REOVIRUS ASSOCIATED WITH NEONATAL HYDROCEPHALUS IN RABBITS

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Abstract

Reovirus was isolated from commercial rabbit colonies in farms at Kafr El-Sheikh and El-Gharbia Governorates with a history of neonatal hydrocephalus with eye affections in form of blindness. The affected neonates exhibited hydrocephalus at age from 3-20 days with seasonal incidence at a late summer and early autumn (August, Sept. and Oct.)1999-2002. The gross lesions observed at necropsy were bulging of the skull, cerebral hemispheres and parenchyma were collapsed and replaced by accumulated colourless, transparent cerebrospinal fluid (CSF). Virus isolation was conducted through chorio-allantoic membrane (CAM) of embryonated chicken eggs. Identification of virus by electron microscope examination revealed presence of a virion particles having a morphological appearance of reovirus. Serological tests were also conducted. Furthermore, the pathogenicity of isolates were studied at different ages of rabbits. Histopathological examination of naturally and experimentally infected rabbits revealed marked loss of neurons, severe oedema, vacuolation with focal gliosis and dilatation of brain ventricles. Eosinophilic intracytoplasmic inclusion bodies were detected in infected CAM of embryonated chicken eggs.

INTRODUCTION

Hydrocephalus is a form of oedema in central nervous system and refers to the slow accumulation of excess CSF within the ventricular system (internal hydrocephalus) or within the subarachnoid space (external hydrocephalus) (*Jones and Koestner, 1997).*

Hydrocephalus was reported as an inherited abnormality or congenital deformity among newborn rabbits *(Lieve, 1988* and *Sandford 1996). Pond et al. (1995)* reported that signs of vitamin A deficiency and toxicity are similar with major

effects on reproduction, low conception rates, fetal resorption, low survival of newborn kits and hydrocephalus in fetuses with toxic level. On the other hand, *(Abd El-Raheem et al. 2000)* attributed neonatal hydrocephalus in rabbits to vitamin A deficiency, while *Fetaih et al. (2001)* recorded that ochratoxin A has adverse effects on reproductivity of rabbits through inducing stillbirth, abortion and teratogenic lesions in the offspring , particularly hydrocephalus.

Some *reovirus* strains may cause abortion and congenital abnormalities including hydrocephaly, ataxia and cerebral hypoplasia in sheep and cattle *(Fenner et. al. 1993 and Murphy et al. 1999). Nathanson et al. (1997)* proved that intracerebral inoculation of *reovirus* into newborn mice, infects ependymal cells lining cerebral ventricles resulting in hydrocephalus.

The aim of this work is to investigate the viral etiology of hydrocephalus in neonate rabbits and its related pathological changes.

MATERIALS AND METHODS

Specimens

A total of 12 rabbit's colonies in farms at Kafer El-Sheikh and El-Gharbia Governorates were investigated during the period (1999-2002). The new born rabbits of 3-20 days of age with seasonal incidence at late summer and early autumn (August, Sept., Oct.) exhibited hydrocephalus with blindness.

Post-mortem examination was conducted and gross lesions were recorded CSF was aspirated aseptically and also brain tissues were collected and processed under aseptic conditions for virus isolation trials.

Fertile chicken eggs were obtained from private farms and Sakha governmental poultry farm; they were used for virus isolation, propagation, titration and identification.

Virus isolation

Embryonated chicken eggs 9-12 days-old were inoculated through CAM with 0.2ml/egg of suspected suspension treated with antibiotic, daily candling of eggs for 7 days, deaths recorded within the first 24 hours post-inoculation were excluded as nonspecific, after that embryos died were examined, embryonic fluids and CAM were harvested for further passages. Five blind passages were carried out before titration and identification of the isolates.

Hemagglutination activity of isolated virus was studied through human (O) type erythrocytes at room temperature according to *Madbouly (2003).*

Virus titration was carried out according to *Villgas and Purchase (1989).*

Avian reovirus positive control serum

Was obtained from KPL proflok® Kirkegaard & perry Laboratories USA (VET LIC. No. 350), for serological studies.

