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Isolation and identification of equine herpesvirus -1 (EHV-1) in Egypt during 2021-2022

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ABSTRACT

Equine herpes virus-1 (EHV-1) is a major cause of economic losses in horse industry all over the world especially in the Arabian countries. The aim of the study is the isolation and identification of the local equine herpesvirus-1 at northern Egypt Governorates (Cairo, Dakahlyia, Qalyubia) during 2021-2022. A total of 50 tissue organs were collected from aborted fetuses of affected mares recently after abortion. The collected samples were screened using consensus PCR for detection of equine herpesviruses (EHVs) using specific primers targeting DNA polymerase gene which revealed that 26 out of 50 samples were positive with percentage of 52%. The molecular positive samples were subjected to conventional PCR for further molecular identification and typing of EHVs using specific primers targeting glycoprotein (gB) gene which confirmed that all amplified products were for EHV-1. Attempts for isolation of EHV-1 were performed through propagation of molecular identified samples on Madin-Darby bovine kidney (MDBK) cell line which revealed that 19 samples developed characteristic cytopathic effect in form of cell rounding and rapidly enlarging grapes like cell aggregation, and then detached of the cell, while 13 samples induced pock lesions on CAMs of SPF-ECE after inoculation for three successive passages. Serological identification of EHV-1 isolates was confirmed by immunofluorescent antibody test (IFAT) which showed that 19 samples induced intracytoplasmic greenish yellow fluorescence. Selected EHV-1 isolate was subjected for transmission electron microscopy (TEM) for demonstration of virus morphology. The obtained results confirmed that EHV-1 is the major cause of abortion among Arabian mares which disrupts breeding and showcasing events. Future considerations about management strategies together with regular and effective vaccine trials are recommended for control of EHV-1.

Introduction

Equine herpesviruses are a group of viruses within the family Herpesviridae including nine EHVs which have been classified to be in *Alphaherpesvirinae* and *Gammaherpesvirinae* subfamilies and none belong to subfamily *Betaherpesvirinae* (ICTV, 2019). Alphaherpesviruses including EHV-1, EHV-3 and EHV-4 are affecting many mammals. EHV-1 was isolated from circulating leukocytes or peripheral blood mononuclear cells (PBMC) in 80% of apparently healthy adult horses due to its ability to establish lifelong latency resulting in several clinical syndromes (Pusterla *et al*., 2012; Hussey *et al*., 2014).

EHV-1 is the main causative agent of abortion, neonatal mortality, neurological disorders (myeloencephalopathy), respiratory dysfunction and chorioretinopathy (Hussey *et al*., 2013: Stokol and Hussey, 2020). EHV-1 is the most clinically and economically significant equine viral agent affecting reproduction and performance in racehorse which may result in significant and negative economic impacts for horse industry (Fortier *et al*., 2010; Kydd *et al*., 2012).

In Egypt, horses play an important role in Egyptian economics and represent around 40% of the total equine population. These horses include pure-bred registered Egyptian Arabian horses mainly used for showcasing, semen collection and export. This includes direct economic impact of the horse industry as breeding, employment and education as well as indirect impact related to horse activities like organization of social events (Animal Wealth development sector, Egyptian Ministry of Agriculture). Despite the continuous circulation of EHV-1 among Egyptian horses there is no official documentation on the health status of horses or vaccination programs (Ghoniem *et al*., 2017). The seroprevalence of EHV-1 in Egyptian horses 62.5% (El-sayyad *et al*., 2015).

EHV-1 was isolated from aborted fetuses in Egypt (Abd El-Hafeiz *et al*., 2010). Moreover, EHV-1 was isolated on CAM of ECEs from aborted fetal organs and developed large pock lesions (Hassanein *et al*., 2002), while EHV-1 was propagated on MDBK cell line with development of characteristic CPE appeared as focal rounding, vacoulation, grapes like cell aggregation and cell degeneration with partial detachment of cells from the culture surface leaving empty spaces (Afify *et al*., 2017).

