STUDY ON THE PATHOGENICITY OF EQUINE HERPESVIRUS AND SALMONELLA ABORTUS EQUI IN LABORATORY ANIMALS

By

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SUMMARY

Attentions were directed to study the pathogenicity of equine herpes virus (EHV-1) and salmonella abortus equi (SAE) in pregnant laboratory animals (Mice and Hamsters) as an available and economic murine model, experimental infection of either EHV-1 and/or SAE in pregnant laboratory animals resulted in abortion, mortalities and deaths. Tissue specimens from the internal organs of aborted animals were taken for reisolation and identification of EHV-1 on embryonated chicken egg (ECE) and agar gel precipitation test (AGPT). SAE was reisolated on specific media and identified biochemically and serologically. Additional tissue samples were fixed in 10% neutral buffered formalin for histopathological examination. The histopathological changes in internal organs of aborted and dead foeti showed massive number of leucocytic blood cells infiltration between degenerated hepatocytes, collapse in air alveoli and focal haemorrhagic area while the placenta showed coagulative necrosis with hyperemic blood vessels.

INTRODUCTION

Abortion in mares is the cause of serious economic losses in horse industry worldwide as well as in Egypt, due to variable etiological agents involving an interaction between microorganisms which may be bacteria or virus infection. EHV-1 and SAE were reported to be the most causative agents of abortion and mortalities in mares (Rooney and Robertson, 1996 and Radositits et al., 2000).

EHV-1 is one of the major pathogen causing abortion in equine with considerable economic losses in horse husbandry and production (Allen and Bryans, 1986).

The other serious agent causing abortion and mortalities in

pregnant mares was SAE (Jones and Hunt, 1983 and Ensik et al., 1993).

Because of the difficulties to study the pathogenesis of abortion in the natural host, and since laboratory animals were infected artificially in which clinical diseases as the natural host (Moore, 1995).

Because of the economic importance of equine especially the Arabian native horses in Egypt and in the world. The present work was designed to study the pathogenicity of EHV-1 and/or SAE in pregnant laboratory animals.

MATERIAL AND METHODS

Strains:

1- Virus strain:

Local viral strain of EHV-1 was isolated from cases of abortion in Equine Research Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (M.M. Hassanein et al., 2002).

2- Bacterial strain:

A local isolate of SAE was kindly supplied from Animal Health Research Institute, Dokki, Giza. The strain was confirmed through gram staining, colonial morphology, biochemical and serological reactions as mentioned by Forbes et al., (1998).

Embryonated Chicken Eggs (ECE):

Specific pathogen free (SPF) embryonated chicken eggs of 11-13 day age were used for viral isolation on chorioallantoic membrane (CAM) obtained from SPF Eggs Farm, Koum Osheim, Fayoum Governorate, Egypt.

Reference antisera:

Hyper immune sera of EHV-1 was submitted from Australia (Janet Wellington Dept. of Biological Science, Macquare University).

Virus titration:

The isolated EHV-1 on CAM was assayed and the titre was calculated according to Reed and Muench, (1938).

Experimental laboratory animals:

The present study was carried out on 24 pregnant mice as well as 16 hamsters and their infants. Moreover, a group of 10 mice were used for passage of bacterial strain.

Preparation of salmonella abortus equi suspension:

The bacterial suspension was made by plate washing technique according to Ouinn et al., (2002), of which the organism was cultured on nutrient agar and then incubated for 24 hours at 37°C.

Such incubated plate was the flooded with 5ml sterile saline and the colonies were removed by gentle rubbing with glass rods. The resulted granular suspension were adjusted to contain average of $1.5X10^8$ colony forming unit (CFU).

Pathogenicity test of EHV-1 and SAE in laboratory animals:

Pregnant mice and hamsters at late stage of pregnancy were used to investigate the pathogenicity of EHV-1 and SAE.

Laboratory animals were divided into 4 groups, each group contained 10 animals (6 mice and 4 hamsters). First group was injected with 0.5ml of 6.2 $\log_{10}$ LD_{50} intraperitoneal (I/P) and intranasal (I/N) of EHV-1. The second group was injected with 0.1ml of 24 hours pure adjusted $1.5X10^8$ C.F.U. culture of SAE suspended in saline intraperitoneally. The third group was injected simultaneously with the EHV-1 and SAE of the same dose I/P and the 4th group kept as control and injected only with sterile saline. Each group was kept separately, under general observation, any abortion or mortalities of infants were recorded, and dead animals (infant and mother) of mice and hamsters were subjected to post mortem examination, lesions of the internal organs (lung, liver and placenta) were taken for reisolation of EHV-1 and SAE and for histopathological examination.