Serological examination

Serum samples were collected from rabbit does whose progeny exhibited hydrocephalus and also serum samples were collected from the progeny which suffered from hydrocephalus for estimation of antibodies against toxoplasma. (TOXO–HAI, Lab. FUMOUZE- France) as well as agar gel precipitation test (AGP) and virus neutralization test for *reovirus* according to methods described by *Beard (1989).*

Transmission Electron Microscopic Examination

Amnio-allantoic fluid (AAF) and oedematous chorio-allantoic membranes with clear multiple pock lesions were collected after five blind passages, and stored at –20 °C then, ground and homogenized in a sterile mortar. Freezing and thawing procedure was repeated three cycles, coarse particles were sedimented by centrifugation at 3000 rpm for 30 min.. The supernatant fluid was collected and checked for sterility to be free bacteriologically.

Ultracentrifugation for supernatant fluid through sucrose cushion to deposit the virions at 30.000 rpm for 30 min was conducted (twice) decant the supernatant

and the sediment was stained with uranyl acetate then, coated the copper grid. Electron micrograph examination was made using TEM ZEISS EM10 (Germany) at HT 60 kv .

Pathogenicity tests

1-Twenty (20) one-day old New-Zealand rabbits from flocks with no history of hydrocephalus were used for testing the pathogenicity of *reovirus* isolates. The rabbits were allotted into four groups (A, B, C, D) five in each and kept in separate units.

-Group A, B, C were orally, intramuscular, intracerebral respectively infected with 0.2 ml/rabbit of *reovirus* isolate containing 10^6 /ml, (ELD, 50) while, group D was used as non-infected control. All infected and control rabbits were observed daily for a period of 20 days for clinical signs and mortality.

2- Five (5) pregnant does New-Zealand, at 10 days of gestation period were experimentally inoculated by intramuscular route with 0.4 ml of *reovirus* isolate containing 10^6 /ml, while, two pregnant does were used as non-infected control. All rabbits were observed until parturition and daily clinical examination for new born rabbits for a period of 15 days.

Histopathological examination

Tissue specimens were collected from the brain of both naturally and experimentally infected neonatal rabbits as well as CAM showing pock lesions harvested from embryonated chicken eggs inoculated with the isolated reovirus. Tissue samples were fixed in 10% neutral buffered formalin and then, processed routinely for paraffin embedding techniques. Embedded tissues were sectioned at 4-6 microns thickness and stained with haematoxylin and eosin (H&E) and examined microscopically (Bancroft *et al.*, 1996).

RESULTS

Clinical & post-mortum examination

The new born rabbits suffering from congenital developmental abnormalities exhibited hydrocephalus with doming of the head dorsally and blindness as well as nervous manifestation in the form of incoordination, torticollis, circling and inability to

stand or walk poorly (Fig.1). The affected rabbits still alive after 15 days showed severe emaciation and poor body condition.

Post-mortem examination of hydrocephalic rabbits showed marked enlargement of skull, the brain tissues were severely atrophied and represented by very thin layer enclosing accumulated CSF, in the form of sac containing serous transparent fluid. The cerebrai hemisphere became collapsed after incision. In addition, some affected cases exhibited xerophthalmia. No characteristic lesions were observed in the other organs.

Virus isolation and titration

The inoculated chicken embryos died 4-6 days post-inoculation showed sub-cutaneous hemorrhages and CAM was odematous, hemorrhagic with characteristic pock lesions (Fig. 2). Embryos died after 7 days showed stunted growth, greenish discolouration of the liver with development of necrotic foci (Fig .3).

The isolated virus agglutinates human "O" type erythrocyte rapidly within one minute at room temp. and the titer of isolated virus was 10^6 / ml (ELD, 50).