In cases of suspected EHV-1 abortions; tissue samples, including the placenta, lung, liver, and spleen, were collected from the aborted fetuses for virus isolation and identification. The diagnosis of EHV-1 must be rapid and sensitive with early intervention policies that aimed to reduce the virus spread. The routine diagnosis of EHV-1 is usually achieved by using virus isolation, polymerase chain reaction and serology from suspected cases (Dynon *et al*., 2007).The common cells used to isolate EHV-1 are rabbit kidney (RK-13), baby hamster kidney (BHK-21), Madin-Darby bovine kidney (MDBK), and pig kidney (PK-15) (OIE, 2012).

Nucleic acid amplification-based detection techniques for EHV-1including conventional, seminested PCR are commonly used for molecular detection and differentiation among, EHVs (Balasuriya *et al*., 2015: OIE, 2015). PCR is a suitable screening method for latent EHV-1 in horses (Pusterla *et al*., 2012). Despite the correlation between the results of virus isolation and PCR, PCR is considered superior to virus isolation in terms of rapidness, sensitivity and specificity in detection of EHV-1 and EHV-4 (Hornyák *et al*., 2006). Virus culture and isolation were identified to be the gold standard for laboratory diagnosis of EHV infections concurrently with the rapid test of polymerase chain reaction (PCR) or Immunoassays particularly IFAT which confirms virus identity using specific antisera are

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well-established standard diagnostic methods for EHV-1 (McBrearty *et al*., 2013; Balasuriya *et al*., 2015; OIE, 2018). Further identification of EHV-1 virions was applied by electron microscopy with demonstration of characteristic herpesvirus morphology which confirmed the EHV-1 specificity of the immunolabeling assays (Jönsson *et al*., 1989; Del Piero *et al*., 2000).

In Egypt, EHV-1 was reported to be prevalent through different diagnostic approaches including isolation on the embryo chicken egg with pock lesions appeared on the CAMs, isolation on tissue cultures with development of CPE as well as PCR assay which considered better approach and substantial technique over virus isolation for confirmation EHV-1due to the benefits of simultaneous detection and differentiation of various types of EHV as well as rapid diagnosis, while virus isolation is time-consuming , laborious and requires the presence of viable virus which can be challenging to achieve in the field (Salib *et al*., 2016; Azab *et al*., 2019; Ali *et al*., 2020).

Stock management through segregation of horses on the basis of age and risk may result in a great reduction of EHV-1 spread. Weaned foals and yearlings that are more susceptible to shed virus, should be separated from horses at high risk such as pregnant mares due to severe EHV1 consequences. These considerations have resulted in reduction of clinical manifestation and nasal shedding of the virus abortion storms, however neurologic disease outbreaks are still occurring (Goodman *et al*., 2012; Pusterla and Hussey, 2014; Wagner *et al*., 2015). This research work was directed for isolation and identification of the local EHV-1 which is responsible for abortions in pure-bred Arabian mares at different Egyptian localities during the period between 2021-2022 through application of molecular assays as consensus PCR for screening of the collected samples for EHVs, then using conventional PCR for typing of EHVs which was followed by further identification and confirmation through propagation and isolation of the virus on MDBK cell line as well as SPF-ECEs. Additionally, serological identification was applied using IFAT and morphological identification using TEM were performed.

Materials and methods

Sampling

A total of 50 specimens from tissue organs including placenta, umbilical cord, liver, lung, spleen freshly were collected from aborted fetuses of 6-10 months age recently after abortion (Fig. 1) at different equine studs within different Governorates in northern Egypt (Cairo, Dakahlyia and Qalyubia) around the years 2021 and 2022 (Table 1).

EHV-1 reference strain

EHV-1strain (Giza-ARRI-USC-VM.EG19) under an accession number of OL505458.1 was provided by Animal Health Research Institute (AHRI), Dokki, Egypt to be used as control positive in the current study for validation of diagnostic assays.

Fig. 1. Aborted fetuses at the last trimester of pregnancy (A) Aborted fetus at the 6th month of pregnancy (B) Aborted fetus at the 9th month.