Re-isolation and identification of EHV-1 and SAE:

Tissue specimens from infected CAM or aborted animals were homogenized, centrifuged, and the supernatants was used for re-isolation of virus by injection of CAM or pregnant mice and cultured on nutrient agar for detection of SAE. Also agar gel precipitation test was done according to Salama et al. (1977) for detection of EHV-1.

Histopathological examination:

For histopathological studies, tissue samples were collected from different internal organs mainly liver, lung and placenta from infected dead mice and hamsters and from aborted foetus. The samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm thick and finally stained with Haematoxylin and Eosin by standard technique of Bancroft et al. (1994).

Results and Discussion

As foal production is limited and seasonal, a murine model as susceptible laboratory animal for EHV-1 and SAE infection were used, where it shows remarkable similarity to infection as in natural host (Field et al., 1992) and Hassanein et al. (2002). In this

respect, Awan et al., (1991) indicated that adult mice are suitable model for infection and studying the pathogenicity of EHV-1. Also, Inazu et al., (1993) and Kirisana et al., (1995) stated that adult pregnant mice can be used as an abortion model for studying the pathogenicity of EHV-1. Moreover, Allen and Bryans (1986) clarify that there is similarity between horse and mice when they infected with EHV-1 where abortions occurred in the late stage of pregnancy.

Van Hoosier and Charles (1987), also found that EHV-1 is highly pathogenic to adult hamsters and deaths occurs 1-5 days after I/P inoculation. The I/P infection in pregnant mice was performed on the hypothesis that a higher viral concentration in that route may have a severe effect on neonates. These results are in agreement with those found in the present study.

Figures (1) and (2) showed the histopathological findings of the internal organs of aborted laboratory mice infected with EHV-1 where figure (1) shows liver of dead aborted mice with severe hyperemic centeral veins and figure (2) illustrate lung of dead aborted mice with hyperemic peri-bronchiolar and peri-alveolar blood vessel and capillaries.

Figures (3) and (4) illustrate the histopathological findings of the internal organs of aborted laboratory hamster infected with EHV-1 where figure (3) shows the liver of dead aborted hamster with mature and primitive leucocytic cells infiltration and degenerated hepatocytes and associated with dilatation of central vein while figure (4) shows the lung of dead aborted hamster with an emphysema in the air alveoli and it collapsed. These results are in complete agreement with those of Banirsckle et al., (1978).

EHV could be re-isolated from the previously infected internal organs of laboratory animals on CAM which showing typical pock lesions, also when these infected CAM injected in pregnant mice, it induced abortion and when subjected to AGPT produced precipitation line. Percy and Barthold (2001) studied the pathogenesis of SAE in laboratory animals. After injection of a virulent culture, invasion of the lymphatic system and multiplication of bacilli and invasion to general circulation producing bacteraemia and secondary invasion to genital tract. Bacteria then multiply in the epithelium of embryonic villi which undergo fatty degeneration and autolysis at connection with maternal placental leading to abortion.

Figures (5) and (6) show the histopathological findings of the internal organs of aborted mice infected with SAE where figure (5)

shows the liver with severe congestion of the central vein. Massive number of primitive and mature leucocytic cell infiltration were observed in between the hepatocytes and figure (6) shows the lungs with hyperemic of the blood vessels and inflammation. These results were also reported by Maronpot et al., (1999) and Percy and Barthold (2001). The bacteria could be re-isolated from the infected tissues of aborted laboratory animals and when reinfect pregnancy mice with SAE, it is aborted. These findings are in parallel with that described by Bayd (1990).

Figures (7), (8) and (9) clarify the histopathological findings of the internal organs of aborted animals infected with both EHV-1 and SAE. Figure (7) shows coagulative necrosis in the central portion with hyperemic blood vessels in placentas, while figure (8) shows karyomegalic nuclei in the affected liver in figure (9) the infected lung shows hyper vascularity and dilatation of blood vessels associated with focal haemorrhagic area. These results are incomplete agreement with these mentioned by Benirsckle et al., (1978); Maronpot et al., (1999) and Percy and Barthold (2001). Group (4) in which pregnant laboratory animals kept as control non infected group showed no histopathological changes. EHV-1 and SAE were not reisolated.

