ISOLATION AND IDENTIFICATION OF SPRING VIREMIA OF CARP VIRUS(S.V.C.V) IN SILVER CARP (HYPOPHATHALMICHTHYS MOLITRIX) AND COMMON CARP (CYPRINUSCARPIO.L) IN EGYPT .

By

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SUMMARY

The spring viremia of carp virus is one of the rhabdoviruse diseases which affecting fish especially cyprinids. It is isolated from diseased carp species(common & silver) in Egypt for first time on specific tissue culture media prepared from carp ovary (C.O .).Electron microscope examination determined the bullet shape of rhabdoriruse and electron dense particle with hallo core .

Immunohistochemistry technique using monoclonal antibody of SVCV and anti mouth IGg revealed the brown coarse granules which indicate virus presence .The histopathological examination showed intranuclear & intracytoplasmic inclusion bodies , eosinoplilic granules which indicate RNA virus in different organs (liver & brain & kidney & gas bladder).

INTRODUCTION

One of the main factors affecting fish production and efficiency allover the world is the fish diseases especially that resulted from viruses. Nowadays, the increased knowledge about the significance of viral diseases in fish populations with the current interests in fish culture and management, lead us to demand further development for viruses and their importance in fish culture. *(Habashi, 1980 and Way et al., 2003).*

The aim of this work was to isolate and identify the S.V.C.V by different methods .

Materials and methods

I-Materials:-

1- Fishes:

Cage cultured fish samples, were collected from Alexandria, Behera and Kafr-El-Sheikh Governorates. They were related to *Silver Carp (Hypophathalmichthys molitrix)* and *Common Carp (Cyprinus carpio-L).*

The fishes were subjected to clinical, bacteriological, mycological and parasitological examination according to (Bucke and Finlay, 1979) .

Samples from liver, kidney, spleen, muscles, intestine, heart, brain and gas bladder were mixed with sterile sand and grinded in sterile morter, freezed and thawed for about 3 times.

Three drops from mycostatin 1000 I.U/ml, penicilline, 1000 µg/ml, streptomycin and anystation 500 I.U/ml were added to the supernatant.

The supernatant was collected in small flasks and kept in refregerator at - 20 C until used, according to the method implied by *Bucke and Finlay (1979).*

-Histopathological examination

From naturally infected fish specimens from, brain, kidney , liver, muscles, gas bladder and intestine were fixed in 10 % neutral buffered formalin and used for histopathological examination according to *Charlton, (1967) and Culling (1983).*

-Electron microscope examination:-

Samples were prepared for electron microscope examination according to *Karlsson, (1976).). Anderson, (1967) and Jerome et al. (1995*

-Immunohistochemistry:

It was done on 20 random samples that preserved in the formaline paraffin by using S.V.C. Monoclonal antibody in addition to Anti-mouse Ig (sigma pharma ceutical comp.) , treated with D.A.B solution for 30 minute. Counter stained for 30 second with fast red, dehydrated in 100 % alcohol, cleared in Xylene and then mounted with DPX matrix according to the method implied by *(Kiernan, 2003).*

Immunohistochemical of anti-SVCV antibodies monoclonal was used on paraffine section of different species of Carp and different organs. Depend on the positivity degree –ve , ++ve moderate , +++ve marked, ++++ve strong, +++++ve intense the results were recorded.

-Tissue culture:

The steps of primary cell layers from sexually mature commom carp gonads was made according to the methods implied of *Habashi (1980)* and used for determination of $TCID_{50}$ according to *Reed and Muench (1938)* .

RESULTS

The clinical signs and PM lesions of naturally infected fish:

The clinical signs and PM lesions of naturally infected fishes, were skin darkening, tail and fin rot, ulceration , haemorrhage on the abdomen and swelling of anal opening , ascitis and congestion of isthmus and head region . While post-mortem lesions were, congestion of all internal organs , distended gall bladder, haemorrhagic gas bladder and congestion of gills and brain with exophthalmia and swelling of anal opening (Fig. 1, 2)

-Bacteriological, mycoclogical and parasitological examination:

The results of bacteriological, mycological and parasitological examination were proved to be negative in all examined samples except two fish from which *A. hydrophila* was isolated .

Immunohistochemistry studies:

The samples from Silver carp number 83, 55 and 76 and number 105, 74 and 101 from Common carp were found to be positive for SVCV according to brown labeled granules of di amino benzidine (DAB) of immunohistochemistry chromogene.

