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Recent isolation and identification of Equine herpes viral abortion (EHV-1) in Egypt-2007

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SUMMARY

The present study was planned to isolate and identify the local Equine herpes viral abortion (EHV-1). So, samples (liver, lung, spleen, placenta and serum) of 5 aborted mares and their foeti were collected from a private stud in Egypt, with a history of recurrent abortion during (2005, 2006). Attempts for isolation of the causative agent were performed on chorioallantoic membrane of SPF embryonated chicken eggs and tissue culture (VERO cells). The highest titre was obtained from the liver of foeti which ranged from 8-9 $\log_{10}$ EID_{50} at 3rd egg passage and 7-8 $\log_{10}$ $TCID_{50}$ at 5th tissue culture passage. These results were confirmed by different serological tests (AGPT, VN test, ELISA and IF) by using reference rabbit anti-EHV-1 sera. In addition, antibodies against EHV-1 were detected in mares sera by AGPT and ELISA.

INTRODUCTION

Equine rhinopneumonitis (ER) is a collective term for any one of several highly contagious, clinical disease of equine that may occur as a result of infection by either of two closely related herpes viruses, Equine herpes virus-1 and 4 (EHV-1 and EHV-4). Both EHV-1 and 4 are worldwide in distributed and constitute an extremely prevalent health risk for domestic horses of all ages and categories.

In Egypt, Matumoto (1965) recorded the presence of EHV-1 antibodies (against Kentucky strain) in horse serum. Recently, Hassanein et al. (2002) have been isolated EHV-1 from equine aborted foeti. Infection by EHV-1 is characterized by (a) Respiratory manifestation (Rhino-pneumonitis) mainly in young horses (Palfi, 1978) (b) Abortion in pregnant mares at late stage of gestation (8-11 months) (Doll and Bryns, 1962b) (c) Neonatal foal disease, foal was borned alive and then

died within few hours or days (Campbell and Staddert, 1983) (d) Paresis or paralysis in aged horses(Jackson and Kendric, 1971).

Due to these highly economic losses which resulted from abortion of pregnant mares and death of newly born foals, the present work was planned for isolation of suspected EHV-1 from aborted foeti carried out on embryonated chicken eggs (ECE) and tissue culture (Vero cell), followed by identification of isolated virus by virus neutralization test (VNT), enzyme linked immunosorbent assay (ELISA) and immunofluorescent assay (IFA).

MATERIAL AND METHODS

Material:

Samples:

In November 2006, clinical specimens were collected from five Arabian mares suffering from abortion (placenta and paired serum samples with 2 weeks interval) and their aborted foeti (liver, lung and spleen). These specimens have been submitted to Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Equine Disease Research Department by Captain Bruce R. Boymton (CAPT, NAMRU-3). The previous specimens were used for virus isolation and identification by using different techniques.

Specific pathogen free embryonated chicken egg:

SPF ECE of 11-13 days old were used for virus isolation, propagation and titration of local isolate.

Cell cultures:

African Green Monkey Kidney (VERO) cells obtained from FADDL Plum Island, USA were used for adaptation and propagation of EHV-1 (O'Callaghan and Osterrieder, 1999 and OIE, 2000).

Antigen:

A partially purified antigen prepared from the local isolate of EHV-1 (Hassanein et al., 2002) according to method described by Azmi (1995).

Antisera:

(a) Reference antisera:

Hyperimmune sera against EHV-1 and 4 were submitted from Australia (Dr. Janet Wellington, Dept. of Biological Science, Macquaire Univ.).

(b) Locally prepared rabbit anti-EHV-1 hyperimmune serum:

Prepared according to Safaa et al. (2005)

(c) Locally prepared rabbit anti-EHV-1 immune serum conjugated with fluorescein isothiocyanate (FITC):

It was prepared by the method described by Safaa et al. (2006).

Methods:

Virus isolation:

(a) On chorioallantoic membrane (CAM) of embryonated chicken eggs (ECE):

Ten percent of tissue emulsions specimen in MEM (lung, liver, spleen and placenta of the aborted foeti) was prepared and centrifuged at 3000 rpm then inoculated on chorioallantoic membrane (CAM) of 11-13 days old SPF-ECE. The inoculated eggs were incubated at 37°C and examined daily for 5 days. The harvested CAMs were examined for characteristic pock lesions (Doll et al., 1956).

(b) On tissue culture:

The harvested CAMs of each case (5 livers of aborted foeti) which showing highest density of pock lesion were used for virus isolation. 10 % suspension of these CAMs were centrifuged and inoculated separately into confluent monolayer Vero cells (0.5 ml / 25 ml tissue culture flask), incubated at 37°C and examined daily for specific cytopathic effect (Walter and Nowotry, 1999). Five passages were carried out.

Virus titration:

The third passage of isolated virus on CAM and the fifth passage on tissue culture were titrated on SPF-ECE and on tissue culture, respectively and the virus titre was calculated according to Reed and Muench, (1938).