The data presenting in table (1) revealed that laboratory mice and hamsters of groups 1, 2 and 3 showing about 80-90 cases of abortion and/or infant mortalities when exposed to experimental infection with EHV-1, SAE and EHV-1 + SAE respectively. Non of the laboratory animals of group 4 (Non infected control) had a case of abortion and/or infant mortalities. Such finding confirm the extreme susceptibility of mice and hamsters to experimental with EHV-1 and/or SAE. Those findings were similar to those described by Maronpot et al. (1999).

From the above study, it could be concluded that:

- The route of inoculation either I/P or I/N, provide similarities in abortion and histopathological changes.
- Mice is more economic and more reliable model than hamster, also they produced abortion in late stage of pregnancy and could be useful in producing vaccines for use in horse in the future.

Acknowledgement

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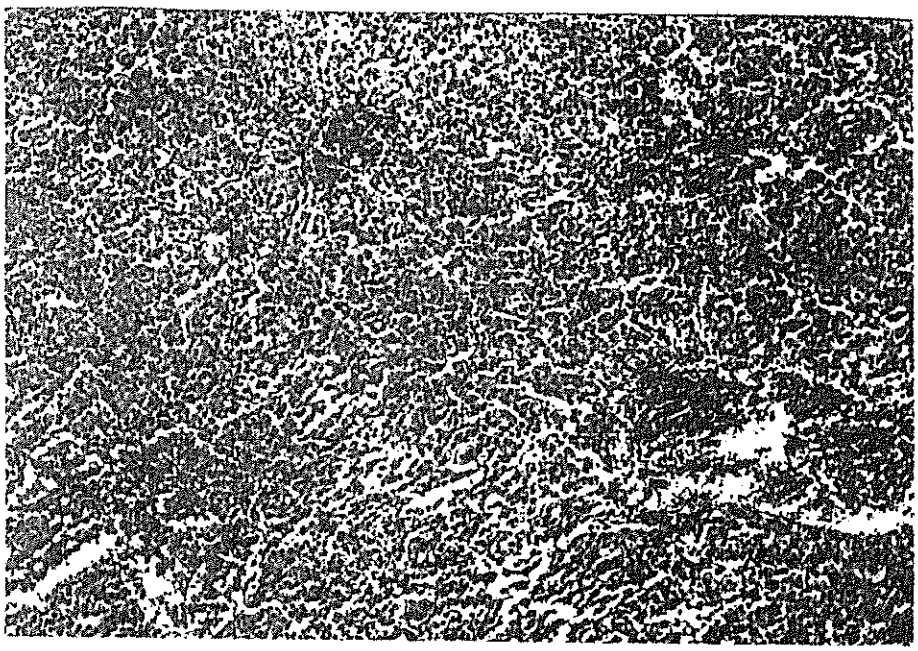

Fig. (1): liver of aborted dead laboratory mice of mother inoculated by EHv-1 showing severe dilatation of the central veins allover the hepatic tissue (H & E X40).

