Research Article

Virologic Detection and Molecular Characterization of Rabies Virus in Delta Region, Egypt

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Abstract | Rabies is a fatal worldwide viral disease and contributes to a great economic load in Egypt through the death of animals and control strategy costs. Due to Egypt's privileged location and friction with neighboring countries and far countries from Europe and Asia throughout the ages, the current research goal is to evaluate the extent of their influence on nucleotide and amino acids variation of rabies virus isolates from the brain tissues of three dogs from 2017 to 2019 at the genetic level of the N and P genes from three governorates of Delta region in Egypt with earlier isolates, locally used vaccinal strains and other used strains internationally. Ten brain tissues from confirmed rabid cases in three governorates; Dakahleya (3), Alexandria (4); and Beheira (3) were diagnosed using viral cell culture and Direct Fluorescent Test (DFT) for the rabies virus. The samples were tested positive by DFT and isolated on baby hamster kidney cell culture. Of them, three confirmed rabies samples were picked to determine their N and P genes by sequencing further (at the level of nucleotide and amino acid). The N and P genes in this study isolates have a great similarity to previous isolates from Egypt as well as adjacent bordering countries. At the N and P genes level, the isolates bear little resemblance to the vaccinal strains (Evelyn-Rokitnicki-Abelseth, Pasteur, the China human rabies vaccine strain AG and others at this study) due to the presence of mutation sites. As the P gene plays a role in viral pathogenicity, the detected variance in nucleotide and amino acid identities of the P gene in our local circulating isolates over the years confirmed the anticipation of distinction in the pathogenicity of such isolates. All vaccinal strains have a genetic distance from field Dakahleya, Beheira, and Alexandria isolates (ranging from 88.2% to 88.9% for the China human rabies vaccine AG):Such results of nucleotide identity among Egyptian isolates over the years confirm the unity of their source. The application of strict quarantine measures on imported rabies vaccinated pets in addition to prospective studies on the pathogenicity of local circulating isolates regarding the N and P genes are strongly recommended in the control strategy.

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Introduction

The rabies virus (RABV) is a member of the *Lyssavirus* genus, which is part of the *Rhabdoviridae* family. Lyssaviruses have seven identified genotypes (G.T.) and unclassified G.T.s. Except for Antarctica and a few islands, the RABV GT1 isolates are distributed worldwide (Nadin-Davis, 2007), Tordo *et al.* (1986) defined the viral genome as a non-segmented single-stranded negative-sense RNA of about 12 kb that encodes five genes: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA polymerase (L). Because the N gene in the entire genome of the rabies virus is highly conserved and can be easily produced, it is commonly employed as a judging index for rabies virus genotyping and population variance (Dhaval *et al.*, 2017).

There are seven recognized genotypes of lyssavirus defined on the basis of their genetic similarity: rabies virus (genotype 1), Lagos bat virus (genotype 2), Mokola (genotype 3), Duvenhage virus (genotype 4), European bat lyssavirus type 1 (genotype 5), European bat lyssavirus type 2 (genotype 6) and Australia bat lyssavirus (genotype 7). Additionally, four novel genotypes: Aravan virus, Khujand virus, Irkut virus, and West Caucasian (Botvinkin *et al.*, 2003) were recovered from bats in Eurasia. Genotype 1 is responsible for classical rabies in terrestrial mammals throughout the world.

The molecular epidemiology of rabies in Africa was described into three phylogenetic lineages of RABV: Africa 1, 2, and 3 (Kissi *et al.*, 1995).

The Africa 1 and 2 lineages were isolated from dogs or humans bitten by rabid dogs, whereas the Africa 3 lineage was found associated with mongoose species, principally the yellow mongoose (*Cynictis penicillata*) from South Africa. The Africa 1 lineage was subdivided into two subgroups: 1a, restricted to North and West Africa, and 1b; limited to Southeast Africa. In general, the Africa 1 lineage was the most similar to current Eurasian RABV lineages, suggesting its recent introduction to Africa (Kissi *et al.*, 1995). The Africa 2 lineage includes wild-type strains that originated from several central and eastern African countries and is phylogenetically ancestral to the cluster that includes the Eurasian and Africa 1 RABVs. The Africa 3 lineage, of mongoose origin, is distant from all dog RABV variants.