Results of Cytopathic effect due to viral infection and $TCID_{50}$:-

The CPE formed within 3 – 4 days post-infection (P/I) in which cells changed from spendle, round, detatched and formed plaque like, finally death of cells was occurred (Fig.3) . The $TCID_{50}$ were $10^{2.75}$ in case of sample 83 and were $10^{3.5}$ in case of sample 55 .

-Results of histopathology:

A- In naturally infected Silver carp(Hypophathalmichthys molitrix):

1- Liver :

The exocrine pancreatic cells are pychnotic , there was pyknotic acinar cells in pancrease with atrophy of paranchyma of hepatic cells. The cells become un-differentiated, the cytoplasmic eosinophilic garnules was decreased and disappeared of stored materials. The nucleus became small and pychnotic. Also, there was focal necrosis and infiltration of RBCs forming congestion and increse no of degenrated bile ducts (Fig. 4).

2-Kidney:

-The main features of the naturally infected Silver carp were dilatation of the blood capillaries , thickning of the walls with alteration of the basal lamina, destruction and fibrosis of the glumeruli. Also, there was a hyaline droplet degeneration of epithelial cells as a typical change in the renal tubule (Fig. 5).

3- Brain :

It showed nerve cells with pale eosinophilic , large nuclei with vacculated cytoplasm. Necrotic and atrophy of granulosa layer. Also, shortage of nerve bunddele, congestion and haemorages of nucleated R.B.C.s in wide blood vesseles (Fig. 6 and 7) were recognized.

4-Gas bladder :

Histopathological changes of posterior chamber of gas bladder in naturally infected Silver carp showed multi-lobular gas gland with oval sphincter, the epithelial layer was desquamated and thick layer of connective tissue degeneration of nuclei and elastic fiber of tunica-externa, dilatation of blood vessels with congestion were found.

The main condition of virus infection showed red nuclei of some epithelial cells of gas gland. (Fig. 8).

B- In naturally infected Common carp (Cyprinus carpio-L). :

1- Liver :

The liver tissue characterized by fatty degeneration showing foamy structure in the cytoplasm of the liver cells where fat was originally present, so, the fatty liver generally refers to pathological condition. Also, there was necrotic hepatic cells. The neuclei appeared pyknotic and undergo karyolysis refered as massive necrosis. On the other hand there was vaccular degeneration. The vaccules contain a dilated proteinus colloid and the other gave red colour that indicated to the virus infection (Fig. 9).

2-Kindey:

It showed extensive damage of the renal tubules replacement by interstatial lymphoid tissue, dilatation of glomerulus capsules lobules and thickness of basal lamina of Bowman's capsules (Fig.10).

-Results of tissue organs examined by electron microscope:-

A- In naturally infected Silver carp :

1- Liver :

Transmission electron microscope of liver of naturally infected Silver carp demonstrating the vaculated cytoplasm with lacking of organells, undifferentiated nuclear inclusion resemble the virus-like particles. The virus particles emerges from the nucleus into cytoplasm. High magnification showed different sizes of virus particles including the heterochromatin and euchromatine-like intranuclear inclusion having electron dense particles with clear halo (Core). The virus particle aggregate in the nucleas, as well as, nuclear membrane and can be indentified by characteristic nucleocapside or envolope (Virions) (Fig. 11).

2- kidney :

Showed vaculated nuclei with dark dense body of virion attached the nuclear envelop (replaced the hetero-chromatine) dense body of virus-like structure depressed in the cytoplasm and other cell has vesicles surrounded of virion containing virus-like particle. Large quantities of virus nucleocapsids with matrix viral precursors materials was found. The viral particles replicating cytoplamic organells. High micrograph from the above figure showed large amount of electron dense bodies or aggregated in groups making (intracytoplasmic inclusion electrone dense) having the same structure of capside viruse like particles of other infected cells. (Fig. 12).

B-Naturally infected Common carp:

1- Brain:

Showed tunica granulosa cell nuclei forming intra-nuclear inclusion bodies and vaculated cytoplasm. The viral particles replicated the cytoplams organlles and absent of myline sheath were shown. At high magnification showed different size and shaped of virus-like particle they consisted of capsid particles with (a) visible core (b)homogenous electron-dense contents and (c) empty capsids (Fig. 13).