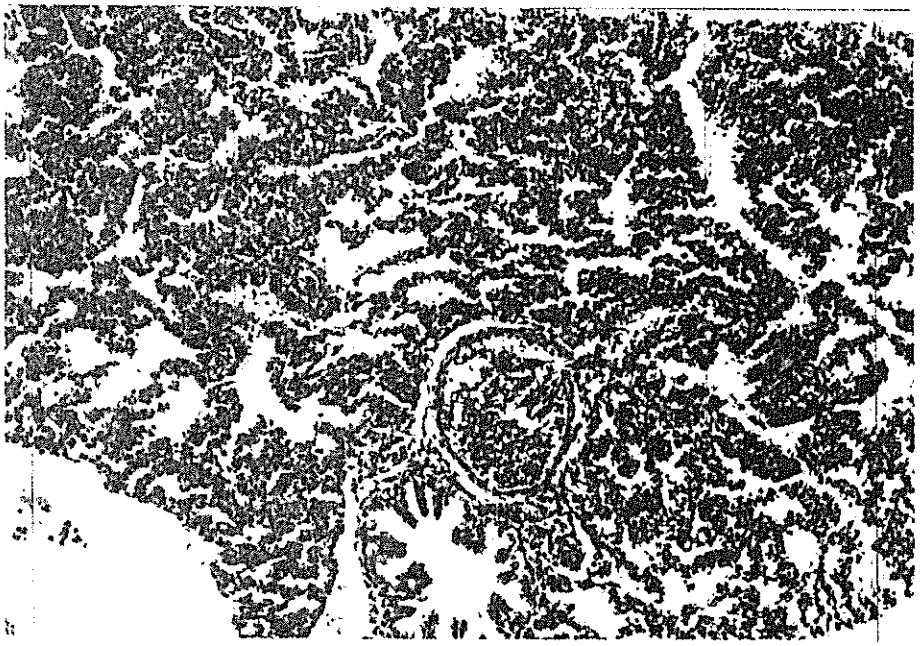

Fig. (2): lung of aborted dead laboratory mice of mother inoculated by EHv-1 illustrate the peribronchoilar blood vessel and perialvealar capillaries were dilated and engorged with blood (H & E X40).

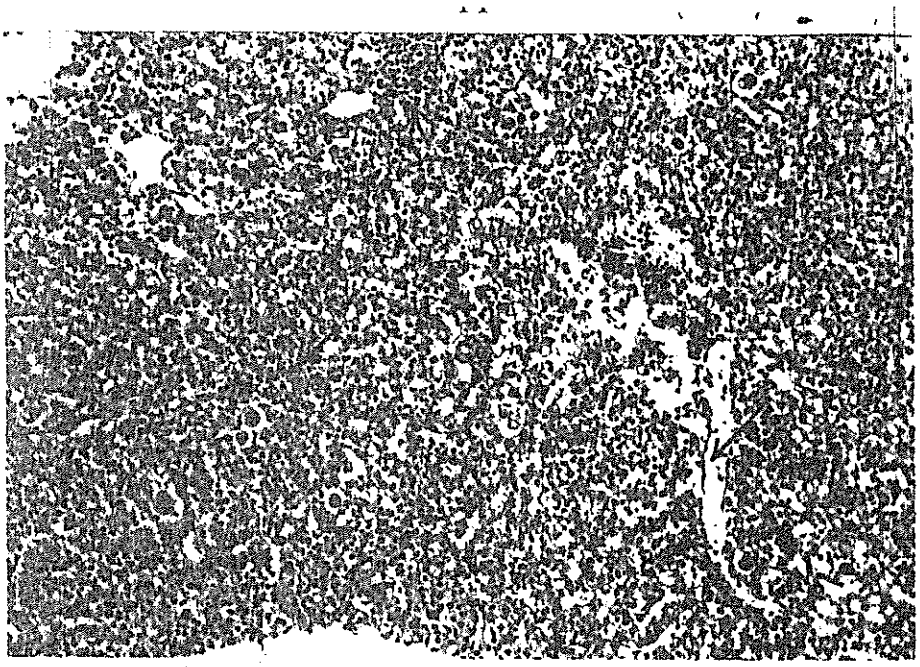

Fig. (3): liver of aborted dead laboratory hamster of mother inoculated by EHv-1 showing number of leucocytic blood cells infilteration (primiative and mature mononuclear cells) in bewteen the dagenerated hepatocytes associated with dilatation of central veins (H & E X40).