Seven clades (I–VII) were identified in Israel and one (Africa 4) in Egypt. This novel clade, Africa 4 was placed ancestral to the cluster of RABV variants that were associated with the Middle East and the middle latitudes of Eurasia, and to Africa 1. This clade is part of the cosmopolitan canine RABV lineage that is believed to have originated in Europe and widely disseminated as a consequence of colonial activity during the 16th to 19th centuries (Smith *et al.*, 1992). Molecular studies were done on Egyptian isolates from different localities as rabid horses from Cairo in 2014 (Yanni *et al.*, 2014), water buffalos from Menofeya in 2015 (Mohamed *et al.*, 2015), and rabid cattle and fox from Elwadi Elgedid in 2021 (Michael *et al.*, 2021). Imported bat from Egypt or Togo to France was diagnosed to be infected with LBV (Lagos bat virus) (Jessica *et al.*, 2021).

The N and P genes are involved in and facilitate the pathogenicity of RABV Gene (Wei *et al.*, 2018). The rabies virus phosphoprotein P5 binding to the BECN1 (Beclin1) ring benefits RABV replication by inducing BECN1 signaling pathway-dependent incomplete autophagy, potentially targeting antiviral drugs against RABV (Lui *et al.*, 2020).

Because of the P proteins ability to bind the signal transducer and activator of transcription (STATs) via the C-terminal domain (CTD) and residues 186–297 of RABV P protein (Brzozka *et al.*, 2005; Vidy *et al.*, 2005; Lieu *et al.*, 2013), P-proteins are considered major interferon (IFN) antagonist of lyssaviruses (Mavrakis *et al.*, 2004; Assenberg *et al.*, 2010). The average reported human rabies cases is around 50 cases per year, 70% of them didn't receive post exposure prophylaxis, mostly in rural areas at the world rabies day celebration 2021 (https://www.fao.org/egypt/news/detail-events/en/c/1441644/).

P gene aids rabies virus replication in muscles by preventing interferon (Satoko *et al.*, 2013). In this current study, it was reviewed the national and regional available data to describe simply the epidemiology of rabies in Egypt focusing on the delta region to present the current state of the rabies control efforts and to determine the nucleotide variation of the N and P genes between the local Egyptian street isolates and the vaccinal stains.

Materials and Methods

Samples

Ten suspected rabid individuals brain tissues of dogs and cattle from the governorates of Alexandria, Dakahleya, and Beheira were euthanized after showing nervous signs (Table 1).

Table 1: *Table showing the full data of examined samples at this study.*

S.	Locality	year	Species	Age	Clinical signs	gender
1	alex	2019	cattle	1y	+	Not specified (n.s.)
2	alex	2019	dog	8m	+	n.s.
3	alex	2019	dog	1y	+	n.s.
4	alex	2019	dog	2y	+	n.s.
5	dakahleya	2018	dog	2y	+	n.s.
6	dakahleya	2019	cattle	4y	+	n.s.
7	dakahleya	2017	cattle	1y	+	n.s.
8	beheira	2018	cattle	3y	+	n.s.
9	beheira	2017	cattle	2y	+	n.s.
10	beheira	2016	cattle	1y	+	n.s.

Methods

The samples were sent to the Biosafety Level-2 Rabies Laboratory of the Animal Health Research Institute (AHRI), Giza, Egypt for rabies diagnosis using Fluorescent Antibody Technique (FAT). The samples were crushed and suspended in 10% MEM (Minimum Essential Medium) for virus isolation on BHK cells via rapid rabies antigen test and molecular research applying PCR and nucleotide sequencing of N and P genes.

Rapid rabies antigen test from China (Zrbio and batch number; 2018061212).

Fluorescence antibody technique (FAT)

Using a rabies polyclonal conjugate supplied by ARC-Onderstepoort Veterinary Institute Batch Number (N4-22), impressed smears from all evaluated cases were analyzed for the presence of rabies antigen (OIE, 2018).

Isolation in tissue culture

Supernatants from 10% crushed brain samples were seeded on monolayers of Baby Hamster Kidney (BHK-21/C-13) cells and monitored at 37°C/72h with 5% CO_2. Three passages of 48 h each were performed according to the techniques specified by (Kanitz *et al.*, 2015). FAT was used to verify the isolates. FAT was performed in a tissue culture chamber slide in which the isolates were fixed in 80% acetone, stained by the conjugate at 37◦/1h, washed 3 times for a duration of 10 min each using phosphate buffer saline and examined under the fluorescent microscope after applying a mounting buffer.

