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ORIGINAL ARTICLE

Molecular and pathological characterisation of genotype VII Newcastle disease virus on Egyptian chicken farms during 2016–2018

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ABSTRACT

Newcastle disease virus (NDV) remains a constant threat to the poultry industry even with intensive vaccination programmes. In the present study, 40 samples were collected from farms showing high mortalities in some Egyptian governorates between 2016 and 2018. Tracheal samples were collected for virus isolation and confirmed by real-time RT-PCR. Molecular characterisation was performed by sequencing, followed by phylogenetic analysis of the novel sequences. Histopathological and immunohistochemical examinations were performed on different organs from NDV-infected broilers. The phylogenetic analysis revealed that the NDV isolates from different areas of Egypt were genetically closely related and all belonged to genotype VII. The histopathological hallmarks included haemorrhagic tracheitis, interstitial pneumonia with syncytia formation, haemorrhagic proventriculitis, necrotising pancreatitis, pan-lymphoid depletion, non-suppurative encephalitis and nephritis. Immunological detection of NDV antigen clarified the widespread presence of viral antigen in different organs with severe lesions. The present study confirmed that a virulent NDV of genotype VII became the predominant strain, causing severe outbreaks in poultry farms in Egypt. The presence of viral antigen in different organs indicates the pantropic nature of the virus. Immunohistochemistry was a very useful diagnostic tool for the detection of NDV antigen.

KEYWORDS

NDV genotype VII, velogenic NDV detection, pathology, immunohistochemistry

INTRODUCTION

Newcastle disease virus (NDV) is one of the main poultry pathogens causing a lethal disease that affects mainly chickens and turkeys, resulting in devastating economic losses in poultry production. In Egypt, the endemic occurrence of NDV necessitated intensive vaccination programmes against NDV (Abdel-Glil et al., 2014). Despite intensive vaccination programmes being implemented, outbreaks still frequently occur in vaccinated poultry flocks (Mohamed et al., 2009; Nabila et al., 2014). The phylogenetic analysis of strains from recent outbreaks affecting Egyptian poultry farms revealed the velogenic genotype isolate, clustered with published class II genotype VII sub-genotype d NDVs, closely related to Middle Eastern isolates (Radwan et al., 2013).

In the past few years, the virus has been evolving rapidly and several new genotypes have been discovered, so molecular analysis and further characterisation of NDV field isolates

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would be valuable in order to recognise the type of strains circulating in a particular geographical area (Chaka et al., 2013). Characterisation of these strains is important for estimating field changes, predicting new outbreaks and developing adequate control measures (Susta et al., 2011).

Velogenic NDV induces severe pathological lesions in organs (Ezema et al., 2016), but the correlation between the residence of virus in tissues and the pathological lesions is insufficiently known.

This study characterises the phylogenetic profiles of NDV strains isolated in Egyptian farms with high mortalities, establishes a correlation between the histopathological changes and the immunohistochemical residence of the virus in different tissues, and clarifies the differences in the susceptibility of different breeds to NDV infection.

MATERIALS AND METHODS

Ethics statement

The study procedures were approved by the Institutional Animal Care and Use Committee (IACUC), Cairo University, Egypt (Approval number of ethics committee: CU/II/F/65/17).

Sampling

In a survey conducted between 2016 and 2018, tracheal specimens were collected from 40 vaccinated commercial broiler, Sasso broiler and Balady broiler flocks located in different Egyptian provinces (El Beheria, Damietta, Qaliubia, Dakahlia, North Sinai, Alexandria, Giza, Fayoum, El Menia and Asyut). The farms had a history of mortalities, the chickens were suffering from moderate to severe respiratory and nervous signs (wing or leg paralysis, ataxia and torticollis), and postmortem examination revealed specific lesions of NDV including haemorrhagic tracheitis, lung congestion, airsacculitis, petechial haemorrhages on the tips of the proventricular glands, distended gallbladder, swollen spleen with scattered necrotic foci, as well as ulcers and haemorrhages on the caecal tonsils and swollen kidneys.

Virus isolation in embryonated chicken eggs

Virus isolation from tracheal samples was performed using the described by the OIE (2012). For all samples, the allantoic fluids from inoculated embryonated chicken eggs were subjected to a rapid and quantitative haemagglutination assay (HA). All of the samples showing haemagglutination were confirmed by the haemagglutination inhibition (HI) assay (OIE, 2012).

RNA extraction

Viral RNA was extracted from allantoic fluid using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instruction manual.

