

FIRST RECORD OF ISOLATION AND IDENTIFICATION OF SPRING VIRAEMIA OF CARP VIRUS FROM OREOCHROMIS NILOTICUS IN EGYPT

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Abstract

First record of isolation and identification of Spring Viraemia of Carp Virus (SVCV) from *Oreochromis niloticus* (*O.n.*) in Egypt is given in the present study. Three hundred samples of *Oreochromis niloticus* from farms in El-Behera, Alexandria and Kafr El-Sheikh governorates were submitted for examination, from them 3 samples were found to be infected with *Aeromonas hydrophila*. On the other hand, all examined samples gave negative results for mycological and parasitological examinations except some fish proved to be slightly infested with monogenetic trematodes. Out of examined samples (30 fish which showed severe clinical signs), 13 fish were found to be positive upon examination with immunohistochemistry by using specific monoclonal anti body for SVCV. Moreover, the isolation of the virus was confirmed by using cell line from carp ovary which gave positive Cytopathic Effect (CPE). As well as Electron Microscope (EM) studies. Clinical signs, post mortem lesions (PM) and histopathological changes were examined. Experimental infection, clinical and histopathological studies were carried out. For the best knowledge of the author, this the first time for isolation of SVCV from *Oreochromis niloticus* not only in Egypt but also in the Middle East area.

Keywords: Spring Viraemia of Carp Virus, *Oreochromis niloticus*, Egypt

INTRODUCTION

The fisheries still have very big interest, not only for being a very important source for food, but also for its valuable incorporation in increasing our ability to understand the wide marine ecological system in all its forms (FAO, 2004). By the year 2030 aquaculture will dominate the fish supplies and less than half of the fish consumed is likely originate in capture fisheries. (FAO, 2000).

One of the main factors affecting fish production and efficiency is the fish diseases especially that resulted from viruses. The fish viruses cause sever losses among different fish especially carp species (*Aquatic Animal Health report, 2005*).

Viral infections cause significant levels of mortality in intensive fish farming and because of no treatment, and vaccination is not yet feasible, prevention is only

possible through fish health control measures (*Mourton et al., 1990*). That makes us in bad need to a further knowledge about viruses and its importance in fish culture (*Habashi, 1980 and Way et al., 2003*).

SVCV is considered one of the most important economic threatening diseases among the cultured fish because of its severe economic losses especially in the carp species. (*Kim et al., 2005*).

It is obvious that, the research on the viral fish diseases in Egypt is scanty due to the lack of the tissue cultures and cell lines which are used for viral isolation and identification. And in spite of being difficult, the SVCV was isolated from Common carp, Silver carp Bighead carp and Grass carp for the first time in Egypt (*Saad, 2005 and Abo eisa, 2007*).

So, to close the circle and to avoid the increase problems occurring by SVCV, the aim of the study was isolation and identification of SVCV from suspected naturally infected *Oreochromis niloticus* which is usually cultured with the carp fish in polyculture system in Egypt. Moreover, evaluation of the dangerous effects of the isolated virus in fish health status upon experimental infection were also done.

MATERIALS AND METHODS

Fish

Three hundred *Oreochromis niloticus* (40 ± 10 gm average weight) showing clinical signs in the form of haemorrhagic patches, fin rot and congestion of internal organs with unilateral exophthalmia in some fish were collected from Behera, Kafr-El-Sheikh and Alexandria governorates cultured in both fish cages and private farms.

-Laboratory examination of collected fishes

According to *Buck and Finlay (1979)* and *Ibrahim, (2002)*, the fishes were subjected to clinical, bacteriological, mycological and parasitological examination. The fish samples were collected from different organs under aseptic conditions.

-Virological examination

According to *Buck and Finlay, (1979)*, tissue samples from suspected diseased fish were grinded in sterile mortar with sterile sand, then subjected to freezing and thawing for 3 times, after each one the tissues were grinded to liberate the intracellular virus, then centrifuged for 10 minutes at 3000 rpm/min. The supernatant containing the virus was collected under aseptic conditions after addition of three drops from mycostatin, 1000 I.U. /ml, penicillin, 1000 µg/ml, streptomycin and anystatin, 500 I.U./ml and kept at -20 C until used.

