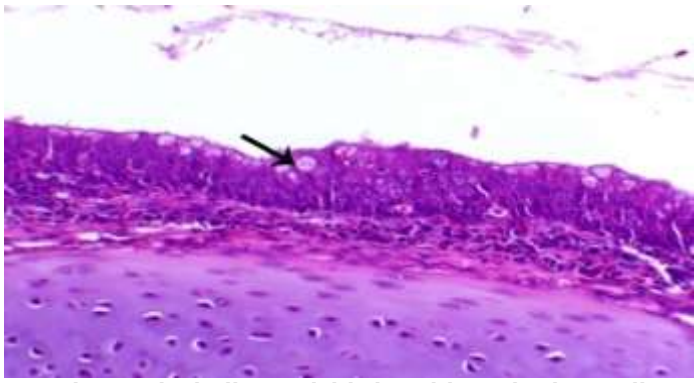


Figure 9: trachea of experimental challenged birds with Dolgoban® live & inactivated vaccine showed decreased the inflammatory lesions.

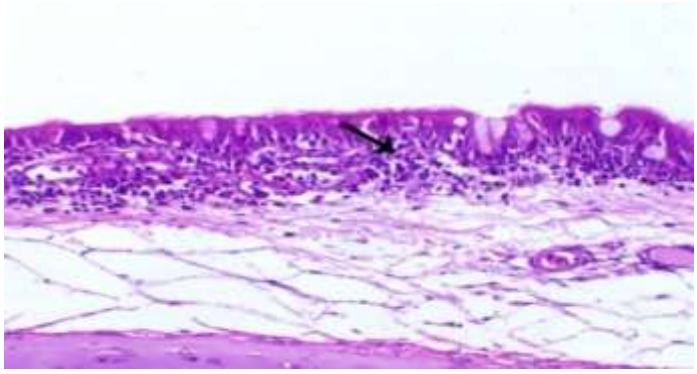


Figure 10: trachea of experimental challenged birds with ND6/10®and Yebio®inactivated vaccine showed mild degree of trachitis

DISCUSSION

Newcastle disease considered as virulent infectious disease which can affect poultry industry (Ke et al 2010.,Lindh et al 2008).Resemble poultry, wild birds are considered a natural reservoir of NDV and several NDV strains with different genotypes were isolated from wild birds (Xie et al 2012). Control spreading of Newcastle disease depends mainly on biosecurity to prevent spreading of NDV to poultry flocks. In this study succefull isolation of Newcastle disease virus via inoculation in 9-11-day emperyonated chicken eggs with isolation rate 2 out of28 pooled samples from Cattle egrets (7.1%), 1 out of 10 samples from sparrows (10%) and 1 out of 10 samples from feral pigeon (10%) .(Miller et al 2009) succeeded in inoculation of three-week-old chickens with NDV wild bird original strain which indicated that this strain may pose a risk to poultry. Moreover, wild birds usually not vaccinated; also, wild birds may have differently immune response against NDV than poultry. Cattle egrets can naturally infect with Newcastle disease as detected by HA test with a titer 22 -24 in their serum (Tan et al 2007) .Moreover three NDV isolates were detected in fecal swabs from Cattle egrets after three blind passages (Guirguis 1983).

Characterization of the isolates wascarried by RT-PCR which considered sensitive and accurate technique for viral identification depending on the partial Fgene sequence (400 bp).homology between the five selected NDV isolates and vaccinal strains showed (90.9-94.9%) identity with NDV chicken/China/, SDWF0712011 and (85.9%) homology to NDV-isolate Hitchner fusion genes, NDV-Lasota-11-fusion and NDV-Clone 30, respectively, and (91 %) to vectormuneNDVasshown in (Fig 3). Phylogenetic alignment revealed that (NDV- CattleEgert -egypt-KFS-1- elhamol2018) and (NDV-sparrows-egypt-KFS- Balteem-2-2018) Clustering of the isolates from cattle egret , sparrow and also from contact chicken farms in NDV Genotype VII (fig 4). The same genotype of NDV obtained from house sparrows was isolated from poultry flocks in southern china (Zhu et al 2010).Although, some NDV strains, from dove or pigeon, with multibasic motif showed mild virulence with in chickens. Some original NDV strains of dove or pigeon may increase by chickens passaging and the virulence increase may related to some amino acid substitutes especially in RNP complex (Dortmans et al 2011). Wild pigeon or dove could be infected by viruses by directly or indirectly contact with pigeons. most of NDV strains from dove or pigeon belongs to genotype VI and a few of these viruses are placed on other genotypes, such as the genotype VII and a new genotype (Tirumurugaan et al 2011).Wild birds were considered the natural reservoirs of NDVs, mostly harbored lentogenic strains, and occasionally carry velogenic strains (Chen et al 2013) In a trail for estimation the efficacy of the commercially produce NDV genotype VII vaccines in protection of the broiler chicks against challenge with NDV genotype VII isolate named Cattle Egert/ Egypt/ KFS- el hamol-1/2018 .the result revealed that the HI titer of the group 4 recorded the highest level of immunity ranging from 2 log2 to 7 log2 and decreased to 5.6 log2 in the day 28 PV when tested against NO 6/1Olive vaccine in combination with A-V11 strain YEBIO . The challenged birds show mild degree of the inflammatory lesions within tracheal tissue, mild heterophilic infiltration. and marked hyperplasia of both epithelium and goblet cells (fig 10) with protection percentage of 99.4% .recent reports confirmed that genotype-homologus vaccines increase the protection against challenge with the

virulent NDV genotype VII and significantly minimize shedding of the virus compared to LaSota vaccine (Yi et al 2011). Because the genetic distances between these isolates and vaccine strains are so long that current, vaccine cannot reduce these viruses shedding in immune chickens (Xiao et al 2012).Since the interaction between poultry flocks and wild birds frequently occur, the wild birds play a critical role in the NDV evolution (Lindh et al 2012). Therefore, surveillance of NDV in wild birds is very important, regardless of controlling outbreaks in poultry farms or identifying circulating NDV.

CONCLUSION

This study ensured that wild birds could play a very important role in the transmission of virulent strain of NDV to poultry farms which cause severe economic loses , so hygienic measures must be done to prevent contact between wild birds and poultry folks to minimize this disease in addition to proper vaccination .

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Author would like to thank Dr. AbdElhalim Hegazy in the animal health research institute for his collaboration during the experiment.

AUTHOR CONTRIBUTIONS

All listed authors have made substantial contributions to the research design, the acquisition, analysis, or interpretation of data; and to drafting the manuscript or revising it critically; and that all authors have approved the submitted version

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