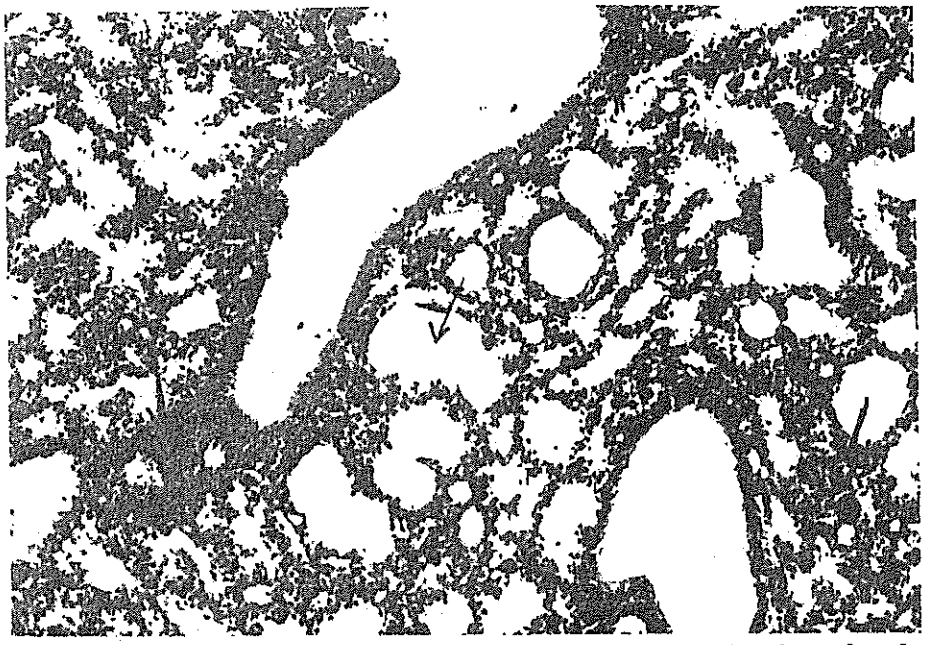

Fig. (4): lung of aborted dead laboratory hamster of mother inoculated by EHv-1 showing emphysema and collapse of air alveoli. (H & EX40).

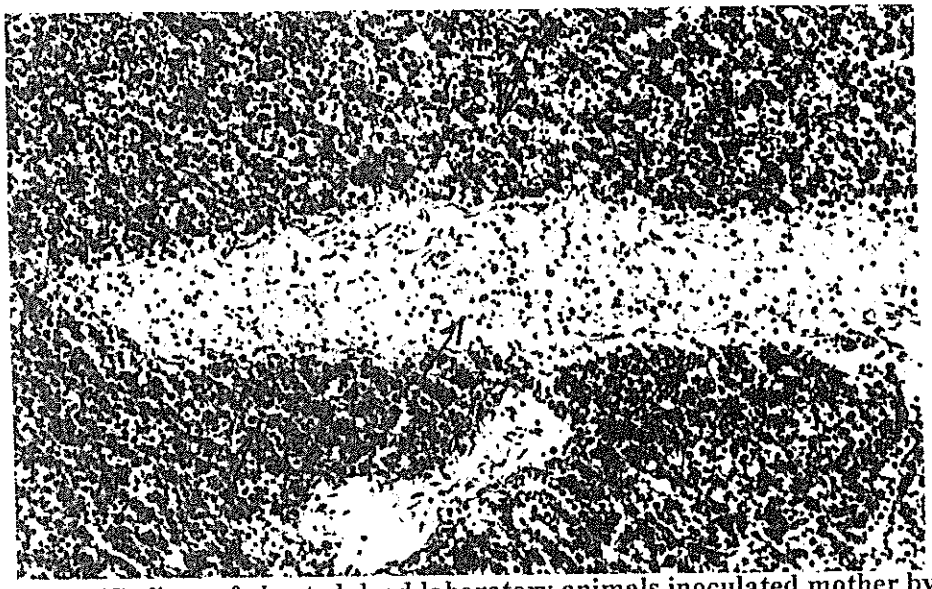

Fig. (5): liver of aborted dead laboratory animals inoculated mother by SAE showing severe dilatation of the central vein which impacted by blood cells associated with massive number of leucocytic cells infiltration (mature and primitive mononuclear cells) in the hepatic tissue (H & E X40).

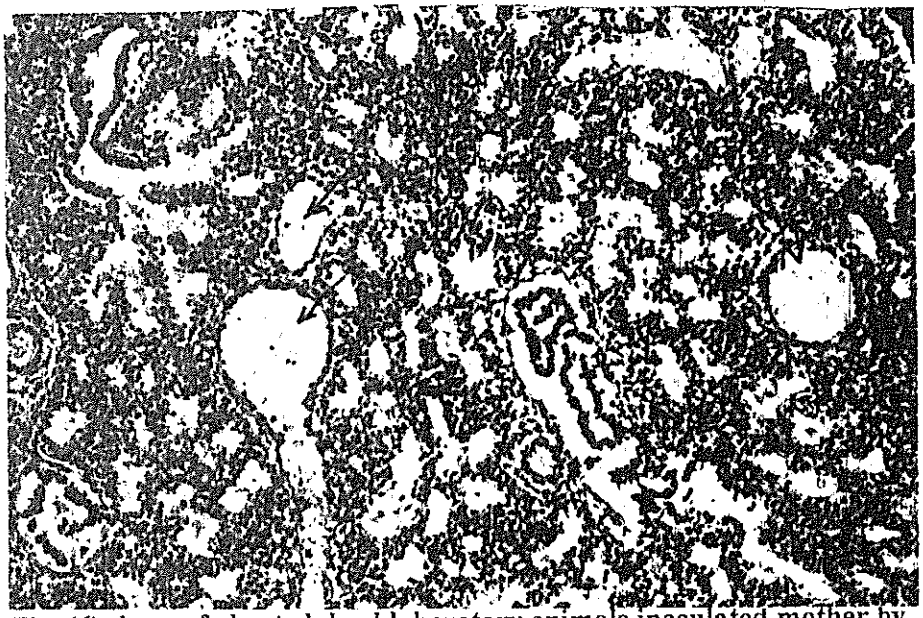

Fig. (6): lung of aborted dead laboratory animals inoculated mother by SAE showing hyperemic blood vessels with inflammation (H & E X40).