-Immunohistochemistry studies

According to *Kiernan (2003) and Saad, (2005)*, samples kept in formalin paraffin by using Spring Viraemia of Carp (SVC) monoclonal antibody (Bio-X Diagnostics Belgium) in addition to anti-mouse Ig.

-Tissue culture

The steps of cell line preparation were made by using the ovaries of sexually mature female Common carp about 1 year old. According to the methods implied by *Habashi (1980) and Saad, (2005)*.

-Histopathological examination

From naturally infected fishes, samples from brain, kidney, liver, musculature, air sac, gills and intestine were fixed in 10% neutral buffered formalin for at least 24 hrs and embedded in paraffin wax at 60 C. Five microns sections were stained by Hematoxyline and Eosin (H. & E.). According to *Culling, (1983)*.

-Electron microscope examination

-Samples were fixed in 3% glutaraldehyde and processed for electron microscopical examination according to *Jerome et al. (1995)*.

-Determination of tissue culture infective dose fifty (TCID₅₀)

-The pre-prepared inoculums of samples were centrifuged for 10 minutes then diluted by 1: 10 in TC199.

-After that ten fold serial dilutions from 10⁻¹ to 10⁻⁶ for measuring Cytopathic Effect (CPE) was done and inoculated in prepared cell lines.

-After 4 subcultures, primary monolayers were inoculated with the samples inoculums.

-The highest two viruses that gave strong CPE were used for measuring the TCID₅₀ according to the methods implied by *Reed and Muench (1938)* by the following equation:-

In each sample (Naturally infected) calculate the proportionate distance (PD):

$$\text{Mortality above 50 \% - 50} \\ = \frac{\text{Mortality above 50 \% - Mortality below 50\%}}{\text{Mortality above 50 \% - 50}}$$

The T.C.I.D50 = P.D + Log Dil. Next above 50%.

- Experimental design

Thirty apparently healthy were divided into three groups (ten fish per group). First group was intra peritoneally injected (IP.) with the TCID₅₀ virus (1 ml TCID₅₀), according to *Saad, (2005)*. The second group was injected with a mixture of 1 ml from SVCV (10^{-2.66} TCID₅₀) and 2 ml dexamethasone according to *Wiik et al. (1989)*. The

third group was injected with 1 ml of sterile tissue culture 199 (TC 199). The fish were kept under observation for 4 weeks and fed with 25% protein commercial fish feed.

RESULTS

The clinical signs and PM lesions of naturally infected fish

Naturally infected *Oreochromis niloticus* showed showing congestion of internal organs and gills with unilateral exophthalmia

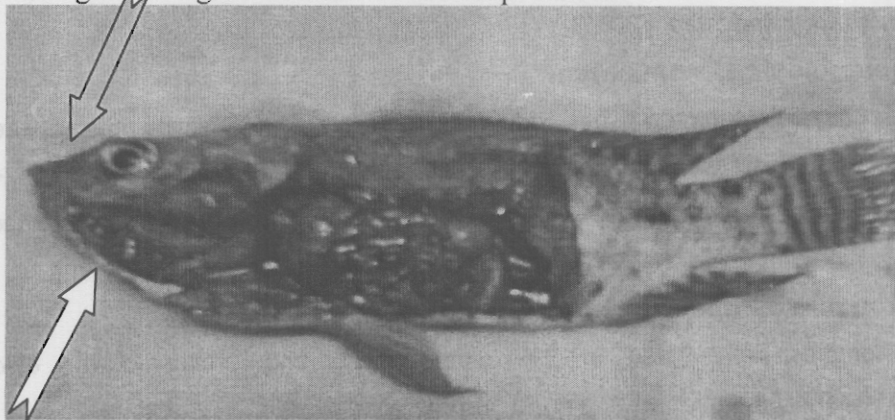


Fig. 1. Naturally infected *Oreochromis niloticus* showing congestion of internal organs and gills (white arrow) with unilateral exophthalmia (green arrow).