Serological examination

The results of serological examination of serum samples from does and hydrocephalic neonates were negative for *toxoplasmosis*, and negative in AGP test for avian *reovirus*, further more, the virus was not neutralized by specific positive avian *reovirus* serum.

Transmission electron microscope examination

Negative staining of the electron microscope revealed that the viral particles were visualized as bright objects against a dark background. *Reovirus* virions are non-enveloped, nearly spherical in outline with icosahederal symmetry, the capsid is characteristically double-shelled and the viruses' aggregation occurs as single or double capsid particles about 80 nm in diameter (Fig.4).

Pathogenicity tests

Experimental oral infection in suckling neonates rabbits with isolated *reovirus* resulted in severe diarrhea and retarded growth within 12 days post-infection without neurological signs. Mortality was 60%, the main gross lesions were catarrhal enteritis, meanwhile, intramuscular infection showed mild diarrhea and mild nervous manifestation including drowsiness, in-coordination and tremors within 10 days post-infection. Mortality was 40%, the main gross lesions were enteritis and the cut section through the cerebral hemisphere showed varying degrees of odema.

Intracerebral infection in suckling rabbits exhibited neurological signs rapidly at 6 days post-infection in the form of in-coordination, tremors, torticollis, spasmodic convulsion and hyper-excitation. Mortality was 60%. The main lesions were mild enlargement of skull after 15days post-infection, the brain tissues were markedly odematous with prominent dilatation of brain ventricles. Experimental I/M infection of pregnant does not induced any abnormal signs or behaviour, although their progenies were free from congenital developmental abnormalities, but severe diarrhea was observed at 7-10 days of age with 80% mortality of neonates.

Histopathological examination

In naturally infected neonates severe atrophy of nervous tissues, most of neurons were completely absent and cerebral tissues were represented by oedematous vacuolated neurepile and remnants of granular cell layer (Figs. 5&6).

In experimentally infected newborn rabbits, the brain cortex showed prominent neuronal degeneration and necrosis represented by condensed deeply eosinophilic cells with loss of demarcation between nucleus and cytoplasm (Fig. 7). The neuronal changes seemed to begin with perineuronal oedema especially the Pyrkinje cells, perivascular oedema was constant findings in both cortex and medulla (Fig.8), and the latter showed marked vacuolation or status spongiosis (Fig.9). Focal gliosis and neuronophagia were occasionally detected (Fig.10). The brain ventricles were severely dilated with focal degeneration, necrosis and desquamation of ependymal cells with focal gliosis of adjacent nervous tissues (Fig.11). Focal meningial hemorrhages were occasionally observed in subarachnoid space of cerebral cortex (Fig.12).

The microscopical examinations for CAM of embryonated chicken eggs inoculated with isolated virus revealed presence of poorly demarcated eosinophilic intra-cytoplasmic inclusion bodies (Fig.13)

DISCUSSION

In the present study, the clinical signs, lesions of the naturally infected new born rabbits, isolation of the viral agent in embryonated chicken eggs, pathogenicity tests and histopathological findings as well as detection of *reovirus* particles by transmission electron microscope, confirm the association of *reovirus* with hydrocephalus in neonatal suckling rabbits.

The epidemiology and seasonal incidence of the disease at late summer and early autumn (August, Sep and Oct.) was not fully documented. However, many mammalian *reoviruses* are transmitted by arthropods and their epidemiology depends on interaction between each of the following host, vector, climate and the virus, which may clarify the common occurrence of this case in the late summer where the vectors are numerous. Some *reovirus* strains may cause abortion and an epidemic of congenital abnormalities in sheep and cattle characterized by hydranencephaly and cerebral hypoplasia (Fenner *et al.,* 1993 and Murphy *et al.,* 1997).

Serological techniques including AGP test and virus neutralization test by using isolated *reovirus* and specific avian *reovirus* serum revealed no serological cross reaction between mammalian and avian *reovirus* strains. This may be attributed to the presence of a group-specific antigen in avian *reoviruses* which is discernible with gel diffusion techniques and a serotype specific antigen demonstrable with neutralizing antibody in plaque reduction or chicken embryo assays (Van der Heide, 1977, Rosenberger and olsen 1997).

