Molecular Identification of Parvovirus in Ducks in Egypt

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Abstract

Parvovirus is the causative agent of Derzy's disease in ducks. The disease appeared in Egypt as a variant of goose parvovirus (GPV) called Short Beak and Stunted Growth Disorder, affecting ducks which causes shortening of beaks, tongue protrusion, and growth retardation, this condition is attributed to the recently identified parvovirus. The goal of this study is to know if there is a new viral mutations of the recent isolated strain in Egypt by, isolation of the virus in fertilized duck egg and fibroblast cells of duck embryo, following by (ELISA) test. Polymerase chain reaction was used to determine the virus's existence followed by gel electrophoresis and sequence analysis of the VP1 gene, comparing the isolated virus to closely related strains from Gene Bank. It was found that the isolated virus is 100% identical to the strains isolated in 2023 in Egypt. It is 98.1% identical with the isolated strain in 2018 with about 10 nucleotide variation at specific locations. Just a single change between them results in a codon shift from Serine in obtained isolated strain into Alanine in the local isolate of 2018.This site of change is very important (receptor binding site of the outer coat of the virus) the change of it will affect viral pathogenicity and antigenicity. While the rest 9 variations result in no codon change (silent mutations).

Keywords: Derzy's disease, PCR and sequence analysis.

Introduction

Derzsy's Disease (DD), was first reported in 1960 the causative agent was the goose parvovirus that made outbreaks in young Muscovy ducklings ***(Derzsy, 1967; Palya, 2013)***. Derzsy's disease in 1978 was known as goose parvovirus GPV. In 1989, Muscovy ducks suffered from similar symptoms to those of GPV and a variant strain of parvovirus was reported, it was

called Muscovy duck parvovirus (MDPV), with more than 85% identity to GPV. This has led to the classification of waterfowl parvoviruses into two main types based on their host specificity: GPV and MDPV ***(CHU et al., 2001)***. Short Beak and Stunted Growth Disorder was found in Egypt in 2017. The causative agent was a variant of goose parvovirus, and characterized by protruding tongues, short beaks, and growth retardation that is regarded as a new GPV strain. One day old ducks are the susceptible host with high morbidity and low mortality. GPV, MDPV are antigenic ally related and classified as members of the family Parvoviridae. The goose parvovirus genome has single-stranded DNA ***(Palya, 2013)***, 5.1 kb. The goal of this study to know if there is a new viral mutations of the Egyptian strain. The virus was identified by isolation in embryonated duck egg and fibroblast cells of duck embryo, following by (ELISA) test and confirmed by PCR. Polymerase chain reaction was used to determine the virus's existence followed by gel electrophoresis and sequence analysis of the VP1 gene, comparing the isolated virus to closely related strains from Gene Bank.

Material and Methods

1. Samples

Nine unvaccinated duck farms were suspected to be GPV-infected farms located in Beheira, Sharqia (Zagazig) and Gharbiya (perma) during 2019-2024 (Table 1). By collecting tissue samples, 11 birds per flock were sampled and polled together (Liver, lung, spleen, heart and intestine). Samples were put in (PBS), with gentamycin (50 μg/ml) as an antibiotic solution, all samples were preserved at -20C°, centrifugation at 5,000 rpm for 5 minute to obtain the clarified supernatant ***(Chen et al., 2016)*.**

2 Control positive goose parvovirus strain

It is a goose parvovirus isolated from Egypt in 2018 was used in the preparation of local Inactivated Derzsy's disease vaccine prepared at veterinary serum and vaccine research institute (VSVRI), that was used as a control positive during full molecular characterization in comparison to the isolated strain.***(Ali Saleh et al., 2024)***

3. Embryonated duck eggs used in virus isolation

Nine day-old embryonated duck eggs (EDE) were used for virus isolation through the allantois' route inoculation. Each sample's supernatant was inoculated at the volume of 0.2 ml. Incubation at 37C° with daily checking of the eggs to see if there is embryonic death or not, the allantois' fluid collected in case of the embryo did not die and then used for making five blind passages. For the identification of virus presence, the fluid we collected was tested by

ELIZA and PCR ***(Chen et al., 2016)*.**

4. Duck Embryo Fibroblasts (DEF)

Duck embryo fibroblast was prepared according to the sundered instructions of cell culture ***(Doyle and Griffiths., 1998)***. Inoculation of Primary DEF cell culture monolayer was ready from 14-day-old Muscovy duck embryos with the supernatant of sample tissue homogenate (liver) and incubated at 37C° for 7 days. The control positive group was made by inoculation of DEF with PBS, then the DEF checked daily for the presence of cytopathic effect (cell detaching and or clumping) ***(Brown et al., 1995)***. The DEF cells then used for assurance of virus presence by using polymerase chain reaction.