-Bacteriological, parasitological and mycological examinations

All examined samples were negative for bacteriological and mycological investigations, except 3 fish from which *Aeromonas hydrophila* was isolated. Moreover, some fish found to be slightly infested with monogenetic trematodes.

-Immunohistochemistry studies

-According to the results of immunohistochemistry to Spring Viraemia of carp (SVCV) for 30 random naturally infected samples, 13 samples were proved to be +ve for the SVCV by the appearance of brown labeled granules of di amino benzidine (DAB) of immunohistochemistry chromogene.

-Cell line formation and Cytopathic effect (CPE) due to viral infection

The cultured ovarian cells from Common carp formed monolayer sheet after 24 hrs post-incubation. The cells became spindle with oval nuclei. Number of cells highly increased and confluent monolayer sheet formed within 48-72 hrs (fig2).

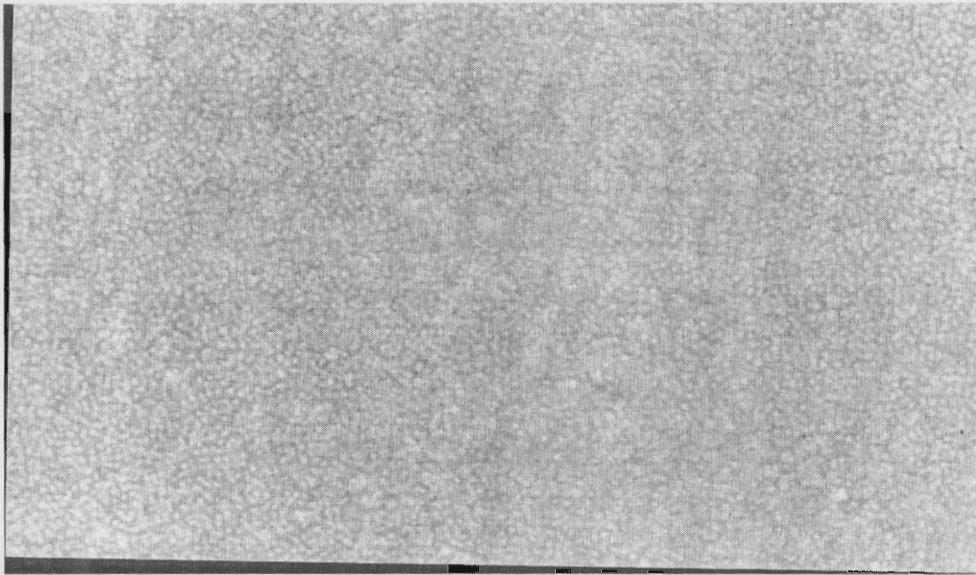


Fig. 2. Normal confluent monolayer of Common carp ovary 24 hrs post-inc.

The CPE formed within 3 days post infection with suspected samples in which cells changed from spindle to round, detached and formed plaque-like, and then death of cells occurred. After 24 hours, the severity and degree of CPE were increased when the virus concentration increased, also, the increase in concentration of the virus decreased the time of virus to cause CPE and vice versa (fig. 3).