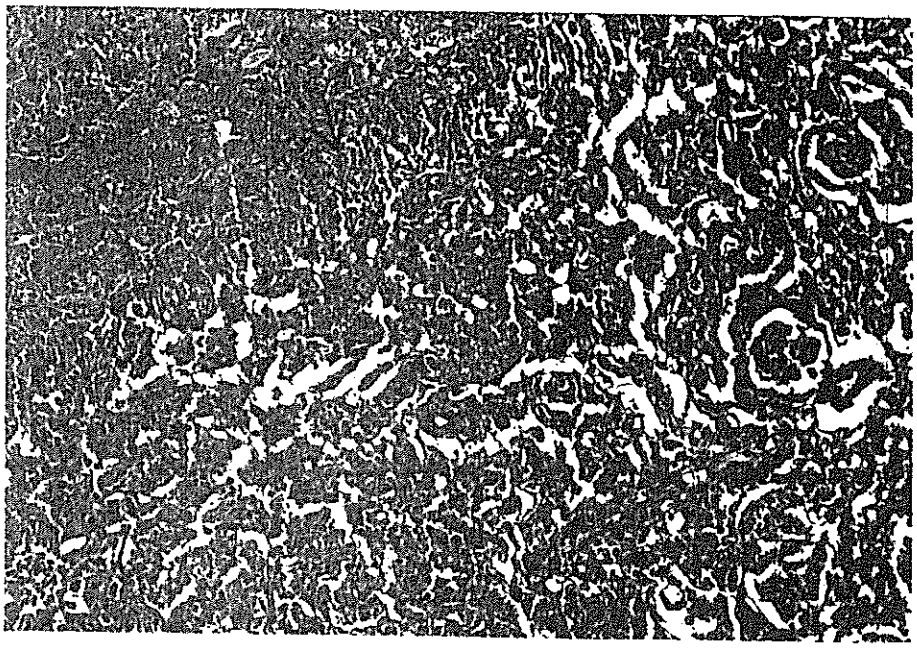

Fig. (7): placenta of inoculated mother by both EHv-1 and SAE showing coagulative necrosis in the central portion with hyperenic blood vessels (H & E X40).

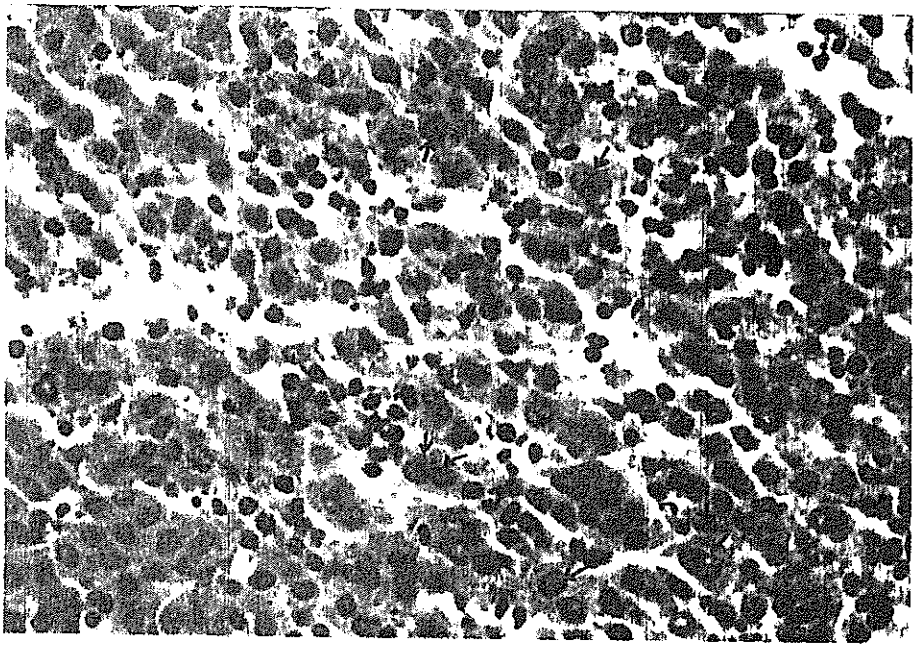

Fig. (8): liver of embryo from aborted inoculated mother by both EHv-1 and SAE showing karyomegalic nuclei (H & E X40).