Reovirus infects ependymal cells lining the cerebral ventricle in newborn mice and hydrocephalus develops as consequence of ependymal cells sloughing and obstruction of the aqueduct of sylvius as well as blockage of cerebrospinal fluid outflow from the fourth ventricle (Nathanson *et al.*, 1997). The neuronal changes

recorded in the present study could be attributed in part to mechanical pressure exerted by accumulated CSF in the brain ventricle (specially in naturally infected cases) as well as to actual infection by the viral agent as it had been reported that *reovirus* had a tropism to both ependymal cells producing hydrocephalus and/or neurons producing meningio-encephalitis in mice, (Weiner *et al.* 1980). Pathological changes in the present study were similar to those recorded by Abd El-Raheem *et al.* (2000) and Fetaih *et al.* (2001). Absence of clinical signs and lesions in the experimentally inoculated pregnant does could be returned to that mammalian *reovirus* exhibited different degrees of neurotropism in suckling mice, but restricted to newborn (Flamand *et al.,* 1991). This result also agreed with that reported by Conrat *et al.* (1988).

On the other hand, experimental infection of newborn rabbits resulted in brain oedema and prominent dilatation of the brain ventricles, but not fully predominate hydrocephalus, as well as absence of hydrocephalus in newborn rabbits from experimentally infected pregnant does. This may be attributed to multiple etiologies inducing congenital hydrocephalus, such factors including vitamin A deficiency and toxicity (Pond *et al.,* 1995), vitamin A. deficiency (Abd El-Raheem *et al.*,2000), intoxication with ochratoxin A(Fetaih *et al.,* 2001). In addition, Dellepiane (1990) and Benko (1991) recorded hydrocephalus in rabbits due to *encephaltozoonosis*, *Toxoplasmosis* and *Listeriosis.* Moreover, Jubb and Huxtable (1993) mentioned that the inducing factors of congenital hydrocephalus are usually obscure. Meanwhile, *Saif (1992)* reported that immuno-suppressive agents have been shown to exacerbate the pathogenesis of *reoviruses* in chickens.

It could be concluded that *reovirus* may be acting in association with other factors (nutritional, immuno-suppresive agents, age, vectors and climate) in induction of neonatal hydrocephalus in rabbits.

According to the available literature, this is the first report of isolation of *reovirus* from neonatal rabbits affected with hydrocephalus.

Further studies are required to clarify more about this infection in rabbits.

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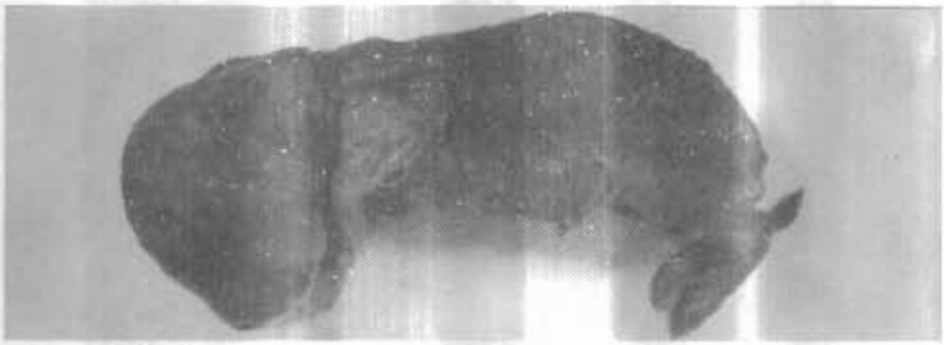
List of figures