Extraction of nucleic acids

The QIAamp viral RNA mini kit batch number 1151234567 was used to extract RNA from three selected positive samples (Qiagen, Germany, GmbH). For 10 minutes, 140µl of the sample suspension was incubated at room temperature with 560µl of viral lysis buffer (AVL) and 5.6µl of carrier RNA. 560 µl of 100 percent ethanol was added to the lysate after incubation. Following the manufacturer's instructions, the sample was washed and centrifuged. The nucleic acid was eluted using 60 µl of the kit's elution buffer (QIAamp Viral RNA Mini Kits, 2013).

To amplify a 684-bp fragment of the N gene of RABV, the primer set (5′-ATGGATGCCGACAA-GATTGT-3′) and (5′-CCCACT CTGATTGCC GAATA -3′), and to amplify a 882-bp fragment of P gene of RABV, the primer set (5′-TTGGTAC-CATCCCAAGTATGA-3′) and (5′-TCT TGCAT-GATTTT GCTTAGCTTGTC-3′) were designed based on (Shoji *et al.*, 2004). The oligonucleotide primers were obtained from Metabion Co., Germany.

PCR amplification

In a 25 µl reaction, 12.5 µl of Quantitect probe rt-PCR buffer (Qiagen, Gmbh), 1 µl of each primer at 20 pmol concentration, 0.25 µl of rt-enzyme, 4.25 ml of water and 6 µl of template were used. An applied biosystems 2720 thermal cycler was used to carry out the reaction. Reverse transcription was performed at 50°C for 30 minutes, followed by a 5-minute primary denaturation phase at 95°C, followed by 35 cycles of 94°C for 30 seconds (annealing: 50°C for 40 seconds for P and N genes) and 45 seconds at 72°C, final extension phase was performed at 72°C for 10 min, (Shoji *et al.*, 2004).

The PCR products were separated using 5V/cm gradients on a 1.5 percent agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature. 15 µl of the products were put into each gel slot for analysis. The sizes of the fragments were determined using the gene ruler 100bp ladder

(Fermentas, Thermo, Germany). A gel documentation system (Biometra, Germany) was used to photograph the gel and the data was processed using computer software. The product of three positive PCR samples was sequenced using a big dye terminator V3.1 sequencing kit on a genetic analyzer 3500 (life technology). Bioedit and CLC's Main Workbench 6 software (2022) are used for the analysis. Bioedit software version 6 based on Clustal W alignment tool has been used to create alignments for each gene containing representative sequences for the different related strains.

A BLAST® analysis (Basic Local Alignment Search Tool) (Altschul *et al*., 1990) was initially performed to establish sequence identity to GenBank accessions. Then, the N and P gene fragments were aligned using Bioedit 6 software (Hall, 1999), and the phylogenetic tree was created using Mega 7 software (Kumar *et al*., 2016).

Results and Discussion

All ten tested brain tissue samples were positive for the presence of rabies antigen (Ag) using the rapid Ag test kit (Figure 1). Using the fluorescent antibody technique, impression smears, and the isolates on BHK cells tested positive for the presence of rabies Ag (Figure 2A). Positive and negative controls (Figure 2B, D) were included.

Figure 1: *Positive brain sample using the rapid antigen test kit C control line /S positive sample line.*

At the level of the N gene, the three recent and old Egyptian isolates in this study originated from the same clade with a high bootstrap (Table 2, Figure 5A). Amino acids analysis of Dakahleya 18 and Alex 19 N protein demonstrated their great resemblance with that of the old isolate CA 99 and their difference from that of the Beheira isolate due to the presence of mutation loci (Table 2, Figure 5B).

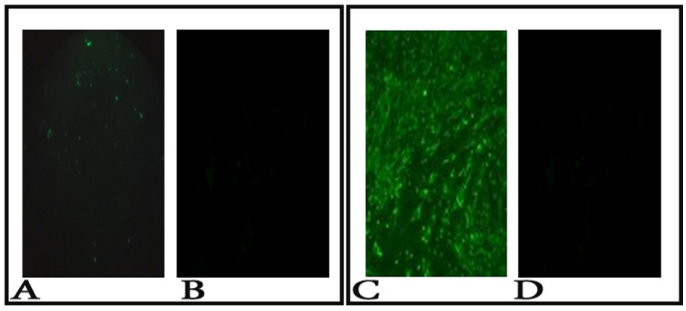

Figure 2: *A: Positive fluorescence on brain impression smear 100x (dust like appearance of apple green color); B: No fluorescence at a negative control brain impression smear; C: Positive fluorescence sample on BHK cells100x (intracytoplasmic fluorescence); D: No fluorescence at a negative control BHK cells.*