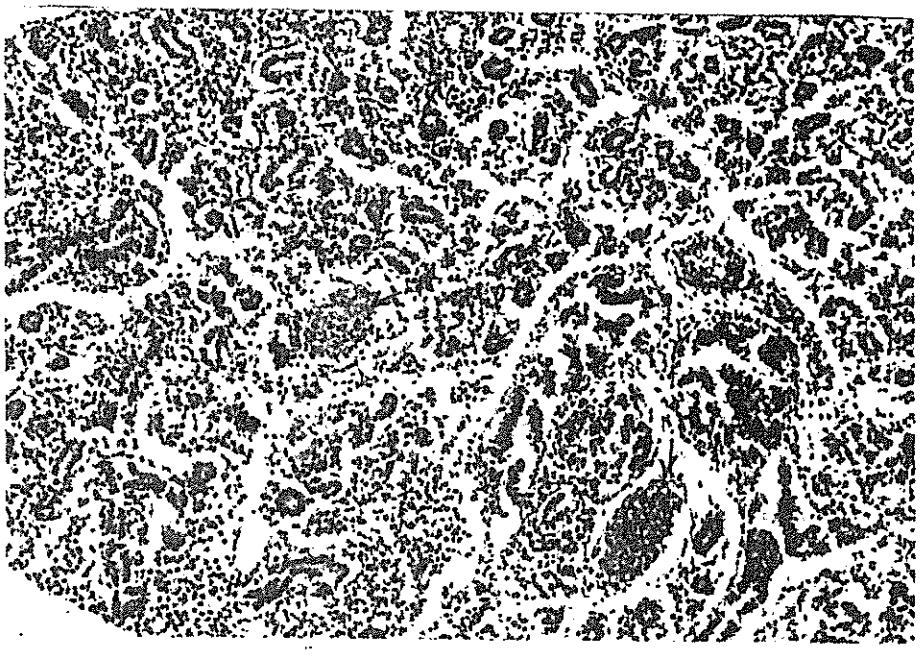

Fig. (9): lung of embryo from aborted inoculated mother by both EHv-1 and SAE showing hyper vascularity with dilatation of the blood vessels associated with focal haemorrhogic area (H & E X40).

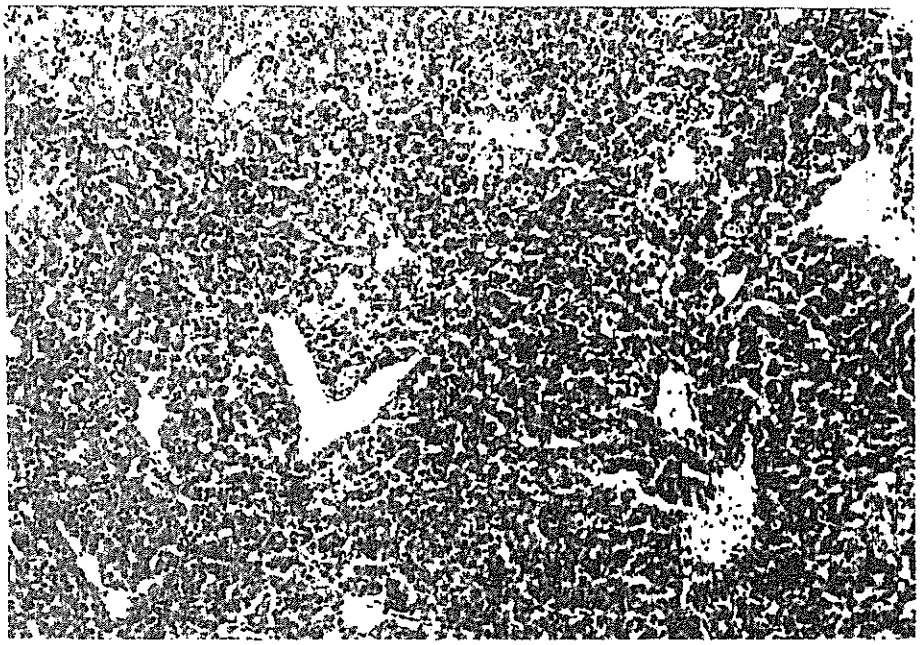

Fig. (10): liver of baby laboratory animals from control group showing the normal histological structure (H & E X40).