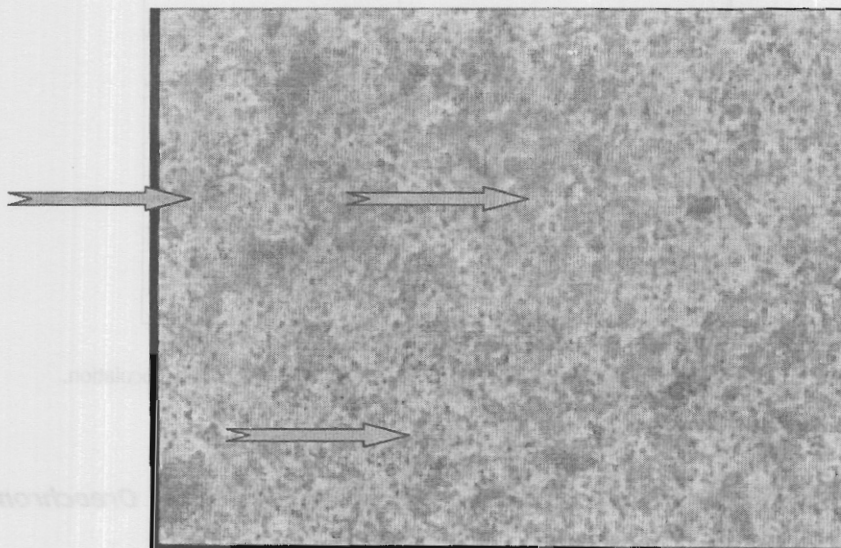


Fig. 3. Sever degree of CPE due to injection of virus appears in the form of rounding and detachment of cells with marked plaque formation (arrows).

-Results of TCID₅₀

After injection of 13 samples which proved to be +ve for SVCV by immunohistochemistry with different dilution (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}) for

each sample, we found that the sample No. (13) gave the highest CPE. The result of TCID₅₀ for sample No 13 was found to be 10^{-2.66} (table 1).

Table (1): Results of calculation tissue culture infective dose fifty (TCID₅₀) for the sample No. 13

Sample No. 13						
*						
6						
5	X	X				
4	X	X				
3	X	X	X			
2	X	X	X	X		
1	X	X	X	X	-	-
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
No. of wells with Plaques/6 wells				Viral titer		
5/6				10 ⁻¹		
5/6				10 ⁻²		
3/6				10 ⁻³		
2/6				10 ⁻⁴		
0/6				10 ⁻⁵		
0/6				10 ⁻⁶		
5/6 X 100 - 50						
** Pd = ----- = 0.66						
5/6 X 100 - 2/6 X 100						
Virus titer = Log dil. Next above 50 % + P.D						
= 2 + 0.66 = 2.66						

*- Indicate no. of wells per sample.

-X indicates well with plaques formation (CPE) due to virus inoculation.

- ** Proportionate distance.

-Clinical signs and PM lesions in the experimentally infected *Oreochromis niloticus*

The clinical signs and PM lesions in *Oreochromis niloticus* infected with viral titer (1 ml X 10^{2.66}) were mild while in case of (1ml X10^{2.66}) of virus plus 2 ml cortisone appeared in the first day post-infection till the 4th week of experiment and found to be pronounced and severe.

The signs appeared in the form of isolation from the herd with erratic swimming and dark discoloration of the body surface with haemorrhag at the base of the anal fin (Fig. 4)), severe congestion of the kidney which was also haemorrhagic (Fig. 5).