Neutralization test:

Virus neutralization test was used for identification of the isolated virus on tissue culture against reference anti-EHV-1 hyperimmune serum and the locally prepared antiserum against EHV-1 (Edington, 1990 and OIE, 2000).

Enzyme linked immunosorbent assay (Solid Phase ELISA):

It was used for detection and titration of EHV-1 antigen in specimens from lung and liver of aborted foeti by using locally prepared anti-EHV-1 hyperimmune serum and EHV-1 antibodies in the paired serum samples of mares against locally prepared EHV-1 antigen (Dutta et al., 1983 and Yasunaga et al., 2000).

Fluorescent antibody technique (FAT):

It was carried out to detect EHV-1 in specimens from different organs (liver, lung, placenta and spleen) by using the locally prepared rabbit anti-EHV-1 hyperimmune serum conjugated with FITC (Gunn, 1992).

Agar gel precipitation test (AGPT):

It was carried out on sera of suspected dams and tissue emulsion from aborted foeti using locally prepared EHV-1 antigen and hyperimmune sera (Tewari and Prasad, 1983).

RESULTS

Virus isolation from different tissue emulsions (lung, liver, spleen and placenta) of aborted foeti on CAM revealed that the appearance of pin headed pock lesions from the first passages which increase in their density from passage to another. The highest intensity of pock lesions was appeared from the liver of aborted foeti than other organs. The titre of isolated viral agent from the liver of aborted foeti at the 3rd egg passage (EP3) was ranged from 8 to 9 $\log_{10}$ EID_{50}/ml (shown in Photo 1 and Table 1).

The isolated viral agents (from the liver of 5 aborted foeti) at EP3 were inoculated separately on VERO cells. CPE were appeared at the first passage after 5 days incubation period (IP) which reduced to 48-72 hours at the 5th passage with the appearance of syncytial formations.

The titre at the 5th tissue culture passage ranged from 7 to 8 $\log_{10}$ $TCID_{50}$/ml as shown in Table (1).

Conducting the VN test by exposing the reference rabbit hyperimmune sera of EHV-1 and 4 to isolated agents proved that all the 5 cases were positive to EHV-1.

Concerning with ELISA test which performed on lung and liver (these producing high density of pock lesions on CAM), confirm the presence of EHV-1 antigen with titre ranged from 64 to 128 in liver emulsions and 32 to 64 in lung as shown in table (2).

Performing ELISA on paired serum samples of mares, revealed that low titre of antibodies were detected in first serum samples ranged from 20-35 while in 2nd serum samples, the titre raised to 120-230 (Table 3).

When direct immunofluorescent test performed on suspension of the collected samples (liver, lung, spleen and placenta) a yellowish green fluorescence granules were observed in all samples. The highest degree of fluorescence granules density were seen in liver suspension while the lowest in spleen suspension as shown in photos 2 and 3.

In AGPT, 1st serum samples of the 5 mares give negative AGPT while the 2nd serum samples of all the 5 mares give precipitin line against the local isolate of EHV-1 antigen (Table 4). Emulsion of tissue samples (liver, lung, spleen and placenta) of the five aborted foeti give precipitin line against locally prepared EHV-1 antisera (Table 5).

Table (1): Titration of isolated virus on ECE and tissue culture

Sample No.	Titre of isolated virus in log_{10} on:	
	ECE (P3)	TC (P5)
1	8	7
2	9	8
3	8.5	8
4	9	7.5
5	8	7

Titre expressed in log_{10} EID_{50} and $TCID_{50}$
ECE P3: Embryonated chicken egg passage 3
TC P5: Tissue culture passage 5

Table (2): Detection and titration of isolated virus in liver and lung by using ELISA

Sample No.	Titre of isolated virus from:	
	Liver	Lung
1	64 *	32
2	128	64
3	64	64
4	128	64
5	64	32

* Reciprocal of viral antigen dilution

Table (3): Detection of EHV-1 antibodies in sera of mares by ELISA

Mare No.	Sample No.	
	Sample (1)	Sample (2)
1	24	150
2	30	200
3	25	160
4	35	230
5	20	120

Table (4): Detection of EHV-1 antibodies in sera of mares by AGPT

Mare No.	Sample No.	
	Sample (1)	Sample (2)
1	-	++
2	-	+
3	-	++
4	-	++
5	-	+

Table (5): Detection of EHV-1 antigen in tissue emulsion of aborted foeti samples by AGPT

Organ	Foetus No.				
	1	2	3	4	5
Liver	+++	+++	+++	+++	+++
Lung	+++	++	+++	+++	++
Spleen	++	+	++	++	+
Placenta	++	+	++	++	+