5. ELISA

ELISA kits was used for identification of goose parvovirus antigen according to the instruction of manufacturers known as (Shanghai Coon Koon Biotech Co., Ltd), Pre-coated plates with specific goose parvovirus antibodies was used for virus identification. ***(Saleh and Khodier, 2020)***.

6. PCR

For virus DNA extraction the liquid layer of DEF, extracted tissue from (liver), and allantois' fluid after centrifugation was used. Genomic DNA Mini Kit (Geneaid) was used in the extraction process, following the manufacture instruction. DNA elution step was made by adding 50 µl TE buffer and preserved in -20 co till use. DreamTaq Green PCR Master Mix 2X (# K1081), was used amplifying 885 bp. The target was VP1 gene. (Table 2): primers of PCR reaction. ***(Ali Saleh et al., 2024)***.

7. Sequencing PCR product

The PCR product was amplified by using high-fidelity nPfu-Forte (Cat.# P410) ***(Lundberg et al., 1991)***. Amplification of segment of approximately 885 bp of the VP1 gene, 1% low melting agarose was used in inoculation of PCR product. Using (Qiaquick gel extraction kit cat #.28704), to extract the PCR product. One sample was sequencing to (allantois' fluid) partial sequencing to VP1 gene by GATC Company, Germany by using ABI 3730xl DNA Sequencer ***(Ali Saleh et al., 2024)***

8. Phylogenetic analysis

The phylogenetic analysis was made using Lasergene DNA Star software; it was used for analysis of the aligned sequences of the 485 bp of VP1. A BLAST was performed to determine sequence identity to Gen Bank published parvoviruses ***(Altschul et al., 1990)***. A phylogenetic analysis was constructed using MEGA11***(Tamura et al., 2013)***.

Table (1): *Examined duck's flocks for parvovirus*

Government	*Number of farms*	*Total number of birds*	*Total number of samples*
Beheira	*3*	*33*	*100 Sample*
Gharbiya,Tanta,Perma	*4*	*45*	
Sharquia,Zagazig	*2*	*22*	

Table 2: *primers used in GPV identification targeting vp1 gene*

Primer	Sequence	Location	Band Size
forward	5^- GTGGGTAATGCCTCGGGAA 3^-	649-667	885 bp
reserve	5^- GACACAGGTCCGGGTTGTAG 3^-	1553-1534	

Results

1. Clinical signs

The observed sings included protrusion of tongue associated with a reduced beak size (Fig.1). Inadequate beak formation led to tongues extending outward, which impaired the duck's ability to feed and drink effectively. Consequently, the affected ducklings exhibited stunted growth and higher mortality rates. Additionally, the ducklings suffered from brittle, easily fractured legs. The prevalence of the disease ranged from 35% to 65 % and the surviving ducks showed significant reductions in feather quality and growth rates.

2. Embryonated duck eggs (EDE)

Examination revealed death of 70% of infected embryos within 4 -5 days' post inoculation in EDE with hemorrhage, congestion and edema of the embryo and deformation of peak that are characteristics for the new strain of GPV (Fig 2).

3. Identification of goose parvovirus by ELISA:

Seven samples of (GPV) were detected from 9 examined samples.

4. Virus isolation in primary duck embryo fibroblast cells (DEFs)

The cytopathic effects were occurred 96 -120 hr post inoculation after 4 passages. By 96-120 hours, DEF cells that were exposed to the infection became visibly rounded and diminished in size, and the entire monolayer was destroyed.

5. Molecular identification

Examined samples were positive for goose parvovirus in 6 samples by PCR, amplification of 885 bp of VP1 gene**.** Control positive virus

strain was used as control positive for our PCR reaction (Fig 3).

6. Result of sequencing

nucleotide identity was 100 % between the local isolates of 2023in Egypt and the isolated strain that submitted to Gene Bank and have Accession number # PP41989 , namely AMN/ Duck/ 2023 (NEW) ,while the isolate of 2018 in Egypt shares 98.1% nucleotide identity with the isolated strain.