Real-time reverse transcription polymerase chain reaction (rRT-PCR)

A rRT-PCR kit (Qiagen, Inc., Valencia CA, USA), with specific primers and probes designed by Wise et al. (2004), supplied by Metabion GmbH (Planegg-Martinsried, Germany), was used. The primers were (F+4839) 5′-TCCGGAGGA-TACAAGGGTCT-3′, (F-4939) 5′-AGCTGTTGCAACCC-CAAG-3′ and the probe was (F+4894) 5′-[FAM] AAGCGTTTCTGTCTCCTTCCTCCA [TAMRA]-3′. rRT-PCR was conducted in the Stratagene 3005P MXpro Real-Time PCR System (Stratagene, CA, USA) according to manufacturer's instructions.

The Mastermix used for real-time RT-PCR was composed of 12.5 µL of 2x QuantiTect Probe RT-PCR Master mix, 0.5 µL (5×10^{-5} µmol) of forward primer, 0.5 µL (5×10^{-5} µmol) of reverse primer, 0.125 µL of probe (3×10^{-5} µmol), 0.25 µL of QuantiTect RT Mix, 4.125 µL of RNase Free Water, and 7 µL of template RNA (QuantiTect probe RT-PCR).

The reserve transcription step was performed at 50 °C for 30 min, while the cycling protocol was 15 min at 95 °C as primary denaturation, followed by 40 cycles of 30 s at 94 °C, 30 s at 52 and 72 °C for 10 s. Six represented rRT-PCR-positive samples were subjected to conventional RT-PCR and sequencing.

Conventional RT-PCR

The used primers were NDV-F330 (5′-AGG AAG GAG ACA AAA ACG TTT TAT-3′) and NDV-R700 (5′-TCA GCT GAG TTA ATG CAG GGG AGG-3′).

Using Thermo Scientific Verso 1-Step RT-PCR Ready Mix Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA), the total reaction mix volume was 50 µL: 1 µL Verso Enzyme Mix, 25 µL of 2X 1-Step PCR ReadyMix, 2 µL Forward primer, 2 µL reverse primer, 2.5 µL RT Enhancer, 12.5 µL RNase-free water and 5 µl template RNA. Thermal cycling RT-PCR conditions included a reverse transcription at 50 °C for 15 min, then an inactivation of reverse transcription enzyme followed by 40 cycles of denaturation at 95 °C for 20 s, 50 °C for 30 s, and 72 °C for 60 s, in addition to the final extension at 72 °C for 10 min using Thermocycler T3 (Biometra, Göttingen, Germany).

The RT-PCR products were subjected to electrophoresis in a 1.5% agarose gel. The DNA bands were excised and purified using QIA quick gel extraction kit (Qiagen Inc., Valencia, CA, USA) according to the user instruction manual.

Sequencing reaction

Purification of the amplified DNA fragments was done using QIA quick gel extraction kit (Qiagen Inc., Valencia, CA, USA) according to the kit instruction manual. The sequencing reaction was done with a Big dye terminator V3.1 cycle sequencing kit (Perkin-Elmer, Foster City, CA, USA) (total volume of 20 µL: 2 µL Big dye terminator v3.1, 1 µL primer, 4 µL purified DNA and 13 µL nuclease-free

Table 1. Number and distribution of NDV-positive rRT-PCR samples in different breeds among different provinces

Provinces	Number of samples	Breed			rRT-PCR-positive samples	% of positive samples
		Broiler	Sasso	Balady		
Alexandria	1	1	–	–	1	100
El Beheira	1		1	–	1	100
North Sinai	1	1		–	1	100
Damietta	1	1	–	–	1	100
Dakahlia	2	1	–	1	1	50
Qaliubia	8	2	2	4	5	62.5
Giza	9	3	6	–	2	22.2
Fayoum	4	1	2	1	1	25
El Menia	11	9	2	–	3	27.3
Assyout	2	–	–0	2	2	100

water) using Applied Biosystems 3,130 genetic analyzer (Thermo Fisher Scientific, MA, USA).

A BLAST analysis was conducted for each sequence (//www.ncbi.nlm. nih.gov/BLAST). Sequence comparisons and phylogenetic relationships through a bootstrap of 1,000 trials were determined with the MEGA version 10 using the Clustal W alignment algorithm and the evolutionary history was inferred using the Neighbour-Joining method (Saitou and Nei, 1987). All obtained partial F gene sequences of genotype VII NDV were submitted to GenBank.

Histopathological studies

At postmortem examination of dead chickens from the 40 broilers farms, tissue specimens from different organs were collected, preserved in 10% neutral buffered formalin and routinely processed, sectioned and stained with haematoxylin and eosin (HE) stain (Suvarna et al., 2013). Tissue sections were examined in an Olympus BX43 light microscope and captured using an Olympus DP27 camera linked to cellSens dimensions software (Olympus).