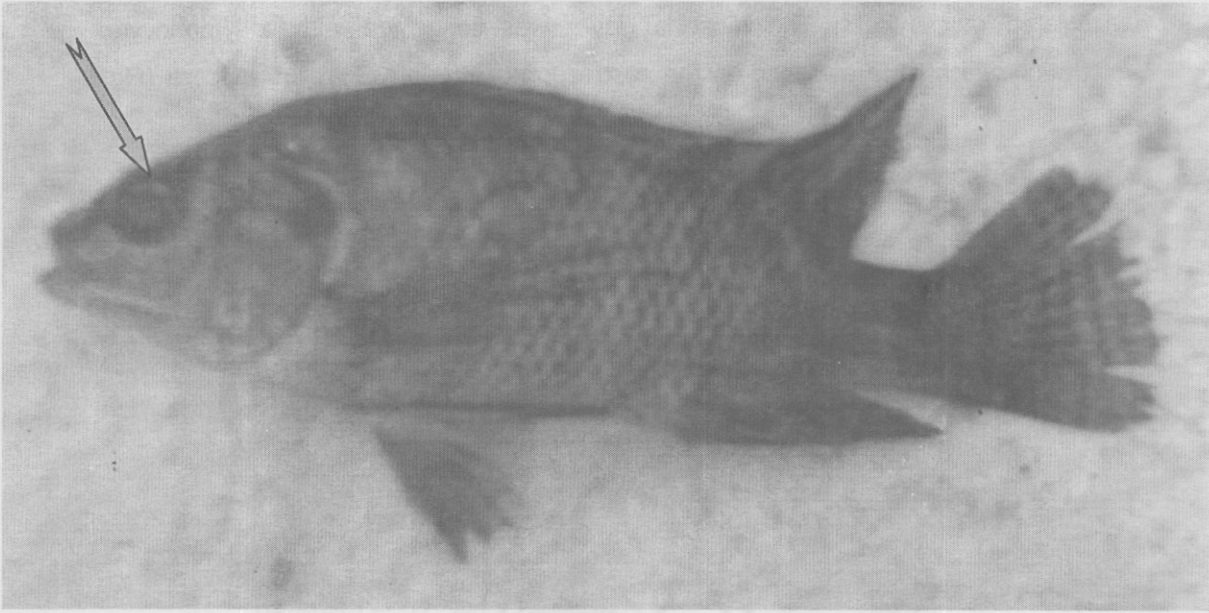


Fig. 4. Experimentally infected *Oreochromis niloticus* 4 weeks post infection with 1 ml X $10^{2.66}$ virus *Oreochromis niloticus* showing erosion of the tail fin, dark discoloration of the body surface with opaqueness of the eye (arrow).

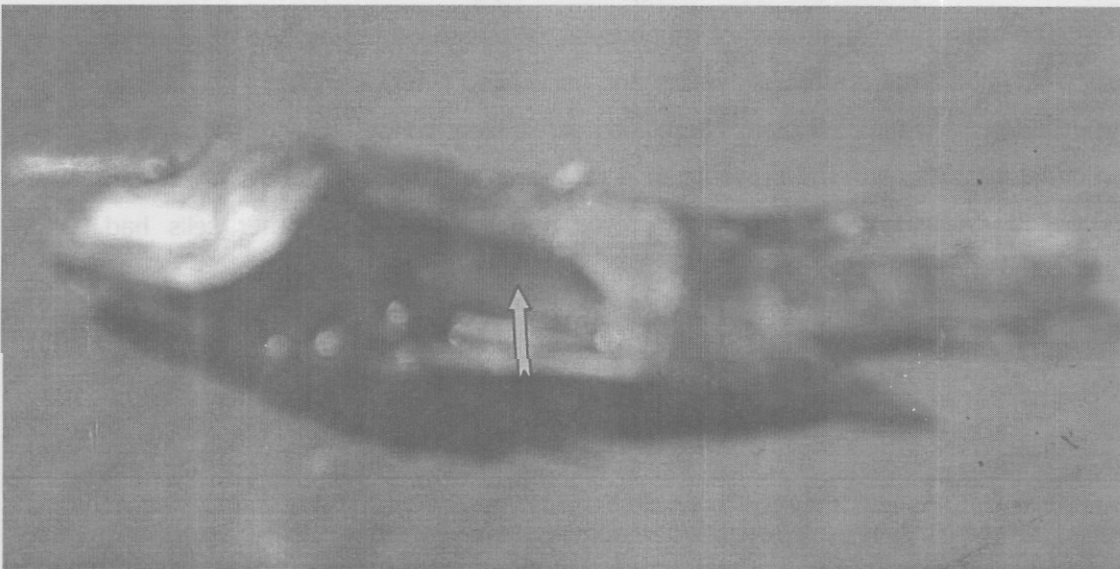


Fig. 5. Experimentally infected *Oreochromis niloticus* 3 weeks post infection with 1 ml X $10^{2.66}$ virus showing severely congested and haemorrhagic kidney (arrow).

-Results of histopathology

The naturally infected intestines showed mild changes, while in experimental infection; the lesions were severe and clear in case of injection of virus and dexamethason only. It showed multiplication of the epithelial cells with large vacuolated mucus cells. The muscular layer was congested with a lymphocytic infiltration which gave evidence on the destruction of the intestinal sub mucosa (Fig. 6).



Fig. 6. Paraffin section of intestine of experimentally infected *Oreochromis niloticus* showing large vacuolated mucus cells (green arrow). The muscular layer was congested with a lymphocytic infiltration (pink arrows) (H & E X 400).