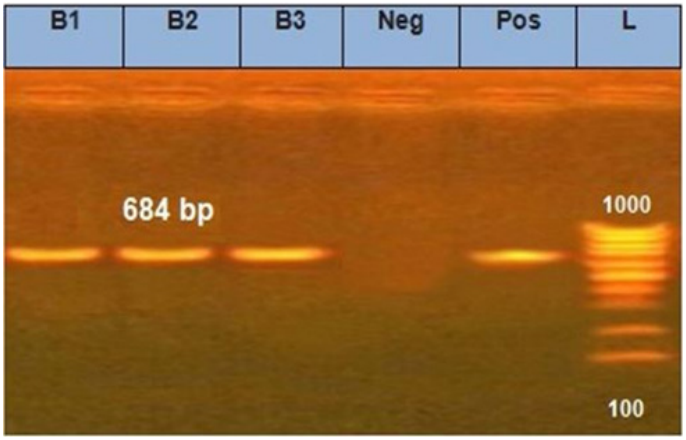

Figure 3: *The electrophoretic pattern of PCR products (684 bp) to the brain samples (B1, B2, B3) for N gene.*

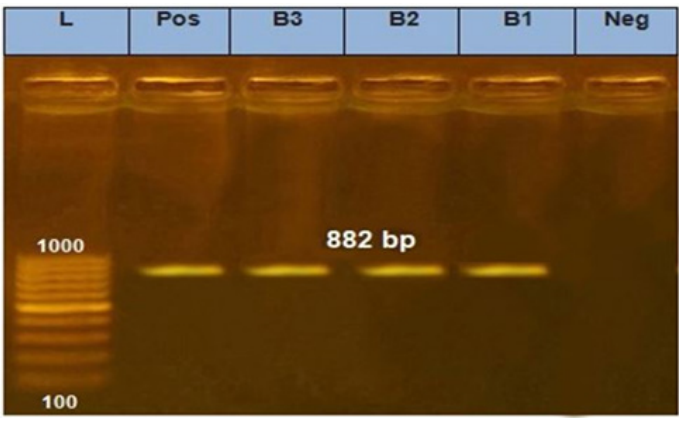

Figure 4: *The electrophoretic pattern of PCR products (882 bp) to the brain samples (B1, B2, B3) for the P gene.*

The % of N nucleotide identity here between Dakahleya isolate and many Egyptian isolates was 99% with (CA98, CA99, and Fay99), 99.1% with (Beheira18), 99.4% with (Alex19), 97.6 % with (Wadi19), and 95.9 % with (Wadi19) (egy79). Alex and Beheira isolates produced a strong correlation (Figure 6).

Table 2: *The description of isolates from NCBI used for comparison in this study at the level of N gene.*

No	Access no	Host	Isolation year	Country	Variant	Abbreviation	% of identity		
							BEH18	ALEX19	DAK18
1	DQ837463.1	DOG	1999	Egypt (Cairo)		CA99	99	99	98.7
2	DQ837462.1	DOG	1999	Egypt (Fayoum)		Fay99	99	99	98.7
3	DQ837461.1	DOG	1998	Egypt (Cairo)		CA98	99	99	98.7
4	MT036064	FOX	2017	Egypt (Wadi)		Wadi17	97.3	97.6	97.3
5	MT036065	CATTLE	2018	Egypt (Wadi)		Wadi18	97.3	97.6	97.3
6	MT036066	CATTLE	2019	Egypt (Wadi)		Wadi19	97.6	97.9	97.6
7	U22627.1	HUMAN	1979	Egypt		Egy79	95.6	95.9	95.9
8	KY12454.1	DOG	2014	Chad		Chad14	89.2	89.2	88.9
9	EV514580		2006	Mauritania		Mor06	90.4	99.4	90.1
10	U22634.1	DOG	1987	Cameron		Cam87	90.7	90.7	90.4
11	EF206707.1				ERA	ERA	92.4	92.4	92.1
12	KM198893.1				MOSCOW3253	MOSC3253	92	92	92
13	GV565704.1				Flury HEP	Hep.	92.6	92.5	92.3
14	GV565703.1				Flury LEP	Lep.	92.9	92.9	92
15	GQ412744.1				AG	Chinese AG	91.4	91.3	91.6
16	GQP18139.1				CVS.11	CVS	92.9	92.9	92.6
17	JQ944709.1				Rabies EPH Vac	ephvac	93.5	93.5	93.2
18	DQ837425.1	cow	1998	Jordan		JOR98	92.9	92.9	92.5
19	DQ837423.1	DONKEY	1999	Jordan		JOR99	92.9	92.5	92.3
20	DQ837465.1	STONE-NARTIN	1997	Lebanon		LEB97	93	92.7	92.4
21	DQ837429.1	DOG	1996	Israel		ESR96	92.7	92.7	92.4
22	DQ837466.1	FOX	2002	Israel		ESR02	93	93	92.7
23	DQ837442.1	FOX	1998	Israel		ESR98	93	92.7	92.4
24	MG011654.1	HOMOSA-PIENS	2016	France		FR16	89.8	89.8	89.5
25	DQ837480.1	DOG	2000	Turkey		TRK00	93.5	93.5	93.2
26	MT134104	CATTLE	2018	Egypt (Beheira)		BEH18	100	99.4	99.1
27	MT134105	DOG	2019	Egypt (Alex)		ALEX19	99.4	100	99,4
28	MT134106	DOG	2018	Egypt (Dakahleya)		DAK18	99.1	99.4	100