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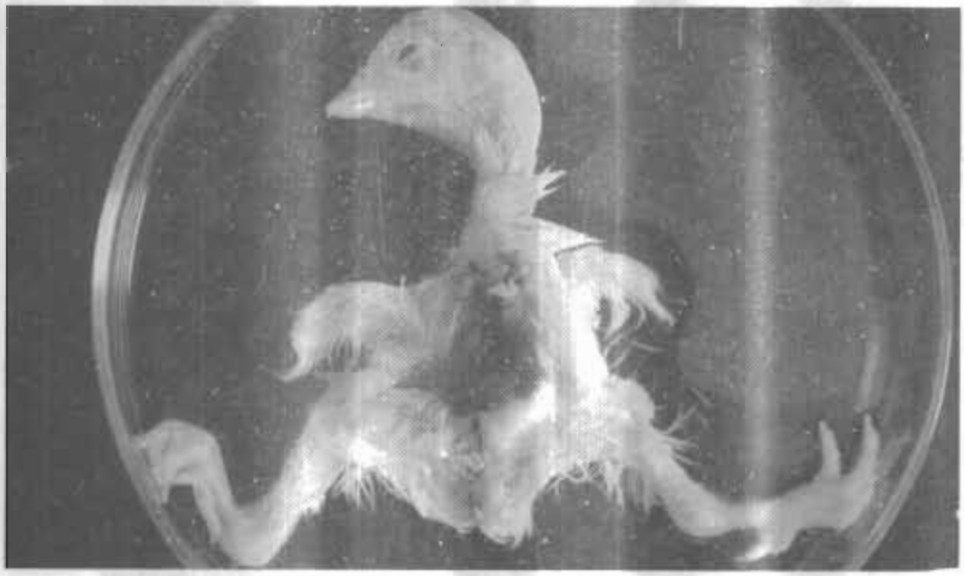

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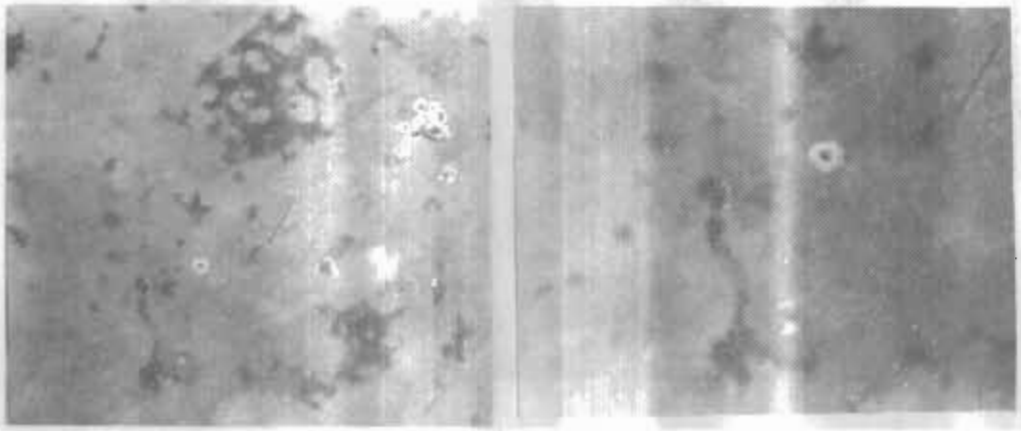

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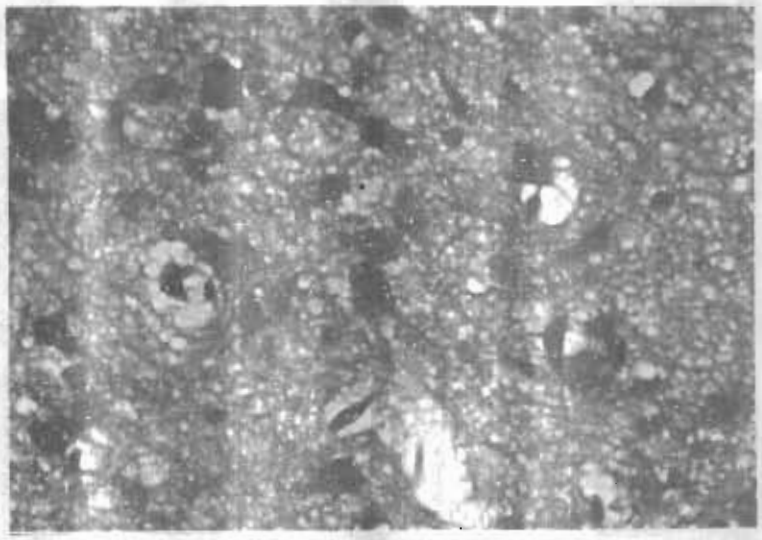