Samples processing and preparation

The processing and preparation samples were applied in coincidence with World Organization for Animal Health (OIE, 2015). Tissue organs were pooled and cut into fine pieces using forceps and scissors then ground in a mortar containing suitable amounts of PBS and sterile sand till homogenization. Tissue homogenate was subjected to freezing and thawing 3 times to disrupt any remaining cells then centrifuged for 15 min at 3000 rpm to remove tissue debris and the supernatant fluid was collected into clean sterile tubes then 5X antibiotic-antimycotic mixtures (Sigma) were added and kept at -80°C till used .

Total viral DNA extraction

EHV DNA was extracted from prepared samples using (QIAamp DNA Mini Kit, QIAGEN, and Germany) following the manufacturer's instructions.

Consensus herpesvirus PCR

Screening of EHVs in the extracted DNA was done by consensus PCR using general primers targeting DNA polymerase gene according to Van-Devanter *et al.* (1996) as listed in Table 2. Primary mixture was prepared as follows; 10 µl master mix, 0.2 µl Primer (DFA), 0.2 µl Primer (ILK),0.2 µl Primer (KG1), 5 µl Template DNA and nuclease free water added up to

Table 1. Details of collected samples used in the study (n=50) samples.

Locality	Type of samples	No. of samples	Date of sampling	Age range of affected mares	Age range of affected mares
Cairo	umbilical cord	4	2021	2-10 y	6-10 months
	placenta	8			
	liver	4			
	lung	4			
Dakahlyia	umbilical cord	3	2022		
	placenta	6			
	lung	5			
	spleen	3			
Qalyubia	umbilical cord	4	2022		
	placenta	5			
	lung	2			
	liver	2			

Table 2. List of primers used in the current study.

Assay/ Target gene	Primer	Primer sequence	Product size	References
Consensus-PCR DNA polymerase gene	DFA-For (first round)	5'-GAYT TYGCNAGYYTNTAYCC-3'	215-315 bp	VanDevanter *et al*. (1996)
	ILK-For (first round)	5'-TCCTGGACAAGCAGCARNYSGCNMTNAA-3'		
	KGI-Rev (first round)	5'-GTCTTGCTCACC AGNTCNACNCCYTT-3'		
	TGV-For (second round)	5'-TGTAACTCGGTGTAYGGNTTYACNGGNGT-3'		
	IYG-Rev (second round)	5'-CACAGAGTCCGTRTCNCCRTADAT-3'		
Conventional PCR assay EHV-1(gB)	For Rev	5'-CACTTCCATGTCAACGCACT- 3' 5'-TCGACTTTCTTCTCGGTCCA- 3'	869 bp	Azab *et al. (*2019)

A: Adenine; C: Cytosine; G: Guanine; T: Thymine; R: Purine (A or G); Y: Pyrimidine (C or T); M: Amino (A or C); S: Strong interaction (C or G); D: (A or G or T); not C, D follows C in alphabet.

20µl. Secondary mixture was prepared as follows; 10 µl master mix, 0.2 µl Primer (TGV), 0.2 µl Primer (IG), 4 µl PCR product of first cycle and nuclease free water added up to 20µl. Primary and secondary mixtures were subjected to amplification in thermocycler (Biometra, Germany) under thermal profile for two cycles as follows: cycle of initial denaturation at 95°C for 5 min followed by 40 cycles including (denaturation at 95°C for 30 s, annealing 46 °C for 60 s and extension at 72°C for 60 s) and one cycle of final extension at 72°C for 10 minutes. PCR products were visualized on 1% ethidium bromide stained agarose, at 120 volts, for 15 min and compared with a 1Kbp DNA ladder (ThermoFisher), amplified products were visualized under ultraviolet transillumination.