The recent Egyptian isolates, the non Egyptian isolates from Africa (Chad14, Moritanya2006, Cameron87) and neighboring countries (Lebanon97, Israel96, 98, 02 and Jordon 98) originated from a common ancestor (Table 2, Figure 5A) with the presence of mutation sites at their N Proteins (Figure 5B).

The degree of nucleotide identity between the Dakahleya isolate as well as certain non-Egyptian isolates at the N gene was reported to be 89.2% with Chad14, 90.4 % with Mauritania 2006, 90.7 % with Cameroon 87 and 92.7% to 93% with Chad, ISR96, ISR98, ISR02, LEB, JORD98 and JORD99) (Table 2).

At the level of the N gene, the % of identity between the Dakahleya isolate and some compared vaccinal strains was found to be 92 % for (EPHVAC and Moscow 3235 strain), 92.4 % for the ERA strain, 92.9 % for the LEP strain, 92.6 % for HEP and 91.4% for the Chinese AG strain. On the other hand, the % of identity between the Dakahleya isolate and some comparative vaccinal isolates were found to be 88.2% with EPHvac, 88.5% with (HEP and Moscow3235 strain), 88.7% with (LEP and ERA), and 88.9% with the Chinese AG strain. Alex and Beheira isolates showed similar figures (Table 2 and Figure 7).

In comparison to the Pasteur reference rabies virus strain partial N gene sequence (Genbank

accession No. GU992320), all the current study Egyptian strains showed the following mutations: [(Thr3Ala), (Val21Ile), (His26Tyr), (Ser61Asn), (Phe80Leu), (Val90Leu), (Gly106Asp), (Asn119Ser), (Pro135Ser)], While strain EgyBEH18 showed another mutation (Lys5Arg.), while in comparison to the lastly published Egyptian strains, sequences were identical except for 2 mutations [(Val22Ala) and (Asn202Ser)] that were recorded in the Egyptian strains MT036064-MT036066.

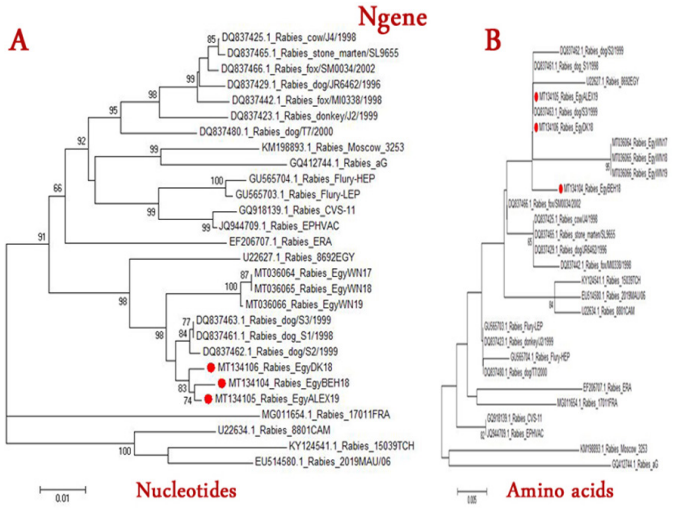

Figure 5: *The Phylogenetic tree of Rabies viruses based on nucleotide (A) or Amino acid (B) sequence of N gene. Phylogenetic tree is based on maximum likelihood calculations (Mega 7 software) under the best fit model. Numbers at nodes represent measures of robustness based on 1000 bootstrap values. The sequences of selected publicly available strains were obtained from GenBank. Strains of current study are labeled with red circles.*