-Results of immuno-histochemistry:-

A- In naturally infected Silver carp:

1-Liver:-

The hepatopancrease gave the main common features which give marked positive (3+ve) of anti- SVCV detection in all exocrine pancreatic cells. Which accounted an intense granulation of virus like precipitated and more vacuolated cytoplasm of morphological virus infection was shown by oil magnification (Fig. 14).

2-Kidney

The good feature of virus detected by immuno-histochemical of anti-virus S.V.C anti-body was visualized in the kidney of (Silver carp). The proximal tubular cells have a large account of virus precipitate. Whereas the high amount of brown granules was detected at base region of proximal tubular basement membrane cells. The coarse brown granules of S.V.C virus detection at base region of proximal tubular cells was shown clearly. (Fig. 15).

B- In naturally infected Common carp :

1- gas bladder :

In naturally infected Common carp, the gas bladder revealed from moderate to marked positively of anti-virus infection detected in epithelial cells and sub-mucosa. It was, markedly in epithelial cell but moderately in sub-mucosa (Fig. 16).

DISCUSSION

- The clinical signs & P.M. lesions of naturally infected fish were in the form of skin darkening , tail & fin rot with the P.M findings of generalized hyperemia and congestion of all internal organs and distended gall bladder in most of examined fish . The above mentioned clinical signs and P.M. lesion which accompanied with naturally infected samples appeared in the fish are due to the causes other than the bacteria and mycotic and parasitic agents . which may be due to viral infection . *(Schaperclaus, 1965 ; Bucke and Finlay, 1979).*

- Bacteriological examination of these naturally infected fish reveled 2+ve farms (No. 47 & 94 infected with A. hydrophila while mycological & parasitological examination indicated negative results .

The presence of the virus in the tissue confirmed by cytopathic effect (CPE) formation. The degree of CPE formation correlated positively with the degree of the virulence and dilution rate of the virus as the high concentration of the viruses the high degree of CPE formation and the low concentration of the virus the lower degree of CPE formation.

These results agreed with those of *Bucke and Finlay (1979)* they found that a cytopathic effect (CPE) was observed after four days with cells becoming rounded and detached from the cell sheet . Faisal and Ahne (1984) reporded that the $TCID_{50}$ of SVCV was $15^{3.8}$, the differences in $TCID_{50}$ values may be due to types of cell lines and virulence of isolated virus .

*** Histopathologicaly In naturally infected fish :**

The changes in the liver which observed in the infected fish of either Common or Silver carp attributed to the destructive effects of the virus.

Jiang and Ahne (1989), studied the virus infection in some marine fish species and clarified that the hepatic tissue in case of such infection showed focal hypertrophy of the liver and multifocal hepatocellular necrosis. The hepatic affection in such cases was constant finding in all fishes.

The destruction and fibrosis of glumeruli may be due to glumerluar nephritis resulted from the effect of SVCV on the kidney. This agreed with those of *Sanders et al. (2003); Way et al. (2003)* they recorded that the interstitial nephritis resulted from affection of SVCV on the kidney.

The changes in both of brain and swim bladder may be due to the multiplication of the virus in these cells causing severe destruction.

Similar results were reported with *Roberts (1989),* who stated that most of the viral diseases cause lymphocytic reaction, plasma cell activation as well as lymphoid hyperplesia.

Electron microscopical observation

Confirmed the presence of the virus in tissue culture , results were in the form of cellular vaculation appeared in ovary cell culture within 3 – 4 days post-infection by visceral organs of common carp *(Bucke and Finlay, 1979).* It also, confirmed the histopathological changes and demonstrated many of the cytopathic effects commonly associated with the presence of viruses in cells, fine structure of virus-like particles.