Conventional polymerase chain reaction

EHV molecular positive samples were subjected to conventional PCR assay for further identification and typing of EHV-1 using specific primer targeting glycoprotein B (gB) gene of EHV-1 as previously described by Azab *et al*. (2019) as listed in Table 2. PCR mixture was prepared as follows; 12.5 µl of 2x Emerald PCR Master Mix (TAKARA-BIO, Japan), 1 µl of forward primer, 1µl of reverse primer, 5 µl of DNA and nuclease free water up to 25 µl (Table 2).Thermal profile used in conventional was as follows: initial denaturation at 95°C for 5 min followed by 35 cycles (denaturation at 95°C for 45 sec, annealing at 55°C for 45 sec and extension for at 72°C for 60 sec) and final extension for at 72°C 10 min. PCR products were visualized on 1% ethidium bromide stained agarose, at 120 volts, for 15 min and compared with a 1Kbp DNA ladder (ThermoFisher), amplified products were visualized under ultraviolet transillumination.

Isolation of EHV-1 into MDBK cell line

Isolation of EHV-1 was applied according to the OIE guidelines (OIE, 2013) using MDBK cell line to isolate EHV-1 from the molecular positive sample. 0.2 ml of each sample was inoculated in recently prepared permanent cell line of Madin Darby bovine kidney (MDBK) cell line monolayer in 25 cm^2 tissue culture flasks supplied by animal health research institute (AHRI). Gentle rotation was done to disperse the inoculums over the cells monolayer. Monolayer of un-inoculated control cell was incubated in parallel with sterile transport medium only. After addition of 5 ml of supplemented maintenance medium (MEM containing 2% new born calf serum and twice the standard concentrations of antibiotics [penicillin, streptomycin, gentamicin, and amphotericin B], the flasks were incubated at 37°C. EHV-1 was isolated after three successive blind passages and inoculated flasks were inspected daily by microscopy for the appearance of characteristic CPE. Inoculated flasks then are subjected to 5 cycles of freezing and thawing for harvesting of the propagated virions. The final passage harvest was stored also in small aliquots at -80°C for further identification processes.

EHV-1 re-isolation into SPF-ECEs

In parallel with propagation of EHV-1 on MDBK cell line, further confirmation was performed through inoculation of the molecular positive samples into chorioallantoic membrane (CAM) of 11 day old SPF-ECE (five ECEs /sample) supplied by animal health research institute(AHRI) according to Warda *et al*. (2003). The inoculated eggs were incubated in horizontal position at 37°C for 7 days with daily examination for non-specific and specific deads, as well as chilling of dead eggs at 4°C /1h and after the end of incubation period, inoculated eggs (dead or not) were chilled for harvesting. CAMs were washed and then examined for detection of lesions.

Serological identification of EHV-1 using immunofluorescence antibody test (IFAT)

EHV-1 infected MDBK tissue culture displayed typical CPE were subjected to IFAT for detection of specific fluorescence of the EHV-1 in the same protocol as previously performed by GUNN (1992). Glass chamber slides Lab-Tek® were inoculated with 50µl/well of each suspected isolate from the 3rd passage and incubated at 37°C for one hour for virus adsorption, and then 200µl/well of maintenance media was added, incubated at 37°C overnight. Then slides were rinsed three times with PBS and fixed with 99% pure HPLC grade cold acetone (BDH Chemicals LTD, England) for 30 min, then acetone was discarded, slides were air dried. EHV positive control antisera (Polyclonal antibody pool; EHV-1, 4), kindly provided by Department of virology, AHRI, Dokki was added by 30µl/well, incubated for 30min. at 37°C. The excess of antisera was discarded, slides were rinsed 3 times with PBS, air dried. Anti-Horse IgG conjugated with FITC (SIGMA, MFCD00163518) was diluted 1/300 and added by 30µl/well also, incubated for 20-30min. at 37°C, Excess of the conjugate was discarded, slides were rinsed 3 times with PBS, then air dried. Evans blue counter stain was freshly prepared at 1% concentration, and added with 30µl/well volume, incubated for 20min. at 37°C. Excess of the dye was discarded; slides were rinsed 3 times with PBS and air dried. Finally, slides were examined for EHV fluorescence under fluorescent microscope with 20 X fluorite lens (OLYMPUS) at Pathology Research Dept., Animal Health Research Institute, Dokki (AHRI). Negative control slides consisted of non-infected cells were stained in the same way.