The brain showed an area of lymphocytic infiltration occupying two large nerve cells which had large eosinophilic nuclei and vacuolated cytoplasm (Fig. 7). While, in the experimentally infected brain, the blood vessels were congested with shortage of the nerve bundles, with the presence of a focal area of large nerve cells which had vacuolated cytoplasm and large eosinophilic nuclei, while some other cells had fragmented chromatin (Fig. 8).

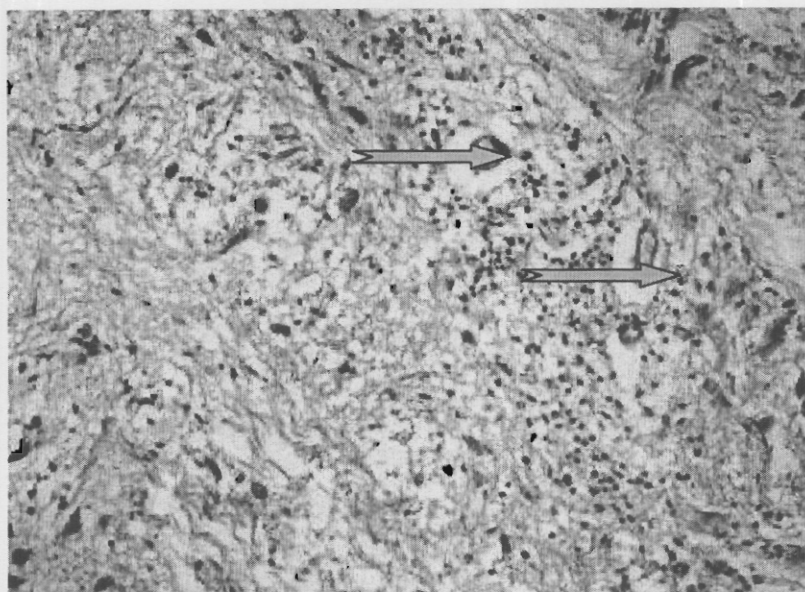


Fig. 7. Paraffin section of brain of naturally infected *Oreochromis niloticus* showing an area of lymphocytic infiltration occupying two nerve cells which had large eosinophilic nuclei and vacuolated cytoplasm (arrows) (H & E X 400).

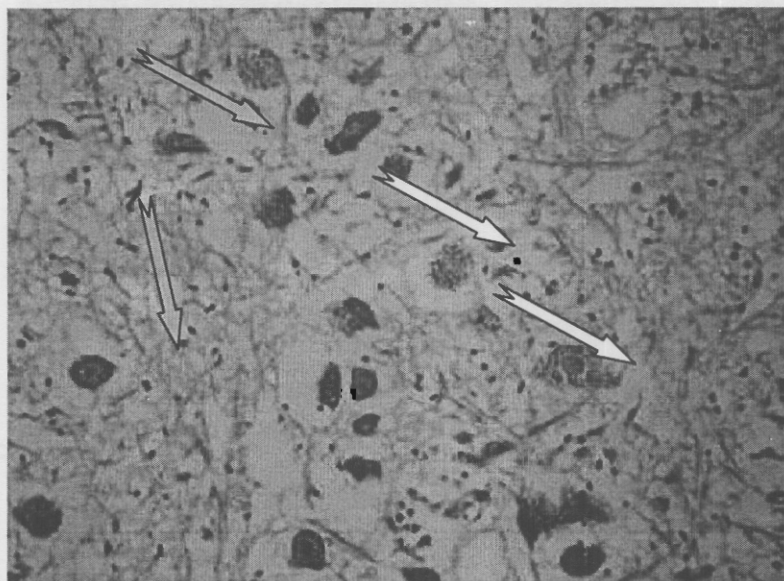


Fig. 8. Paraffin section of brain of experimentally infected *Oreochromis niloticus* 4 weeks post-infection with the virus showing congestion of blood vessels, with the presence of a focal area of large nerve cells which had vacuolated cytoplasm (green arrows), some other cells had fragmented chromatin (white arrows) (H & E X 400).