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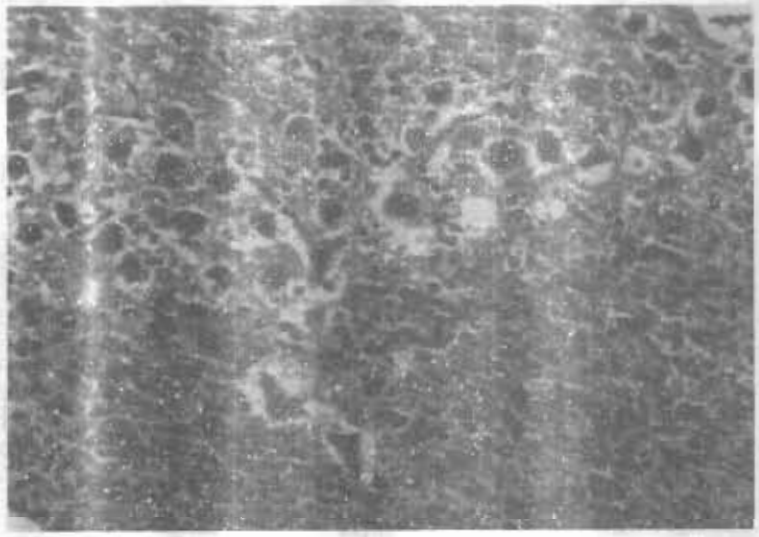