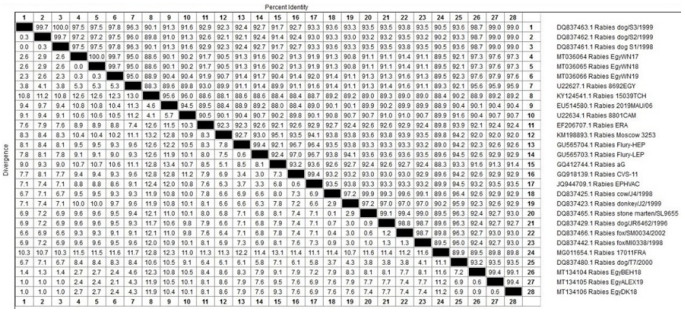

Figure 6: *Figure illustrating the % of nucleotide identity of some Egyptian rabies isolates with other rabies virus isolates and strains at the level of the N gene.*

At the level of the P gene, the % of identity of the Dakahleya isolate with (Behe18) was 98%, 97.9% with (Alex19), 95.7% with (Egy79), 88.7% with Wadi18, and 88.5 % with (Wadi19) (Table 3). The P genes of the old and recent isolates all originated from the same clade (Figure 8) with the presence of a mutation at the AA composition of their P proteins (Figure 9).

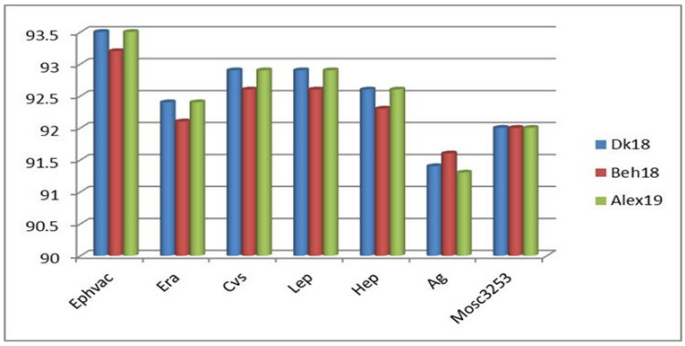

Figure 7: *Comparing the nucleotide identity between each of the new Egyptian isolates and some vaccinal strains at the level of the N gene.*

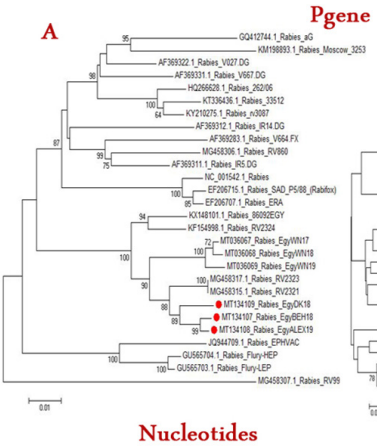

Figure 8: *The phylogenetic tree of Rabies viruses based on nucleotide (A) or Amino acid (B) sequence of P gene. Phylogenetic tree is based on maximum likelihood calculations (Mega 7 software) under the best fit model. Numbers at nodes represent measures of robustness based on 1000 bootstrap value. The sequences of selected publicly available strains were obtained from GenBank. Strains of current study are labeled with red circle.*

The % of nucleotide identity between the Dakahleya isolate and some non-Egyptian isolates at the P gene were 95.6 % with ESR50, 90.4 % with TUN88, 90.7 % with Ethio, 89.5 % with the South Africa06 and the Israeli isolate ESR01, 89.5 % with the South Africa02, 87.6 % with Tanzania and 85.5 % with South Africa 00. Alex isolates and Beheira isolate both yielded similar findings.

At the level of the P gene, the % of identity between the new Egyptian isolates and some compared vaccinal strains was found to range from 88.2 % to 89.8% for AG vaccine (Figure 9 and Table 3).

Table 3: *The description of isolates from NCBI used for comparison in this study at the level of P gene.*