Immunohistochemical studies

Hyperimmune serum against NDV was raised in rabbits using a series of injections following the schedule described by Samiullah et al. (2006). Antibody purification was performed using Magne™ Protein G Beads for Antibody Purification (Promega Corporation, Madison, Wisconsin, USA) according to the manufacturer's instructions. Tissue sections on Poly-L-Lysine-coated slides were deparaffinised and rehydrated as usual, heat-induced antigen retrieval was performed, blocking of non-specific protein binding and endogenous peroxide was followed by overnight incubation in primary antibody (rabbit anti-NDV Ig as mentioned previously), then incubated with horseradish peroxidase-conjugated goat polyclonal secondary antibody to rabbit Ig (SM802 EnVision™ FLEX/HRP). Colour was developed with 3,32-Diaminobenzidine (DAB) substrate (DM827 EnVision™ FLEX DAB + chromogen) (Burns, 2005).

RESULTS

Virus isolation and frequency

All tested samples were subjected to virus isolation by inoculation into SPF embryonated chicken eggs and the collected allantoic fluid was tested by HA and NDV HI tests. Detection of vNDV using rRT-PCR showed only 18 positive samples out of the 40 samples used for virus isolation (Table 1).

Viral sequences and genomic analysis

The six positive samples selected for sequencing and phylogenetic analysis of the partial sequence F gene showed that the isolated viruses belonged to genotype VII. The sequences of the vNDV isolates were deposited in the GenBank under accession numbers MK568510, MK568511, MK568512, MK568513, MK568514 and MK568515 (Fig. 1).

The analysis showed that our isolates are closely related to Egyptian isolates recorded between 2015 and 2017 from different provinces in Egypt. The vNDV isolates in this study were 97.1–100% identical to each other based on nucleotide sequences and 99–100% identical based on amino acid sequences. Compared to other recent Egyptian strains isolated during 2015–2017, the identity ranged between 96.8–99.3% and 98.1–100% on nucleotide and amino acid levels, respectively (Table 2).

Histopathology

The microscopic examination of tracheal sections revealed tracheitis with variable inflammatory reactions in the studied cases. Acute severe haemorrhagic and fibrinous tracheitis was detected (Fig. 2a). The pulmonary lesions included severe depletion of bronchus-associated lymphoid tissue (BALT) in secondary bronchi in which the lymphocytes showed abundant eosinophilic cellular and karyorrhectic debris (lymphocytolysis and lymphorrhexis). Interstitial pneumonia was more prevalent in infected flocks. In complicated incidental cases, the lesion was associated with the presence of caseous material in the parabronchi in which syncytial cells were detected (Fig. 2b). Moreover, acute