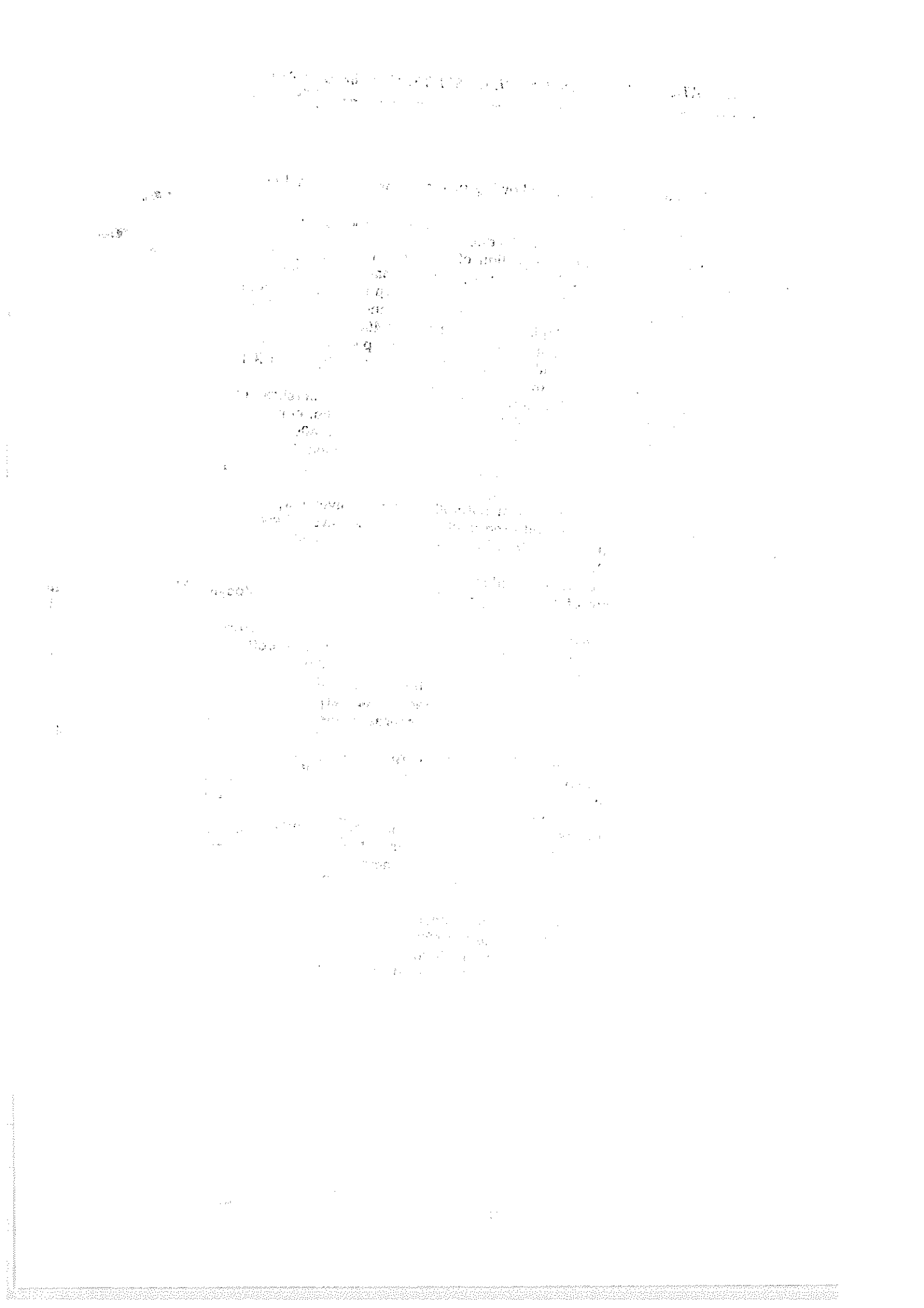
The E.M results confirmed the SVCV infection in silver and common carp fish and consider as one of the most important tools for identification and diagnosis of SVCV in case of infectious dropsy in Carp fish which supported by the finding of *(Bekesi and Szabo, 1979)*

Immunohistochemistry studies

Using specific monoclonal antibody against SVCV 6 out of 20 random samples collected from naturally infected fish were positive, the infected samples from Silver Carp were 83, 55 and 76 and the samples number 105, 74 and 101 from Common carp. According to the presence and the strength of the brown pigmentation of immunohistochemistry indicated the severity of infection and concentration of the virus in the tissues.

Ibrahim (2002) Diagnosed SVCV antigen from C. carpio by using antigen capture ELISA.

Based on the results of CPE on cell line, immunohistochemistry by using specific monoclonal antibodies, E.M and PCR we can confirmed the positive isolation of SVCV from both of C. carp and S. carp in Egypt for the first time.