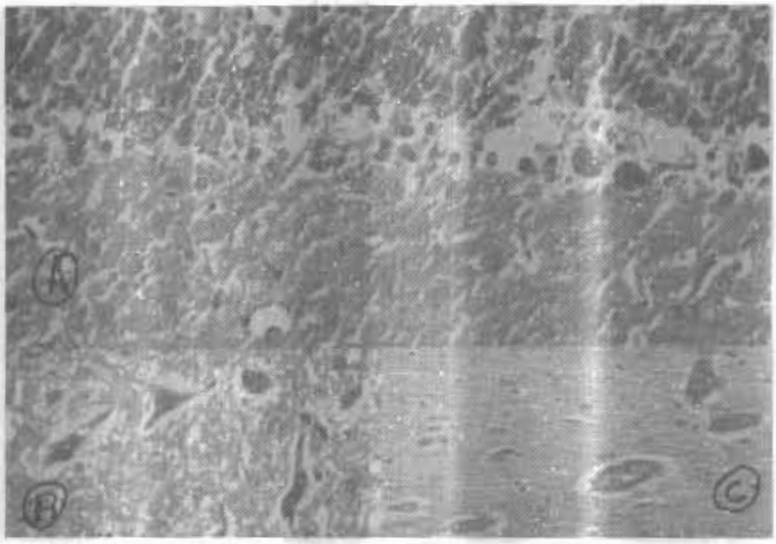

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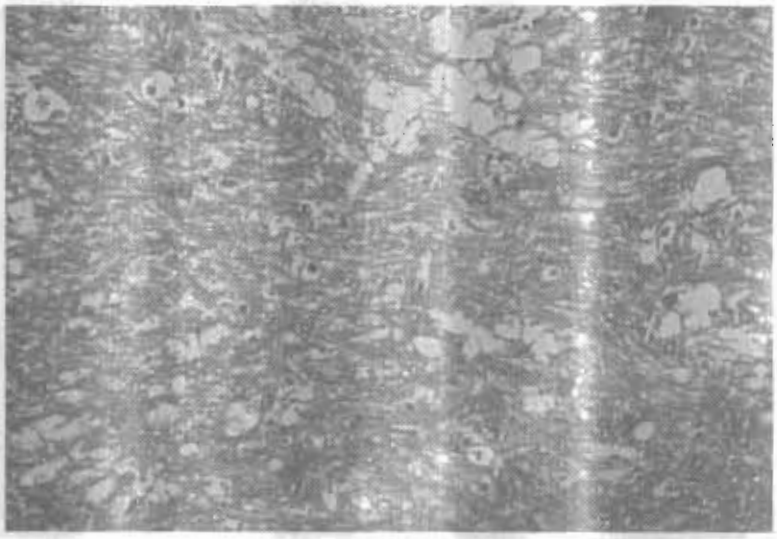

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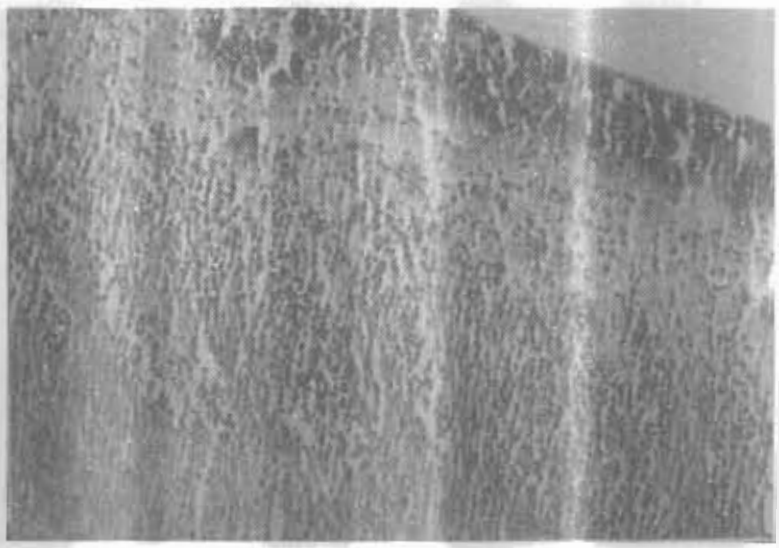

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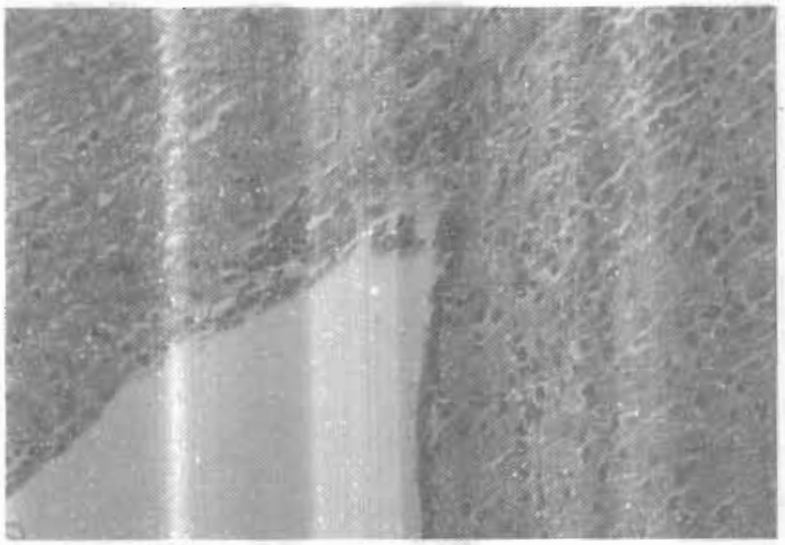