No	Access no	Host	Isolation year	Country	Abbreviation	Variant	% of nucleotide identity		
							Beh18	Alex19	Dkh18
1	MG458317.1	DOG	1999	Egypt	CA99		97.6	97.4	97.6
2	MG458315.1	DOG	1998	Egypt	CA98		97.6	97.4	97.6
3	MT036069	CATTLE	2019	Egypt (Wadi)	Wadi19		95.5	95.4	88.5
4	MT036067	FOX	2017	Egypt (Wadi)	Wadi17		95.7	95.8	88.9
5	MT036068	CATTLE	2018	Egypt (Wadi)	Wadi18		95.7	95.6	88.7
6	KX148101.1	MOUSE	1979	Egypt	Egy79		95.4	95.2	95.7
7	HQ266628	DOG	2006	South Africa	Sa06		89.8	89.9	89.5
8	KT336436.1	DOG	2012	South Africa	Sa12		89.4	89.7	89.3
9	MG458307	CAT	2000	South Africa	Sa00		84.9	85.1	85.5
10	KY210275.1	COW	2013	Tanzania	Tanz13		87.9	88	87.6
11	MG458306.1	FOX		Chekoslovakia	chkosl		89	89	89.3
12	AF369331	DOG		Ethiopia	eth		90.4	90.6	90.7
13	AF359322		1988	Tunisia	Tunis98		90.5	90.6	90.4
14	NC001542				Pasteur	Pasteur	89	89.2	89
15	EF206715.1				Rabifox (SAD)	SAD	88.6	88.9	88.9
16	JQ944709				EPH VAC		87.8	87.7	88.2
17	GQ412744.1				AG	AG	89.4	89.8	88.9
18	EF206707.1				ERA	ERA	88.5	88.7	88.7
19	KM198893.1				MOSCOW3253		88.5	88.4	88.5
20	GU565704.1				FLURY HEP	HEP	88.3	88.2	88.5
21	GU565703.1				FLURY LEP	LEP	88.5	88.4	88.7
22	AF369312	DOG	2001	ISRAEL	Esr01		89.7	89.9	89.5
23	AF369311	DOG	2001	ISRAEL	Esr01		89.5	88.7	89.5
24	AF369283	FOX	2001	ISRAEL	Esr01		88.3	88.4	88.3
25	KF154998	DOG	1950	ISRAEL	Esr50		95.4	95.2	95.6
26	MT134107	CATTLE	2018	Egypt (Beheira)	Beh18		100	99.4	98
27	MT134108	DOG	2019	Egypt (Alex)	Alex19		97.9	100	97.9
28	MT134109	DOG	2018	Egypt (Dakahleya)	Dkh18		98	97.9	100

Presence of AA divergence among the recent Egyptian isolates and the vaccinal strains in this study is due to the presence of mutation sites (Figures 8B, 10).

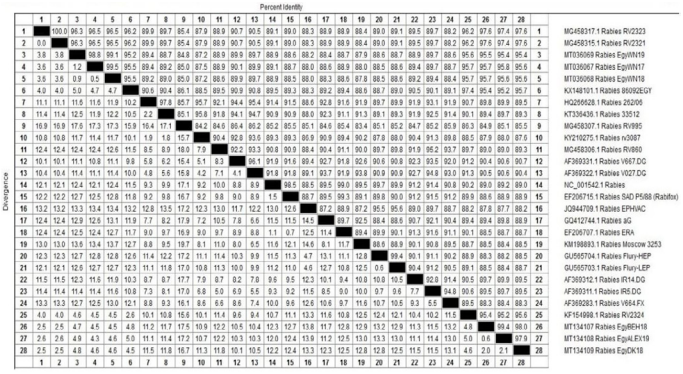

Figure 9: *Figure illustrating the % of nucleotide identity of some Egyptian rabies isolates with other rabies virus isolates and strains at the level of P gene.*

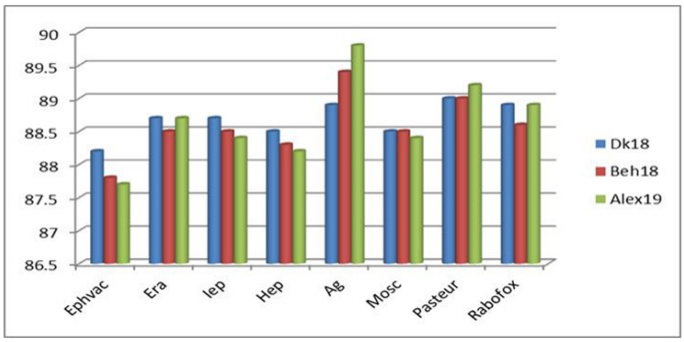

Figure 10: *Chart comparing the nucleotide identity between each of the new Egyptian isolates and some vaccinal isolates at the level of the P gene.*

When compared to the Pasteur reference rabies virus strain full P gene sequence (Genbank accession No. NC_001542), all the current Egyptian strains showed the following mutations; [(Gly54Arg), (His57Gln),

(Pro64Ser), (Pro66Leu), (Met69Val), (Ala70Thr), (Lys71Ala), (Ile108Met), (Ala130Thr), (Pro137Ser), (Leu153Pro), (Lys154Arg), (Thr158Pro), (Pro159Ser), (Ala171Val), (Thr177Val), (Leu223Phe), (Arg252His), (Ser280Ala) and (Ser284Asn)]. However, MT134109 showed 2 extra mutations [(Ser90Gly) and (Ser140Val)]. However, Ser140Leu was detected in MT134107 and MT134108.