Morphological identification of EHV-1 by transmission electron microscopy (TEM)

The examination of one selected EHV-1 isolate was carried out according to Del Piero *et al*. (2000) at the TEM lab, FA-CURP, Faculty of Agriculture, Cairo University, Research Park. The sample suspension was dropped cast on the carbon grids for 10 minutes then fixed by using 3%

phospho-tungstate (Sigma Aldrich, USA) for 10 min. The material was examined with the electron microscope (JEOL JEM 2100, Japan) and the images were captured by Mega View III camera and using the software ITEM version E 23082007(Olympus Soft Imaging Solutions, Hamburg, Germany), employing a voltage of 80 kV and a constant current of 1 A and a total magnification of 60,000–120,000X.

Results

Molecular detection of EHVs using consensus herpesvirus PCR

Screening of the extracted EHVs DNA from pooled tissue organs homogenates by consensus PCR identified EHVs in 26 out of 50 samples with percentage of 52%. The positive PCR products are at the size of approximately 250 bp as illustrated in Fig. 2.

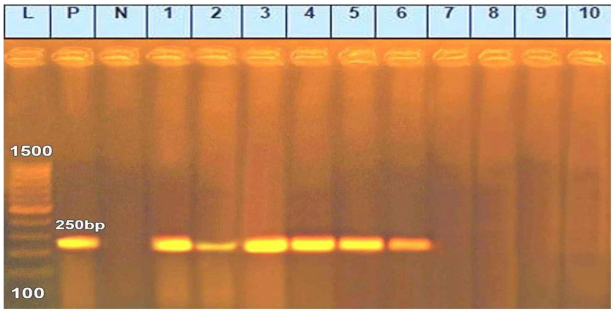

Fig. 2. Consensus PCR amplified DNA products of DNA polymerase gene of field EHVs, (Positive PCR products at the size of approximately (250 bp). Lane L: 100bp DNA marker, Lane P: positive control, Lane N: negative control, 1-6: field samples.

Molecular identification and typing of EHVs using type specific conventional PCR assay

Molecular typing of the positive samples by conventional PCR using specific primer targeting glycoprotein B (gB) gene of EHV-1 revealed that all molecular positive samples specifically amplified at (869 bp) fragment of EHV-1 as shown in Fig. 3.

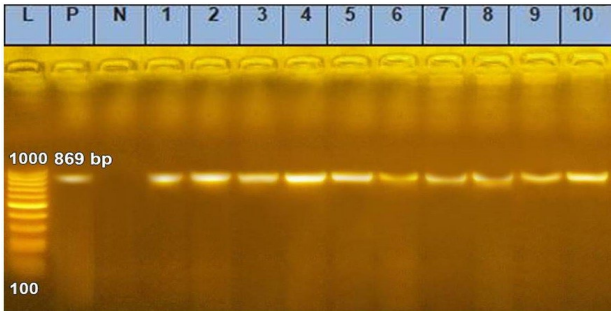

Fig. 3. Electrophoresis of the amplified products by conventional PCR assay for glycoprotein B (gB) gene of local EHV-1 strain: positive PCR products are at the size of approximately (869 bp): Lane L: 100bp DNA marker, Lane P: positive control, 1-10: field samples.

EHV-1 isolation into (MDBK) cell line

EHV-1 molecular identified samples were propagated on MDBK cell line and 19 samples developed CPE within two days after inoculation with rapid progression through three successive blind passages. The infected cell culture characterized by focal rounding and increases in refractivity at the 2nd day post inoculation (dpi) of 1st passage. Cell aggregation and rapidly enlarging grapes like cell aggregates were observed at the 3rd dpi of the 2nd passage, while cell degeneration and partial detachment of cells from culture surface leaving empty spaces were found at the 4th dpi of the 3rd passage which is characteristic for alphaherpesviruses as demonstrated in Fig. 4.