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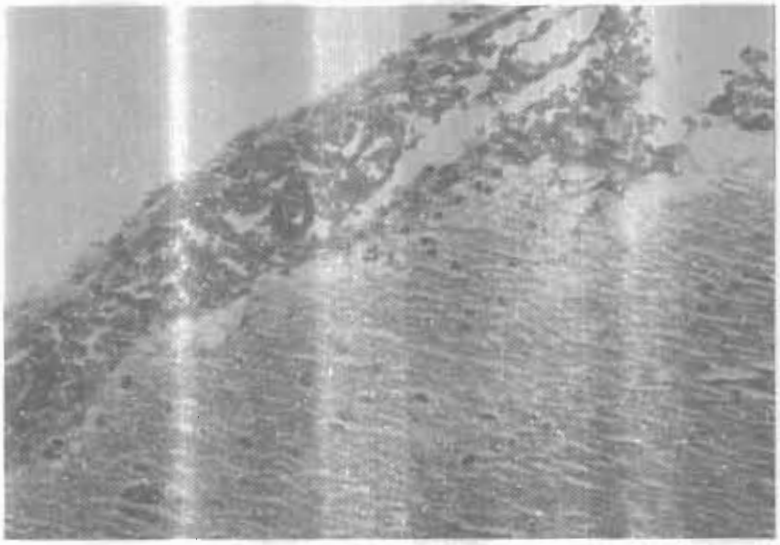

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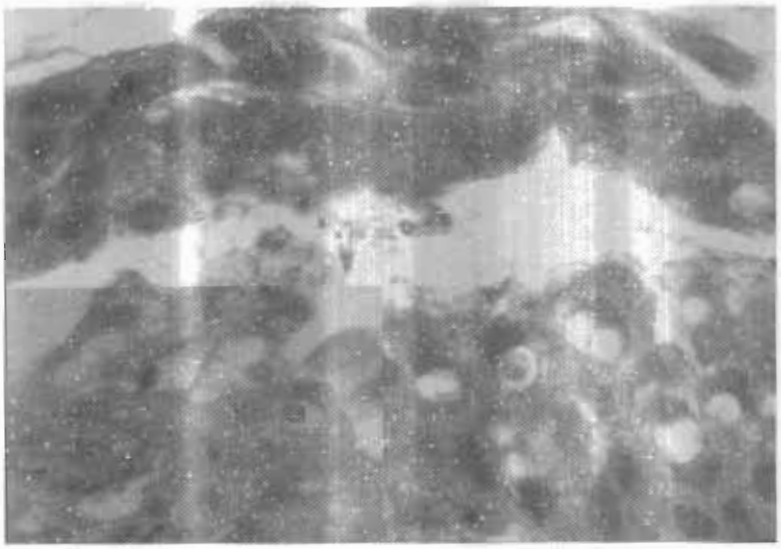

فيروس الريو كعامل مسبب في تضخم الرأس المائي في الأرانب الوليدة

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في هذه الدراسة تم عزل فيروس الريو من مزارع الأرانب في محافظتي كفر الشيخ و الغربية من الأرانب الوليدة من عمر ثلاثة حتى عشرين يوما وبها تجمع مائي في المخ وعمي كلي في بعض الحالات . وكانت الإصابات جميعها في نهاية فصل الصيف وبداية الخريف خلال أشهر أغسطس و سبتمبر وأكتوبر من عام ١٩٩٩ حتى عام ٢٠٠٢ وقد تم عزل الفيروس في أجنة بيض الدجاج المخصب وتم التعرف علي الفيروس من خلال الفحص الميكروسكوبي الإليكتروني والعدوى الصناعية في الأعمار المختلفة من الأرانب وكانت نتيجة الفحص النسيجي الباثولوجي عبارة عن استحالات مرضيه وتنكرز خلوي في الخلايا والأنسجة العصبية في كل من القشرة المخيه والنخاع وكذلك تم الاستدلال علي وجود أجسام احتوائية داخل سيتوبلازم الخلايا المصابة في الأغشية الجنينيه لأجنة الدجاج.