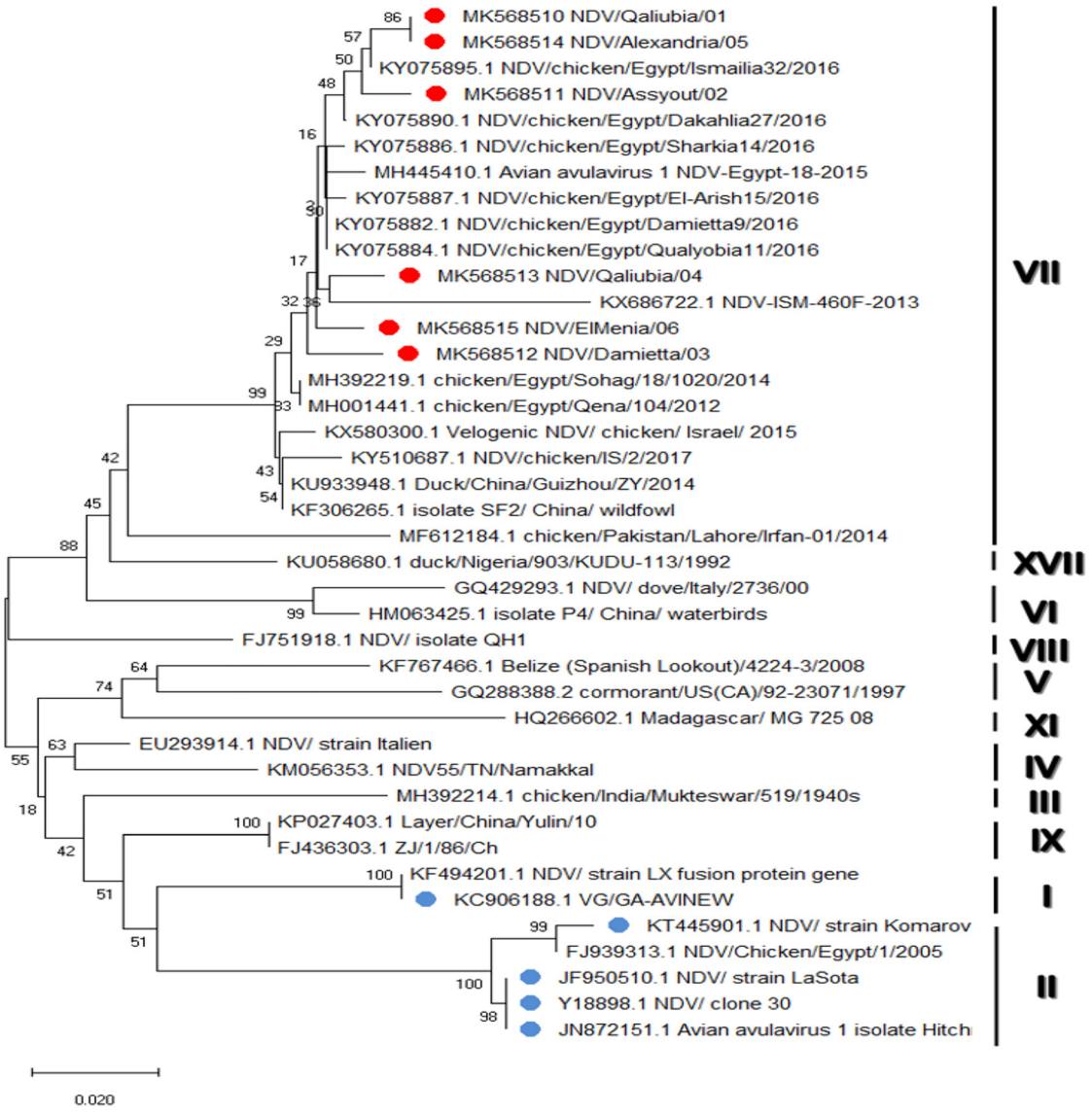

Fig. 1. Phylogenetic analysis of the partial F-gene sequence of isolated vNDV strains (red circle) and vaccine strains (blue circle). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. This analysis involved 39 nucleotide sequences. Evolutionary analyses were conducted in MEGA X (Saitou and Nei, 1987)

fibrinous bronchopneumonia was evident in some cases. Microscopic examination revealed diffuse fibrinous exudate admixed with heterophil granulocytes in the bronchial wall extending to the interstitial tissue separating the parabronchus and the pleura.

Most studied cases showed haemorrhagic proventriculitis that extended to the junction between the oesophagus and the proventriculus (Fig. 2c) with ulceration of the epithelial layer on the mucosal surface and mononuclear inflammatory cellular infiltration admixed with red blood cells (RBCs) in the submucosal layer. The reaction was extended to the proventricular glands associated with diffuse infiltration of heterophil granulocytes.

Necrotising pancreatitis and peripancreatitis were evident, the pancreatic acini showed focal apoptosis to diffuse necrosis of the acinar epithelium with infiltration of mononuclear inflammatory cells in the necrotic tissue (Fig. 2d).

Concerning liver lesions, mild acute portal hepatitis characterised by portal congestion and infiltration of the portal area with mononuclear cells admixed with heterophil granulocytes as well as hepatocellular necrosis were seen. Few examined cases revealed cytoplasmic vacuolation of hepatocytes and formation of Kupffer cell granuloma (Fig. 2e).

In the immune organs (thymus, caecal tonsils, spleen and bursa), there was severe lymphocytic depletion. Additionally, thymic lobular atrophy with reduction of cortical width and hyperplasia of the reticular cells were noticed (Fig. 2f). Fibrinoid necrosis of the splenic red pulp associated with hyperplasia of reticular cells was detected (Fig. 2g). The mucosal surfaces of the caecal tonsils showed necrosis with

Table 2. Nucleotide and amino acid identities of the isolated ND strains in this study in comparison to recent Egyptian isolates and vaccinal strains commonly used in Egypt