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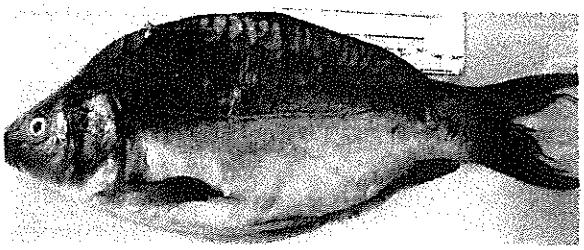

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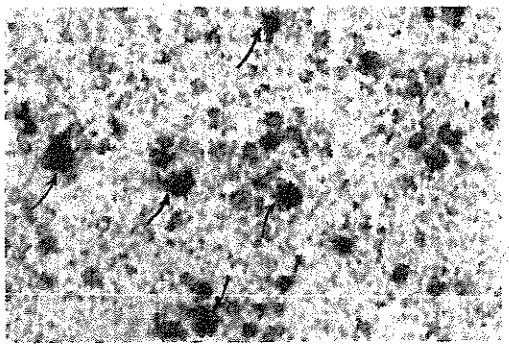

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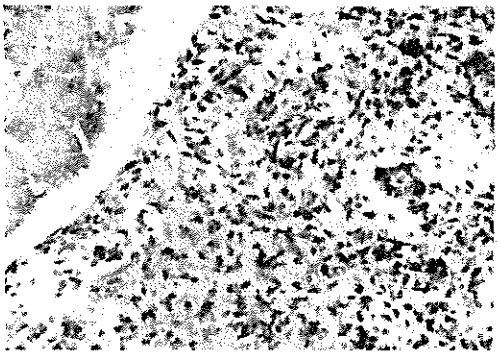

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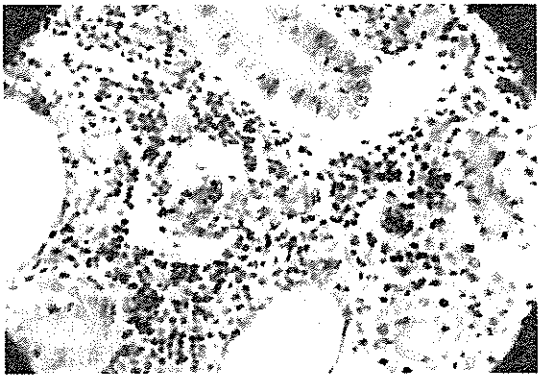

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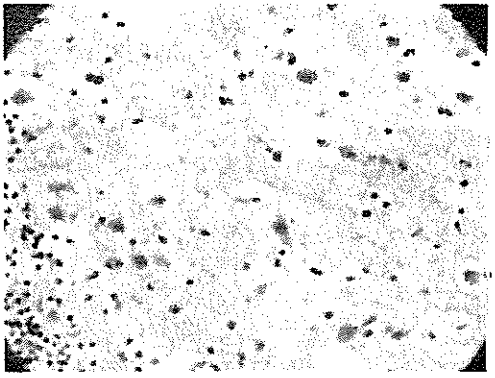

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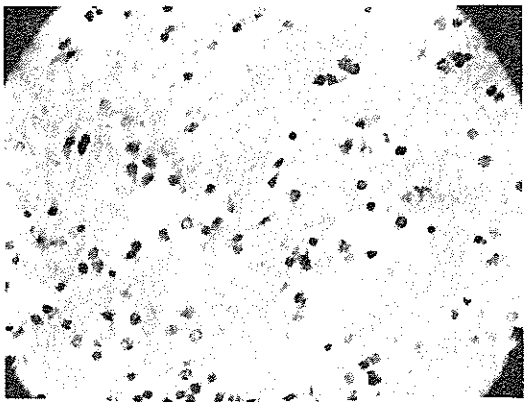