Photo (1): CAM of ECE showing pock lesions

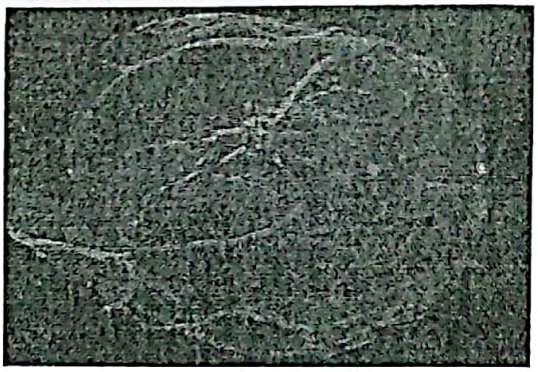

Photo (2): Strong yellowish greenish illumination with shining granules in liver of aborted foeti using FAT (Mag. 40x)

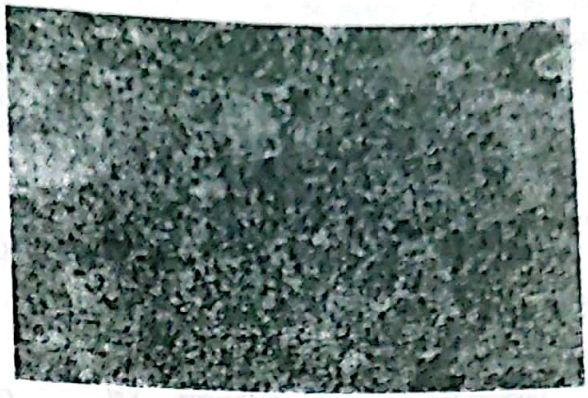

Photo (3): Diffuse yellowish greenish illumination in spleen of infected foeti using FAT (Mag. 40x)

DISCUSSION

EHV-1 disease is an important disease affecting equine, the dangerous of this disease appeared through a storm of abortion in pregnant mares at last trimester of pregnancy, deaths of newly born foals within hours or days of age. So, this study deals with isolation and identification of EHV-1 from aborted foeti.

The results of tissue emulsion inoculation on CAM come in accordance with Hassanein et al. (2002) who concluded that the presence of pin headed pock lesions on CAM which increased by progressive passage considered the first step for virus isolation.

The pattern of CPE appeared on VERO cells inoculated with the isolated virus agreed with Plummer and Waterson (1963) and Safaa (2007) who said that syncytial formation is characteristic CPE for EHV-1.

Results of serological investigation (Virus Neutralization, ELISA, Immunofluorescent test and AGPT) is in coincidence with OIE (2000) who stated that VN test is the chief test for typing the isolated virus, Dutta et al. (1983) who detected EHV-1 antigen by ELISA. This result was similar to that obtained by De-Simon and Lodetti (1971) who stated that antibodies labeled with fluorescein isothiocyanate showed efficiency for rapid detection of EHV-1 in field samples and Tewari and Prasad (1983) who applied AGPT direct to field samples from cases of equine abortion.

From the previous work and results, we can conclude that the viral agents which isolated from all cases were identified as EHV-1 by using standard and locally prepared antisera and this is considered the 2nd record of EHV-1 in Egypt as the 1st record was by Hassanain et al. (2002). This work considered a preliminary step for tissue culture vaccine preparation from the locally identified isolated strain.

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عزل وإستبيان لفيروس الإجهاض المعدى فى الخيول فى مصر 2007

نهال صالح عبد الرحمن ، إيمان محمد عبيد ، ماجدة أنيس قلد ، إبراهيم محمد أحمد سليمان

معهد بحوث الأمصال واللقاحات البيطرية - العباسية - القاهرة

أجريت هذه الدراسة لعزل وتصنيف العترة المحلية من فيروس الإجهاض المعدى فى الخيول. تم تجميع عينات (كبد، رئة، طحال، مشيمة ومصل) من خمس فرسات وامهارها المجهضة من مزرعة خيول خاصة فى مصر. مع وجود شكوى فى هذه المزرعة من تكرار الإجهاض فى الخيول الحوامل خلال الثلث الأخير من الحمل خلال عامى 2005، 2006. تم عزل فيروس الإجهاض المعدى فى الخيول على الأغشية الجنينية (CAM) للبيض المخصب الخالى من المسببات المرضية وكذلك على خلايا الزرع النسيجى VERO. وقد لوحظ أن أعلى تركيز للفيروس فى عينات الكبد المأخوذة من الأمهار المجهضة وكذلك نتائج معايرة الفيروس على البيض المخصب تتراوح بين لو 8 الى 9 جرعة نصف معدية لأجنة البيض المخصب عمر 11-13 يوم عند التمريرة الثالثة ولو 7 الى 8 جرعة نصف معدية للخلايا النسيجية (VERO) عند التمريرة الخامسة. تم تأكيد هذه النتائج بالاختبارات السيرولوجية المختلفة (اختبار الترسيب فى الآجار، اختبار التعادل المصلى، اختبار الاليزا واختبار الفلورسين المشع) باستخدام السيرم المرجعى المضاد لفيروس الاجهاض المعدى فى الخيول EHV-1 المحضر فى الأرانب (Rabbit anti-EHV-1 sera).