	Virus isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	MK568510 NDV/Qaliubia/01	ID	98.4%	97.1%	97.4%	100%	97.7%	99.3%	98.7%	96.8%	98.1%	86.7%	86.7%	85.2%	86.7%	87.4%	
2	MK568511 NDV/Assyout/02	99%	ID	97.4%	97.1%	98.4%	97.4%	99%	98.4%	97.1%	97.7%	86.1%	86.1%	85.2%	86.1%	87.4%	
3	MK568512 NDV/Damietta/03	99%	100%	ID	97.7%	97.1%	98.1%	97.7%	98.4%	97.1%	97.7%	86.4%	86.4%	85.5%	86.4%	87.7%	
4	MK568513 NDV/Qaliubia/04	99%	100%	100%	ID	97.4%	98.4%	98.1%	98.7%	96.8%	98.1%	87.4%	87.4%	85.8%	87.4%	88%	
5	MK568514 NDV/Alexandria/05	100%	99%	99%	99%	ID	97.7%	99.3%	98.7%	96.8%	98.1%	86.7%	86.7%	85.2%	86.7%	87.4%	
6	MK568515 NDV/ElMenia/06	99%	100%	100%	100%	99%	ID	98.4%	99%	97.1%	98.4%	87.7%	87.7%	86.7%	87.7%	88.9%	
7	KY075895.1 Ismailia32/2016	99%	100%	100%	100%	99%	100%	ID	99.3%	97.4%	98.7%	86.7%	86.7%	85.2%	86.7%	88%	
8	KY075884.1 Qualyobia11/2016	99%	100%	100%	100%	99%	100%	100%	ID	98.1%	99.3%	87.4%	87.4%	85.8%	87.4%	88.6%	
9	KY510687.1 NDV/IS/2/2017	98.1%	99%	99%	99%	98.1%	99%	99%	99%	ID	97.4%	86.7%	86.7%	85.2%	86.7%	87.4%	
10	MH445410.1 Egypt-18-2015	99%	100%	100%	100%	99%	100%	100%	100%	99%	ID	86.7%	86.7%	85.2%	86.7%	88.3%	
11	JF950510.1 NDV/strain LaSota	94.3%	95.2%	95.2%	95.2%	94.3%	95.2%	95.2%	95.2%	94.3%	95.2%	ID	100%	98.1%	100%	90.5%	
12	Y18898.1 NDV/clone 30	94.3%	95.2%	95.2%	95.2%	94.3%	95.2%	95.2%	95.2%	94.3%	95.2%	100%	ID	98.1%	100%	90.5%	
13	KT445901.1 NDV/Komarov	92.4%	93.3%	93.3%	93.3%	92.4%	93.3%	93.3%	93.3%	92.4%	93.3%	98.1%	98.1%	ID	98.1%	89.3%	
14	JN872151.1 isolate Hitchner	94.3%	95.2%	95.2%	95.2%	94.3%	95.2%	95.2%	95.2%	94.3%	95.2%	100%	100%	98.1%	ID	90.5%	
15	KC906188.1 VG/GA-AVINEW	93.3%	93.3%	93.3%	93.3%	93.3%	93.3%	93.3%	93.3%	92.4%	93.3%	96.2%	96.2%	94.3%	96.2%	ID	Nucleotide identity %
		Amino acid identity %															