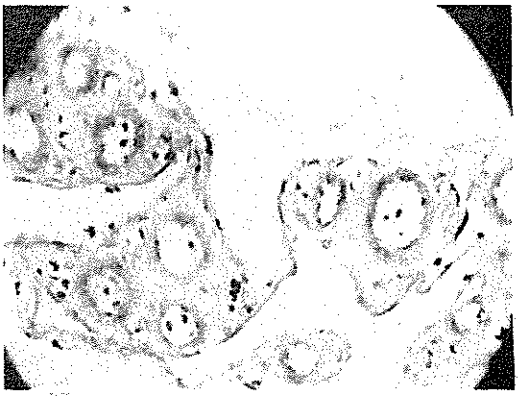

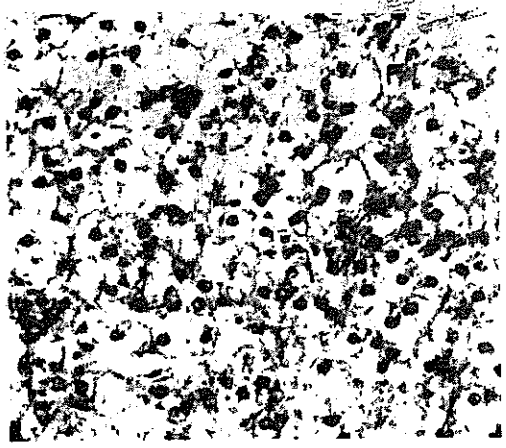

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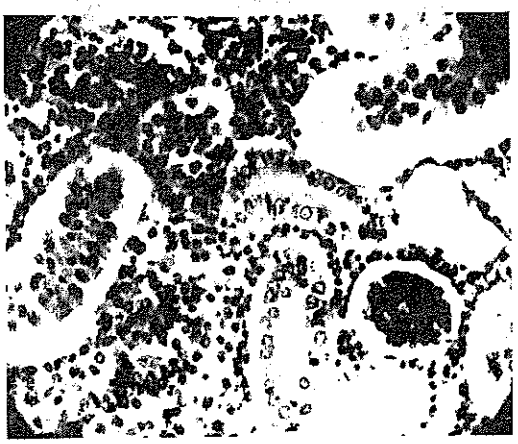

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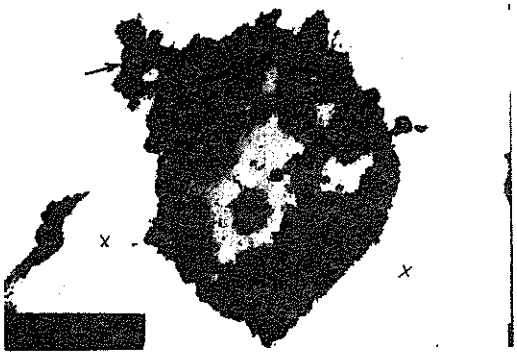

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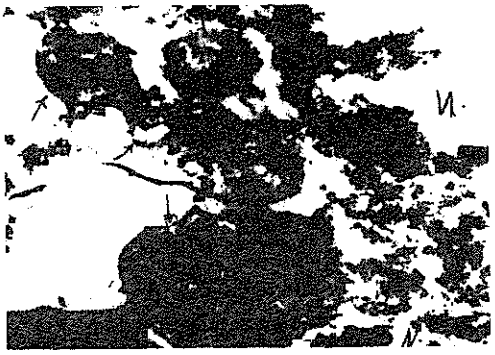

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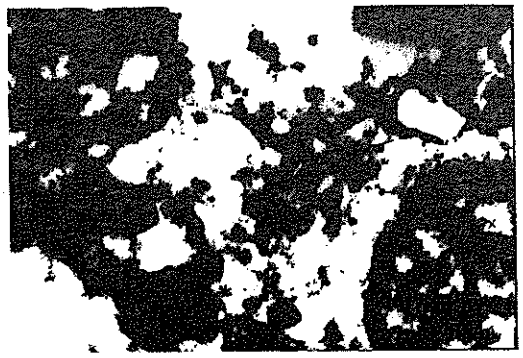

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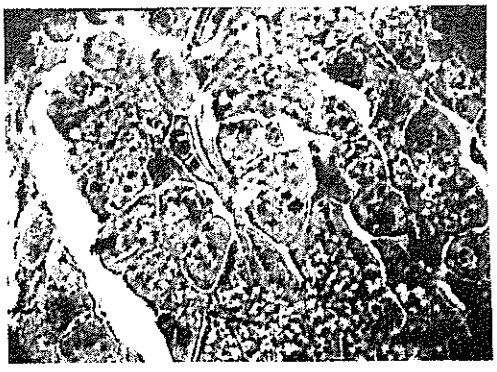