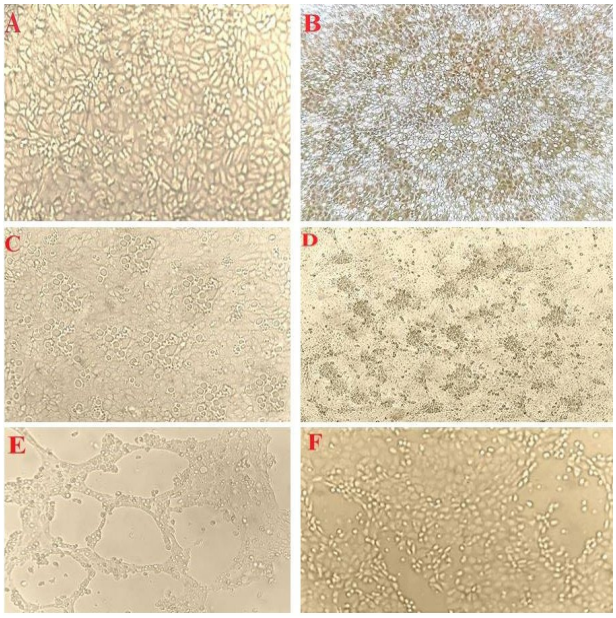

Fig. 4. Characteristic CPE of EHV-1 on MDBK cell line (A). Control negative: non-infected cell culture showed an epithelial-like morphology of MDBK cells (B). Early CPE after 1st passage; glistening and increase in refractivity (C). Second passage: cell rounding and vacoluation (D). Grapes like cell aggregation, cell degeneration and partial detachment of cell sheet (E&F).

EHV-1re-isolation on CAMs of SPF-ECEs

EHV-1 molecular identified samples were subjected to further inoculation on CAMs of SPF-ECE through three successive blind passages. Thirteen samples induced pock lesion on CAM. Pock lesions were demonstrated at the 1st passage which increased in their number through successive passages in comparison with normal CAM. The CAMs showed lesion were collected and examined as shown in Fig. 5.

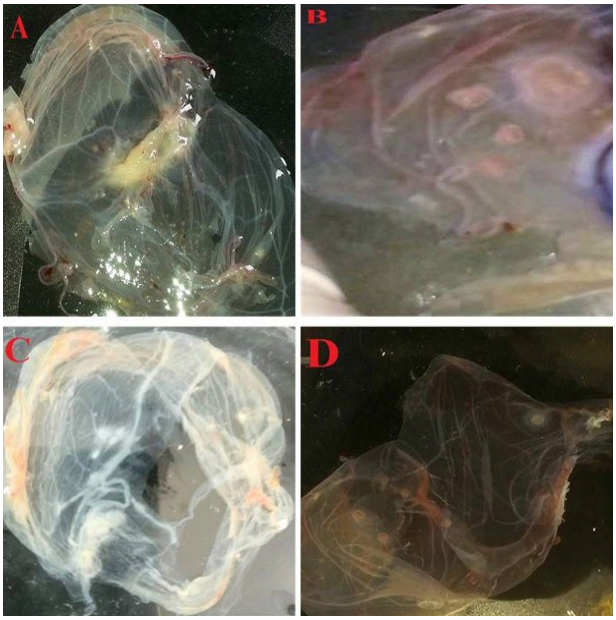

Fig. 5. Pock lesions on CAM of SPF-ECE of 11 days age after inoculation with EHV-1 isolates through three successive blind passages (A). Control negative: non-inoculated normal CAM (B). Early pock lesion development after 1st passage with increase in number and distribution of pock lesions(C&D).

Serological identification of EHV-1 isolates using IFAT

Glass chamber slides of infected MDBK cell line were examined for EHV-1 fluorescence with observation of green fluorescence with illumination in 19 slides, while control negative appeared as pale green without fluorescence as shown in Fig. 6.