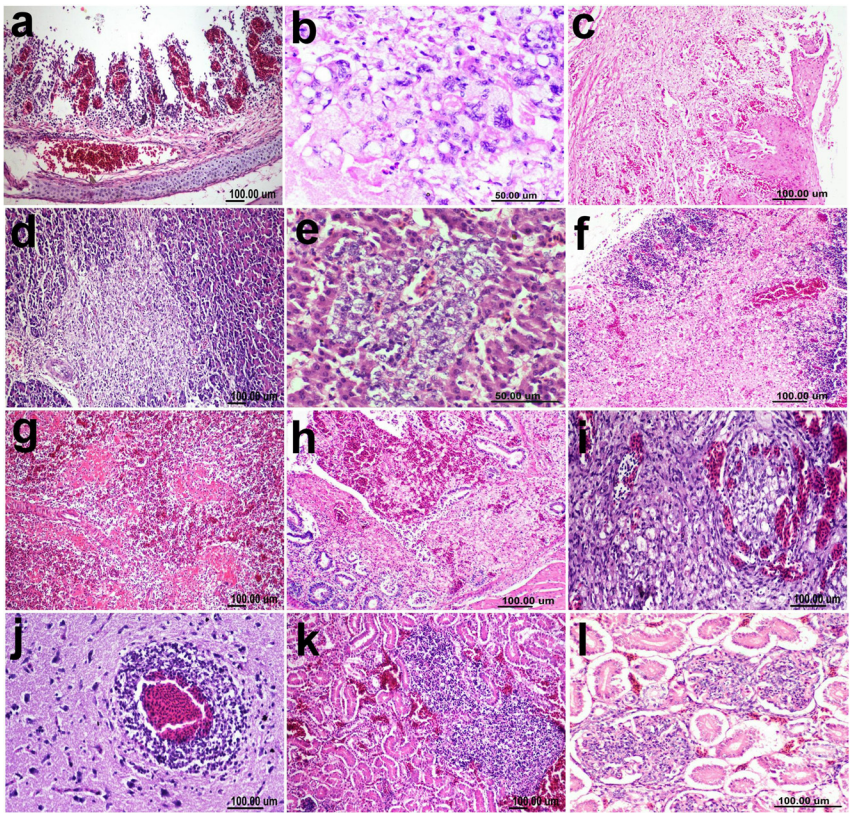

Fig. 2. (a) Trachea of commercial broiler showing ulceration of tracheal mucosa, exudation of red blood cells admixed with lymphocytes, congestion and oedema of the underlying submucosa. (b) Lungs of Sasso broiler showing the presence of syncytial cells within the bronchial lumen. (c) Proventriculus of Sasso broiler showing massive haemorrhage at the junction between oesophagus and proventriculus. (d) Pancreas of commercial broiler showing necrosis of pancreatic acini replaced by eosinophilic cytoplasmic and nuclear debris as well as mononuclear cell infiltration. (e) Liver of commercial broiler showing focal Kupffer cell micronodule (microgranuloma) formation with pyknosis of hepatocyte nuclei. (f) Thymus of Sasso broiler showing severe cortical atrophy associated with marked lymphocyte depletion and congestion. (g) Spleen of commercial broiler showing noticeable paucity of lymphocytes observed due to marked lymphocytic necrosis. (h) Caecal tonsils of Sasso broiler showing severe mucosal necrosis with red blood cell exudation and inflammatory infiltrates the lamina propria. (i) Bursa of Fabricius of commercial broiler showing haemorrhagic bursitis with congestion of the inter- and intrafollicular blood vessels. (j) Cerebral cortex of commercial broiler showing perivascular lymphocytic cuffing with congestion of blood vessel. (k) Kidney of commercial broiler showing interstitial mononuclear inflammatory cell infiltration with necrosis of the renal tubular epithelium associated with oedema, haemorrhage and congestion of peritubular capillary sinuses. (l) Kidney of commercial broiler showing proliferative glomerulopathy characterised by glomerular hypercellularity and obliteration of Bowman's spaces by mesangial cell proliferation

exudation of free RBCs and inflammatory reaction (mononuclear inflammatory cellular with heterophil granulocytic infiltration) (Fig. 2h) in the lamina propria. Individual cases showed haemorrhagic bursitis (Fig. 2i).

The microscopic picture of virus-induced meningoencephalitis was evident in NDV-infected flocks. Concerning histopathological lesions in the cerebrum and cerebellum, the lesions included perivascular lymphocytic cuffing, vasculitis (Fig. 2j), capillary endothelial proliferation, neuronal necrosis with neuronophagia and focal to diffuse gliosis. Additionally, the cerebellar nuclei (the dentate nucleus) showed chromatolysis. Areas of malacia involving the medulla oblongata were evident in individual cases, the lesion was microscopically characterised by necrosis of heterophil granulocytes with glial cell proliferation, mainly microgliosis admixed with astrocytes, as well as vasculitis and microthrombosis of the cerebral blood vessels.

The main lesions detected in the kidney were interstitial nephritis and glomerulonephritis. Interstitial nephritis of acute severity was observed, the lesion was characterised microscopically by multifocal infiltration of mononuclear cells associated with interstitial oedema, haemorrhage and congestion of peritubular blood capillary sinuses (Fig. 2k).

Glomerulonephritis of proliferative nature was evident, the lesion was characterised by glomerular hypercellularity with the obliteration of Bowman's space by proliferating mesangial cells and infiltrating leukocytes (Fig. 21).

Percentage distribution of histopathological lesions in different breeds

The percentage distribution of lesions in different breeds is summarised in Fig. 3. Sasso broilers exhibited a higher percentage of haemorrhagic tracheitis (60%), while Balady broilers showed the lowest percentage of affected birds