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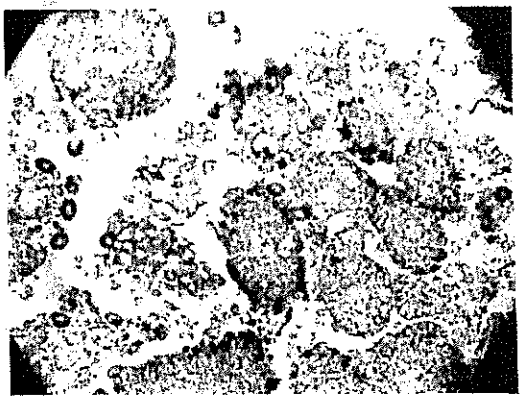

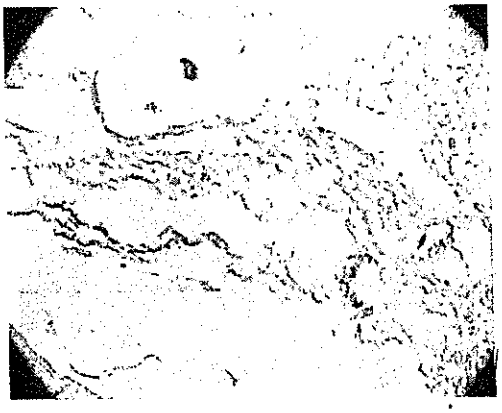

الملخص العربى

عزل والتعرف على حمى الربيع الفيروسى فى المبروك الفضى والعادى لأول مرة فى مصر

مجدى خليل سليمان*و رياض حسن خليل *و .طلعت طلعت سعد*و صافيناز جمعة شحاته**
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**معهد علوم البحار و الاسماك فرع الأسكندرية.

أجريت هده الدراسة على عينات تم تجميعها من محافظات الأسكندريةوالبحيرة وكفر الشيخ فى أسماك المبروك الفضى والعادى حيث تم أخد عينات من الكبد والمخ والكلى والمثانة الهوائية .
أجريت على هده الأسماك اختبارات خاصة لتحديد الأسماك المصابة بالأمراض الطفيلية والفطرية والبكتيرية حيث تبين وجود عينتين فقط حاملتين لمرض التسمم الدموى ,بينما كانت الاختبارات الطفيلية والفطرية سالبة.
-لتشخيص الفيروسات وعزلها تم عمل زرع نسيجى (cell line) من مبايض أسماك المبروك لدراسة تأثير الفيروس المعروف ب (CPE) واستخدام أجسام مضادة خاصة (monoclonal anti body) وذلك لعمل اختبار ال(I.H.Ch) وأيضا فحص الأنسجة ومشاهدة الفيروس من خلال الميكروسكوب الالكترونى وأيضا دراسة الأعراض الداخلية والخارجية للأسماك الميتة والمصابة من خلال تلك الدراسة وجد أن الأعراض الداخلية والخارجية كانت فى صورة اسوداد لون الجسم وتاكل الزعانف ,انتفاخ البطن وجحوظ العينين و احتقان الأعضاء الداخلية ووجود أنزفة نقطية على المثانة الهوائية. من خلال الزرع النسيجى (cell line) كانت درجة حدوث ال (CPE) تتناسب طرديا مع كمية الفيروس ودرجة تخفيفة حيث كلما زادت كمية الفيروس زاد ال(CPE) .
-التغيرات الهستوباثولوجية كانت عبارة عن : ضمور واضمحلال وصغر حجم الخلايا الكبدية وظهور عينات حمراء اللون تدل على وجود الفيروس . الكلى و بها زيادة فى حجم الأوعية الدموية وتهتك بالخلايا وأنزفة وتورم جدار الأمعاء وتورم الألياف العضلية ,احتقان الأوعية الدموية وانتفاخها فى المثانة الهوائية بالاضافة الى اضمحلال واحتقان خلايا المخ وفقدان صبغة الكروماتين .
ومن خلال الميكروسكوب الالكترونى وجد حويصلات داخل الخلايا تكونت فى خلال 3-4 أيام وظهر شكل الفيروس الذى يشبه الرصاصة أو حدوة الحصان .
أما اختبار الخلايا المناعية فمن خلاله تم فحص 6عينات تحمل فيروس حمى الربيع لأسماك المبروك, حيث أثبت وجود الفيروس فى الأعضاء الداخلية بدرجات مختلفة فى الكبد والكلى والمخ والمثانة الهوائية والألياف العضلية .
ومن هنا نؤكد أنه قد تم ولأول مرة فى مصر عزل وتصنيف فيروس المبروك الربيعى من أسماك المبروك العادى والفضى.-

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