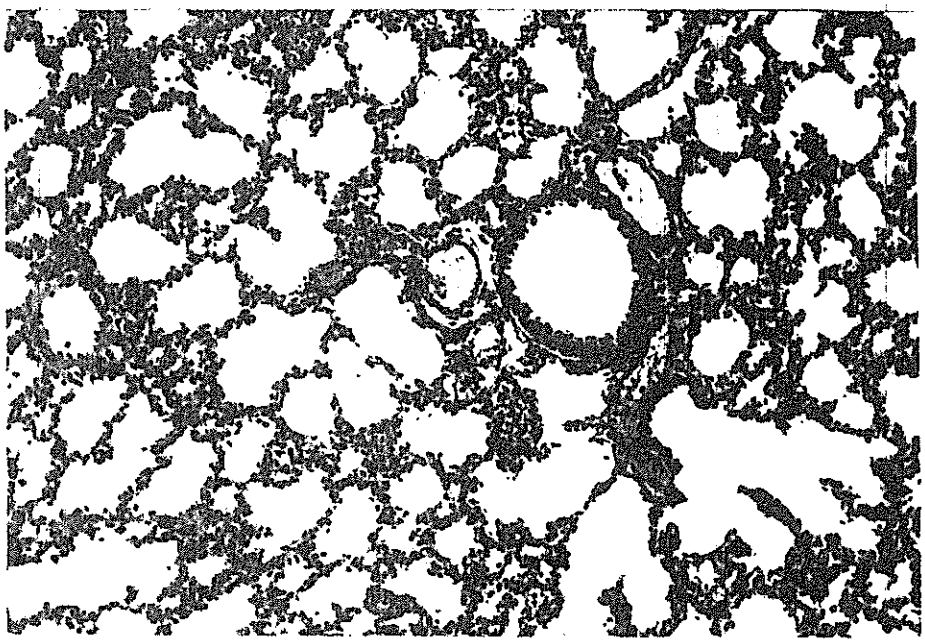

Fig. (11): lung of baby laboratory animals from control group showing normal histological structure (H & E X40).

Table (1): Gross pathological responses of mice and hamsters to experimental infection with EHV-1 and/or SAE

Group	Infecting agent	Pathological response post infection			
		Mice		Hamster	
		Abortion	Infant mortalities	Abortion	Infant mortalities
1	EHV-1	5/6	8/10	2/4	6/8
2	SAE	5/6	9/10	2/4	5/8
3	EHV-1+SAE	6/6	10/10	3/4	5/8
4	Non infected control	0/6	0/10	0/4	0/8

N.B. Number of mice used are 24 (6 of each group).
Number of hamsters used are 16 (4 of each group).

الملخص العربى

دراسة الحالة المرضية لكل من فيروس الاجهاض المعدى للخيول والسالمونيلا المجهضة للخيول فى حيوانات التجارب

ماجدة أنيس قلد و صفوت كمال روفائيل

معهد بحوث الأمصال واللقاحات البيطرية – مركز البحوث الزراعية – الجيزة

أجريت المحاولات لدراسة الحالة المرضية لفيروس الاجهاض المعدى للخيول والسالمونيلا المجهضة للخيول على حيوانات التجارب العشر (الفئران والهامستر) كنموذج اقتصادى متاح0 وأثبتت العدوى التجريبية لكلا الميكروبين على حدة أو مجتمعين فى حيوانات التجارب العشر حدوث إجهاض وموت. وأخذت عينات من الأعضاء الداخلية للحيوانات النافقة والمجهضة لعزل فيروس الإجهاض المعدى للخيول باستخدام البيض المخصب، اختبار الترسيب فى الأجار وايضاً لعزل السالمونيلا المجهضة للخيول بزراعتها على الأوساط البكتيرية المختلفة والتعرف عليها بالاختبارات الكيميائية والسيرولوجية، وجد أن التغيرات الباثولوجية فى الأعضاء الداخلية لحيوانات التجارب النافقة والمجهضة هى وجود ترسيب متجمع من خلايا الدم البيضاء فى الكبد. أما الرئتين فأوضحت إنضغاط فى الشعب الهوائية مع وجود إحتقان بالأوعية الدموية بالإضافة إلى تنكرز فى المشيمة مع أرتشاح دموى.

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