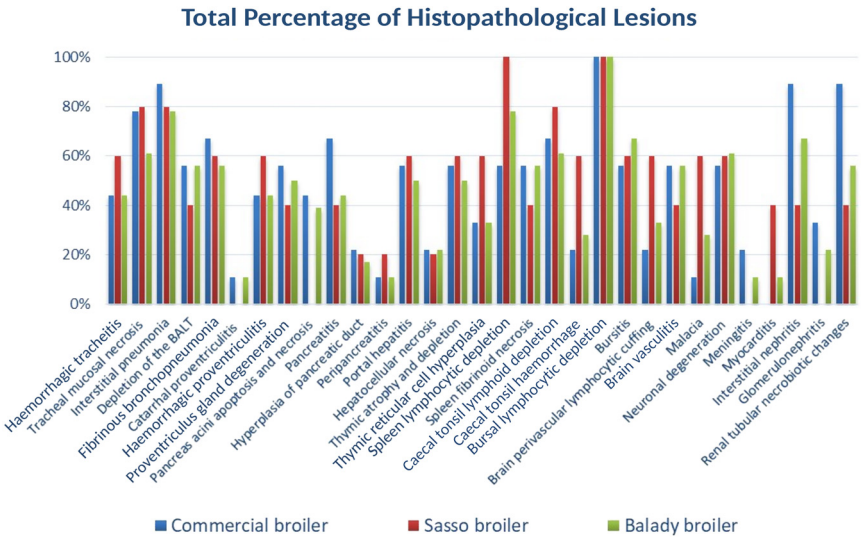

Fig. 3. The percentage of histopathological lesions in positive samples ($n = 18$) in different breeds: commercial broiler ($n = 9$), Sasso broiler ($n = 5$) and Balady broiler ($n = 4$)

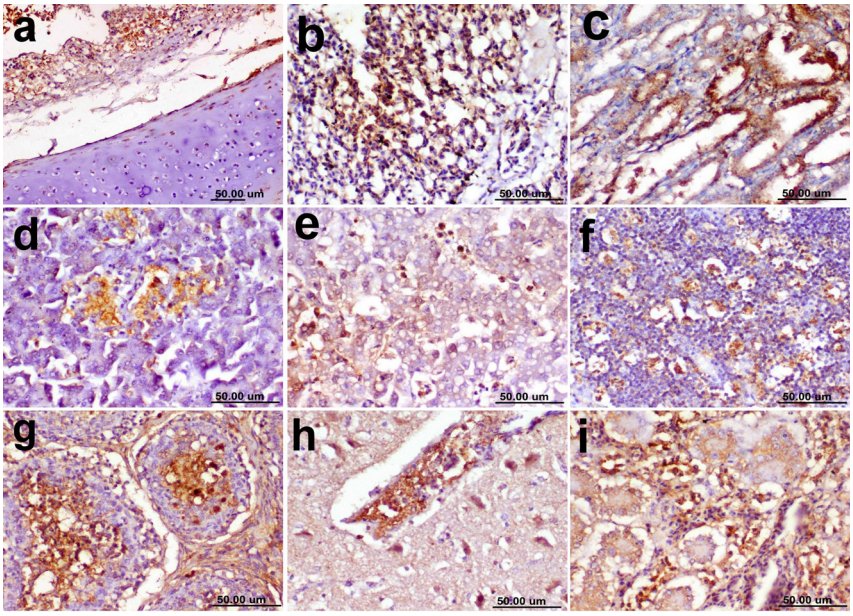

Fig. 4. Immunohistochemistry of different organs showing positive expression of NDV antigen in (a) tracheal mucosa, (b) lung air capillaries, (c) glandular epithelial lining of the proventriculus, (d) the exocrine acini of the pancreas, (e) Kupffer cells and monocytes in the liver, (f) depleted lymphocytes comprising the cortical zone of the thymus, (g) depleted lymphocytes within the medulla of atrophied bursal follicles, (h) the lymphocytes infiltrating the blood vessel wall, vascular endothelium and degenerated neurons of the cerebral cortex, and (i) degenerated renal tubules of the kidney

(25%). Concerning lung pathology, commercial broilers demonstrated a higher percentage of all pulmonary lesions while Balady broilers showed the lowest percentage except lesions related to the depletion of BALT that showed the highest incidence in Balady broiler (75%). In the proventriculus, most lesions were encountered in commercial broilers, followed by Sasso broilers, while the Balady broiler was the least affected breed.

As regards pancreatic necrosis, Balady broilers showed the highest incidence (75%), while pancreatitis occurred most frequently among commercial (67%) and Sasso broilers (22%). Sasso broilers showed the highest incidence of peripancreatitis (20%). As for liver changes, Sasso broilers exhibited a high incidence of hepatitis (60%), while hepatocellular necrosis was detected more frequently in Balady broilers (25%).

The pathological changes of lymphoid organs showed the highest incidence among Sasso broilers, while in Balady broiler such lesions were less commonly detected. On the other hand, vasculitis (75%) and neuronal degeneration

(75%) in the brain showed the highest incidence in Balady broilers, while perivascular cuffing was more frequently encountered in Sasso broilers (60%). Regarding the pathological changes of kidneys, a higher incidence was recorded in commercial broilers, followed by Sasso broilers.