7. Results of Multiple Sequence Alignments

The result revealed the presence of about 10 nucleotide variation at specific locations. Just a single change between them results in a codon shift from Serine in obtained isolated strain into Alanine in the local isolate of 2018 .The mutation occurs at amino acid number 28 in the obtained isolated strain which is opposite to the amino acid number 261 of the whole VP1 capsid protein, this change lead to change in the outer capsid of Vp1 protein that will affect viral pathogenicity and antigenicity. While the rest 9 variations lead to silent mutations.

Fig. 1: short beak and protruding tongue

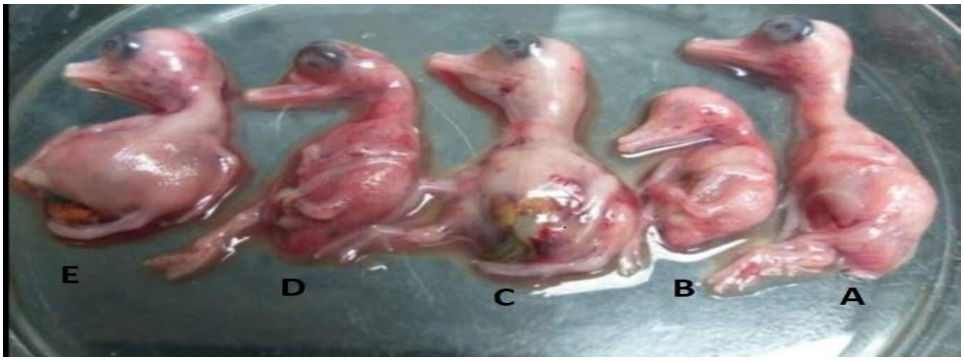

Fig.2: Pathological changes of goose parvovirus in EDE
A: Control none inoculated, B: Stunted growth, C&E: Short beak,
D: Hemorrhages of the embryo.

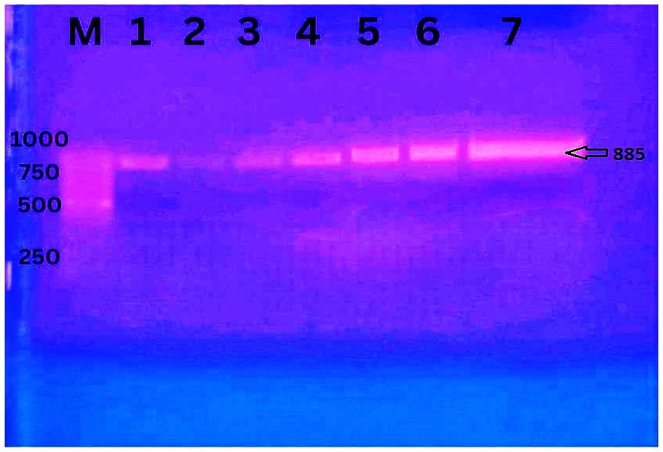

Figure 3: gel electrophoresis of PCR products of VP1 gene (885 bp band)

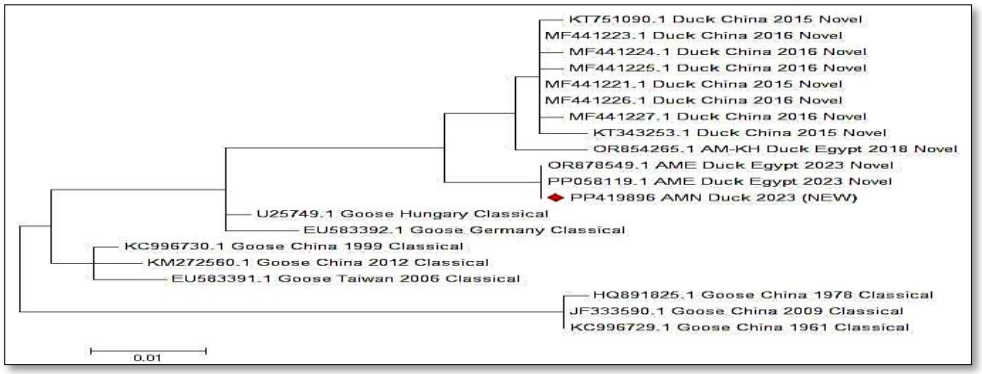

Figure 4. Phylogenetic analysis of goose parvovirus in ducks related to other strains in Gene Bank showing clustering of the isolated strain with the local isolated strains previously isolated in Egypt during 2023.