As the rabies virus does not produce the appreciable cytopathic effect (CPE) in BHK-21 cells, the presence of virus antigen was confirmed in tissue culture by using the florescent antibody technique (FAT).

This research aimed to characterize rabies virus (RABV) strains that were circulating in Egypt. Phylogenetic analysis of this study indicated clustering of Egyptian field rabies isolates at the N gene-level into comparable phylogenic clusters, showing close genetic distance (from 99% to 95.9%). The tested field rabies Dakahleya isolate exhibited a genetic distance of 92.7% or more with isolates from nearby countries (Israel, Lebanon, and Jordan) and of 90.7% and less compared to isolates from remote nations (Chad, Mauritania, and Cameron). The genetic difference between the field Dakahleya isolate and the vaccinal strains in this study ranged from 91.4 % (Chinese AG strain) to 92.9 % (LEP vaccinal strain), including the ERA strain (The used strain in Egypt in veterinary rabies vaccines).

In the current study, a phylogenetic analysis of the tested Egyptian field rabies isolates at the P gene revealed that the field Dakahleya isolate was genetically close to the majority of Egyptian isolates (from 98% to 95.9%). It has a large genetic distance from El-Wadi governorate isolates, ranging from 88.5 % to 88.7 % (wadi19 and wadi18, respectively). The field rabies Dakahleya isolates shared a genetic distance of 90.7 % or more with isolates from nearby countries (Israel, Tunisia, and Ethiopia) and a genetic distance of 89.5 % and less compared to isolates from far away countries (South Africa and Tanzania). At the P gene level, all vaccinal strains in this investigation have a genetic distance from our field Dakahleya isolate ranging from 88.2 % to 88.9 % (for the Chinese AG vaccinal strain). Beheira and Alex isolates produced similar results.

It was notable that all Egyptian field isolates shared a high level of genetic similarity. The following previous studies supported these findings. Lineage 1 from Africa RABVs are quite similar to RABVs seen in Europe and Asia and they are assumed to have just arrived in Africa from Europe as a result of colonial activity from the 16th to 19th centuries (Smith *et al.*, 1992). it is a partner of the cosmopolitan canine RABV lineage.

Isolates from Middle Eastern and European countries have a significant correlation, but they differ from isolates from Asian and African countries (Kissi *et al.*, 1995). The tight genetic similarity between isolates from European and Middle Eastern nations shows that the viruses circulating in foxes in both zones may have emerged from the same source (David *et al.*, 2000). According to a previous study, the three Egyptian isolates (CA98, CA99, and FAY99) belonged to Africa 4, a novel clade with a high bootstrap value (99 %). It was reported to be ancestral to the cluster of RABV variants associated with the Middle East and Eurasia's middle latitudes, as well as Africa 1 (David *et al.*, 2007).

Dog trading and the porosity of the border areas that promote and facilitate rabies virus transmission according to Silberfein *et al.* (2006), Sunday *et al.* (2014) explain and support that finding.

Conclusions and Recommendations

The examined rabies Egyptian field isolates, most of the previous Egyptian isolates, isolates from nearby countries (Israel, Lebanon, and Jordan), and the vaccinal strains used in this study, particularly the Chinese AG strain and the LEP vaccinal strain, have a small genetic distance with each other and a significant genotypic variation with isolates from far countries to Egypt at the N gene level. Except for isolates from El-Wadi governorate, all the analyzed Egyptian field isolates and isolates from nearby countries had a close genetic distance at the P gene level (Israel, Tunisie, and Ethiopia). Isolates from far-flung places (South Africa and Tanzania) and vaccine strains employed in this study have a wide range of genetic distances. It could be concluded that dog trading and the porosity of the border areas have a great role in the spread of the disease in Egypt. Strict quarantine measures on the importation of unvaccinated animals, border follow-up, and control are recommended.

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Novelty Statement

Our results shoed the importance of dog trading and the porosity of borders on Rabies virus control.

Author's Contribution

Bothe auhtors prepared the manuscript and planned the work. MY conducted the experiments and wrote the manuscript. EA was responsible of sampling and revision of the the manuscript.

Conflict of interest

The authorts have declared no conflictof interest.

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