Immunohistochemistry

ND antigen was detected in the different tissues with strong positive peroxidase reaction involving the trachea (Fig. 4a), lung tissue (Fig. 4b), proventriculus (Fig. 4c), pancreas (Fig. 4d), liver (Fig. 4e), lymphoid tissues (Fig. 4f, g), brain (Fig. 4h) and kidneys (Fig. 4i).

DISCUSSION

Newcastle disease is an economically important disease of poultry for which vaccination is applied as a preventive measure in many countries to alleviate the detrimental effect of NDV on the growth rate and the efficiency of weight gain (Wang et al., 2015).

In the present study tracheal samples were used for viral isolation because virus isolation rate was found to be higher from tracheal samples than from cloacal swabs and serum. This could be attributed to the presence of maximum virus load in the trachea during the course of infection (Haque et al., 2010).

The present study confirmed that the serological tests (HA and HI) and RT-PCR were as accurate, precise and quick diagnostic and pathotyping tools of ND as described by Manzoor et al. (2013).

The data obtained in this study by sequencing and phylogenetic analysis confirmed the high circulation of genotype VII ND causing severe outbreaks in poultry farms, highlighting the role of illegal transport of live animals and their products in disease transmission because of the lack of governmental control as described previously by Brown and Bevins (2017). In Egypt, virulent NDV class II genotype II, VI and VII had been frequently isolated and clustered in 1947, while genotype VII was assumed to be predominant among chickens inducing outbreaks in Egyptian commercial poultry farms (Mohamed et al., 2009; Radwan et al., 2013).

The incidence rate of histopathological lesions observed in different breeds indicates that susceptibility to infection could vary by breed, and this was clearly noticed in the Balady breed which generally showed less pathological alterations when compared to the other breeds. This factor was previously discussed in various studies focussing on the resistance and susceptibility of different breeds to infectious diseases and stressful conditions (Hassan et al., 2004).

Haemorrhage occurred in the trachea, proventriculus, caecal tonsils and kidneys due to vascular damage induced by viral replication in the endothelial cells which was confirmed by strong expression of the viral antigen in the vascular endothelium, leading to vascular damage with subsequent increased vascular permeability resulting in haemorrhage and oedema. These findings are consistent with the findings of Alexander et al. (2003). In addition, Galindo-Muniz et al. (2001) reported that thrombocytopenia and endothelial damage are considered the probable causes of haemorrhage in ND infection. Moreover, Julian et al. (1996) mentioned that endotheliotropic viruses affect endothelial cells causing vasculitis that eventually leads to necrosis.

The inflammatory reaction observed in various organs was characterised by lymphocytic and mononuclear cell infiltration indicative of virus replication in the infected tissue and direct replication in the macrophages as confirmed by immunohistochemistry, which is consistent with the findings of Kommers et al. (2002) and Manzoor et al. (2013).

Syncytial cell formation in the respiratory epithelium recorded in our results is considered a confirming characteristic feature of NDV infection as previously mentioned by Dortmans et al. (2011). In the present study, the massive mononuclear cell infiltration in the air capillaries was previously explained by Nakamura et al. (2001) who described a 'virus-associated haemo-phagocytic syndrome' due to severe proliferation of lung macrophages.

In present study, proventriculitis, degenerative and necrotic changes as well as mononuclear and heterophil granulocytic infiltrations were commonly recorded lesions in the proventriculus. Sun et al. (2008) attributed those lesions to the detrimental role of mast cells due to their ability to release a wide variety of pro-inflammatory mediators which enhance the recruitment of lymphocytes and macrophages. Proliferative glomerulopathy mentioned in our results was less commonly detected in previous studies. It was characterised by the hypertrophy and hypercellularity of glomeruli, and was confirmed by demonstration of the viral antigen within the glomeruli by immunohistochemistry.

The severe lymphocytic depletion in the lymphoid tissues found in this study might reflect some degree of immune depression leading to other infections as previously reported by Bergfeld et al. (2017). Immunohistochemical detection of NDV in the lymphocytes and macrophages may indicate that infected macrophages play a role in inducing the apoptosis of non-infected cells, through immune signalling as suggested by Harrison et al. (2011).

As regards the brain, the neuropathology of ND is peculiar. Malacia and neuronal degeneration observed in damaged areas of the brain are attributed to vasculitis and thrombosis. The virus induces hypoxia and ischaemia in the brain which has a direct effect on the endothelial cells of capillaries, leading to their elongation and proliferation. The presence of the virus antigen in vascular endothelial cells was confirmed by IHC. These results support those reported by Dombrowski et al. (2008).

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