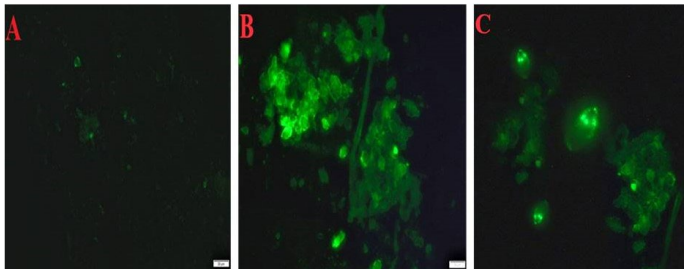

Fig. 6. Examination of glass chamber slides of EHV-1 infected tissue culture under fluorescent microscope with 20 X fluorite lens: (A) Control negative: normal without any fluorescence. (B) Control positive: green fluorescence with illumination. (C) Infected MDBK cell culture suspension showed fluorescence with illumination.

Morphological identification of EHV-1 isolates using TEM

EHV-1 infected MDBK cell line was examined using TEM which revealed that the infected cells contained few virus particles enveloped with condensed dark center and some appears star like or rounded with electron translucent center. The characteristic virus particles were observed at nuclear membrane and enveloped in the cytoplasm as shown in Fig. 7.

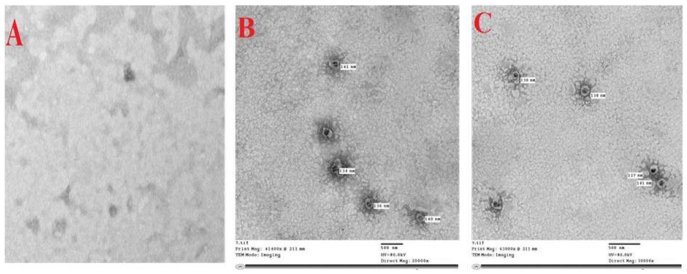

Fig. 7. Electron micrograph of selected MDBK cell line infected with EHV-1: (A) Control negative: normal non-inoculated MDBK cell line. (B) Control positive: MDBK cell line inoculated with reference strain. (C) Selected MDBK cell line with EHV-1 infection: characteristic virus particle appears ovoid or rounded with condensed dark center.

Discussion

EHV-1 is ubiquitous alphaherpesvirus affecting many mammals' especially equine populations all over the world. EHV-1 infects immune cells through which the virus can spread rapidly resulting in respiratory disorders with systemic infection, abortion, myeloencephalopathy and chorioretinopathy (Hussey *et al*., 2013). The survival of EHV-1 in horse populations is related to its ability to establish lifelong latency in a large proportion of affected animals resulting in several clinical syndromes (Hussey *et al*., 2014). EHV-1 has the ability for establishment of lifelong latency in infected animals resulting in the survival of herpesviruses in equine populations with sporadic shedding of the virus throughout the lifetime of the host (Gilkerson *et al*., 2015). EHV-1 was reported to be among the most frequent causes of respiratory disease (Hussey and Landolt, 2015). The clinical signs associated with EHV-1 include respiratory disorders, reproductive problems, central nervous system disorders and ocular disorders (Hussey *et al*., 2013). EHV-1 can cause death in horses with a course of disease of 2-15 days. In some cases; the foal will be born alive at term and will die shortly after birth. The abortion rate may approach 100% in a herd of susceptible mares. The vaccines that are currently available are the best we have but are of questionable value in preventing abortion (Amer *et al*., 2011).

In Egypt, there is no official documentation on the health status of horses or vaccination programs and EHV-1 was identified in clinical samples collected from horses which mean the continuous circulation of EHV-1 among Egyptian horses (Ghoniem *et al*., 2017).

The molecular approaches have advantages of rapidity and accuracy in detection and identification of EHV nucleic acid in field samples in comparison with the traditional methods such as virus isolation, immunohistochemical (IHC), and direct immunofluorescence method. Traditional virological methods also are very laborious and time-consuming which shed the light on the requirement for the application of nucleic acid amplification-based detection techniques, such as consensus PCR, which has already been applied in this current study as a screening assay for EHV and conventional PCR which was used for EHV typing in agreement with Balasuriya *et al*. (2015) and Ohta *et al*. (2020).