Discussion

The virus isolation resulted death of 70% of infected embryos. Cell changes were observed at 96 -120 hr after infection by 4 passages in duck embryo fibro blast. While new GPV was growing successfully in duck embryos and duck embryo fibroblast cells, it could not be growing in goose embryo fibroblasts ***(Palya et al., 2009)***. Identification of viral isolate by ELISA revealed that; (GPV) was detected in 7 samples from 9 examined samples. It was found that the isolated GPV is 100% identical to the strains isolated in 2023 in Egypt and is 98.1% identical with 2018 isolated strain with about 10 nucleotide variation at specific locations***(Ali Saleh et al., 2024)***

Conclusions

The presence of GPV mutations in Egyptian duck farms reported, that is responsible for the increasing of Short Beak and Stunted Growth Disorder infections. The causative virus was successfully isolated using fertilized duck eggs and DEF cells. The virus existence was confirmed through EIIZA for of the allantois fluid, indicating that the isolate was positive for GPV. PCR and partial VP1 gene sequencing further placed the isolated virus within the GPV strains with newly some viral mutations.

References

Ali Saleh, A., MS El-Nagar, E., Sayed, M.I. (2024). Molecular Characterization and Sequence Analysis of VP1 Gene of Duckling Short Beak and Dwarfism Syndrome Isolated In Egypt during 2023. Egyptian Journal of Veterinary Sciences 56, 457-468.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. (1990). Basic local alignment search tool. Journal of molecular biology 215, 403-410.

Brown, K.E., Green, S.W., Young, N.S. (1995). Goose parvovirus—an autonomous member of the dependovirus genusVirology 210, 283-291.

Chen, H., Dou, Y., Tang, Y., Zheng, X., Niu, X., Yang, J., Yu, X., Diao, Y. (2016). Experimental reproduction of beak atrophy and dwarfism syndrome by infection in cherry valley ducklings with a novel goose parvovirus-related parvovirus. Veterinary microbiology 183, 16-20.

Derzsy, D. (1967). A viral disease of goslings. I. Epidemiological, clinical, pathological and aetiological studies.Acta Veterinaria Academiae Scientiarum Hungaricae 17, 443-448.

Doyle, A., Griffiths, J. B. (1998). Cell and tissue culture: laboratory procedures in biotechnology.

Lundberg, K.S., Shoemaker, D.D., Adams, M.W., Short, J.M., Sorge, J.A., Mathur, E.J. (1991). High-fidelity amplification using a thermostable DNA polymerase isolated from Pyrococcus furiosus. Gene 108, 1-6.

Palya, V., Zolnai, A., Benyeda, Z., Kovács, E., Kardi, V., Mató, T. (2009). Short beak and dwarfism syndrome of mule duck is caused by a distinct lineage of goose parvovirus. Avian Pathology 38, 175-180.

Palya, V.J. (2013). Parvovirus infections of waterfowl. Diseases of poultry, 444-451.

Saleh, A.A., Khoudeir, M.H. (2020). Preliminary studies on the virus causing outbreak of duckling short beak and dwarfism syndrome (SBDS) in Egypt. Journal of Applied Veterinary Sciences 5, 55-60.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution 30, 2725-2729.

الملخص العربى

التعرف الجزيئي على فيروس البارفو في البط بمصر

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مرض الدرزي في البط هو مرض شديد العدوي يسببه فيروس بارفو الاوزظهرمرض جديد يسمي متلازمة المنقار القصير والتقزم في مصر يسببه بارفو الإوزالجديد الذي يؤدي إلى ظهور مناقير قصيرة، وألسنة بارزة، وعظام هشة، وتأخر النمو في البط ويعتبر نوعًا مختلفًا من بارفو الاوز .في هذه الدراسة قمنا بجمع وتحضير العينات ، عزل الفيروس،عمل تفاعل البلمرة المتسلسل متبوعًا بالفصل الكهربائي الهلامي وتحليل التسلسل الجيني والمقارنة أيضًا بالسلالات ذات الصلة الوثيقة من بنك الجينات وتكوين شجرة العائلة.ووجد ان الفيروس المعزول متطابق 100%مع السلالات المعزولة في عام 2023ويتطابق بنسبة 98.1%مع سلالة 2018