In the current study, A total of 50 tissue organs including placenta, umbilical cord, liver, lung, spleen freshly collected from aborted fetuses of 6-10 months age recently after abortion at different studs of pure-bred registered Arabian horses at different localities around Cairo, Dakahlyia and Qalyubia Governorates in the years 2021 and 2022. The collected samples were screened by consensus PCR for detection of EHVs DNA polymerase gene which showed that 26 out 50 samples with percentage of 52% were positive for EHVs. The obtained results illustrated the continuous circulation of EHVs through Egyptian northern governorates, with significant economic losses due to disruption of major horse events, horse breeding and sports industry which could threaten the stability of Egyptian horse industry as documented by Salib *et al*. (2016), Ghoniem *et al*. (2017); Azab *et al*. (2019) and Ali *et al*. (2020).

Later, molecular identification and typing of EHVs in collected specimens using conventional PCR assay was performed and revealed that all molecular positive samples were positive for EHV-1 and this explains that the incidence of abortion among equine populations was mainly attributed to EHV-1 (Equine virus abortion) as reported by Amer *et al*. (2011) and Azab *et al*. (2019) .

Tissue culture is still the golden technique for virus isolation so that in the current study the molecular detected and typed samples were propagated on MDBK cell line for three successive blind passages and showed that 19 samples developed characteristic CPE including focal rounding, increase in refractivity, and grapes like aggregates, degeneration and partial detachment of cells. These findings are in coincidence with OIE (2000) and Afify *et al*. (2017).The isolation of EHV-1 on MDBK cell line was performed in parallel with isolation of the same molecular identified and typed samples on CAM SPF-ECE of 11 days age for three successive blind passages with observation of pock lesions in 13 CAMs that increased with progressive passages as mentioned by Hassanein *et al*. (2002) and Warda *et al*. (2003). The obtained results confirmed that PCR technique on the tissues of aborted fetuses is more efficient for diagnosis of EHV-1 abortion in the aborted mares than EHV-1 isolation from fetal tissue organs using traditional cell culture techniques. World Organization for Animal Health (OIE), recommended PCR assays for the confirmation of the virus in the samples of aborted fetuses, for diagnosis of active EHV infection (Galosi *et al*., 2001; Salib *et al*., 2016). It worth mentioning that, EHV-1 could be successfully isolated on MDBK cell culture and SPF-ECE which is considered as an alternative method for isolation of EHV-1 in case of unavailability of cell culture.

EHV-1MDBK tissue culture wells were subjected to IFAT for further identification as previously done by Gunn (1992) which showed green fluorescence with illumination. Concerning with examination of selected MDBK cell line infected with EHV-1 using TEM, virus particles were demonstrated as enveloped with condensed dark center and some appears star like or rounded with electron translucent center as mentioned by Bozzola and Russel (1999). The characteristic virus particle was observed at nuclear membrane and enveloped in the cytoplasm which agreed with previous descriptions of EHV by Galosi *et al*. (1989). Demonstration of EHV-1 virions using TEM with characteristic herpesvirus morphology confirmed the EHV-1 specificity of the immunolabeling assays as previously described by Jönsson *et al*. (1989) and Del Piero *et al*. (2000). The results of the current study illustrated the high prevalence of EHV-1 which is mainly incriminated in abortion among Arabian horse populations resulting in disruption of major Arabian horse events and high economic losses that threaten the stability of the equine industry in Egypt as previously reported by Azab *et al*. (2019) and Khattab *et al*. (2022).

Conclusion

The current study confirmed that EHV-1 is mainly responsible for incidence of abortion among Arabian mares in northern Egypt in the period 2021-2022 which resulted in disruption of breeding or training schedules and horses showcasing events leading to great economic impacts on equine industry that necessitates a series of regular, comprehensive and objective measures in combination with successful vaccination strategy to combat the virus spread and shedding through equine Arabian populations in Egypt.

Conflict of interest

The authors declare that there are no conflicts of interest.

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