The naturally infected liver showed rounded red nuclei of hepatocytes indicating marked viral infection (Fig. 9). While in the experimental infection, infected hepatocytes showed eosinophilic nuclei and vacuolated cytoplasm, dilatation of the sinusoids with red nuclei cells infiltration. Also, there were congestion and pyknosis of acinar cells of the hepato-pancreas (Fig. 10).

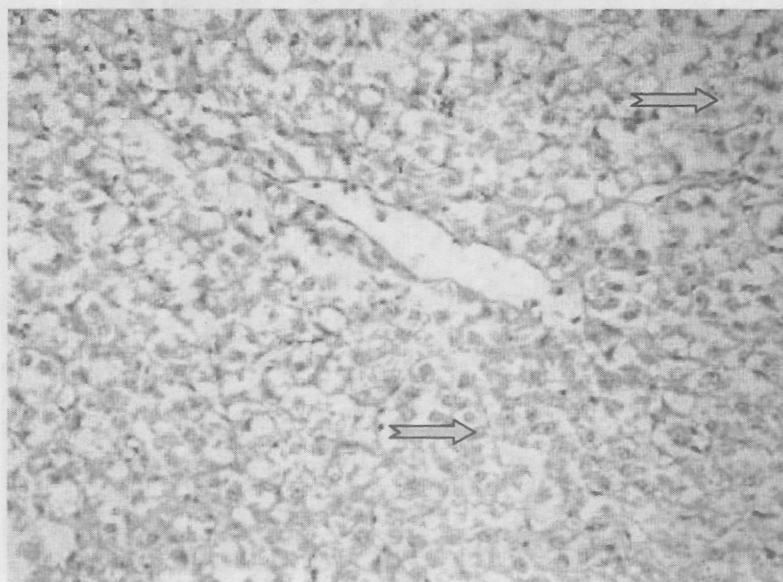


Fig. 9. Paraffin section of liver of naturally infected *Oreochromis niloticus* 4 weeks post-infection with the virus showing rounded red nuclei (arrows) (H & E X 400).

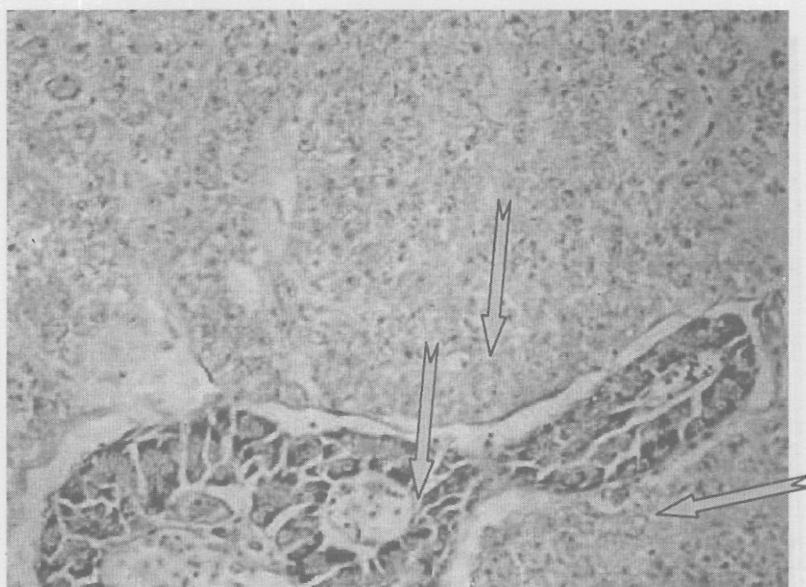


Fig. 10. Paraffin section of liver of experimentally infected *Oreochromis niloticus* 2 weeks post-infection with the virus showing eosinophilic nuclei and vacuolated cytoplasm, dilatation of the sinusoids with red nuclei cells infiltration. (Arrows) (H & E X 400).

The muscular tissue showed muscle fibers with enlarged nuclei embedded in between the damaged muscle fibers, some myofibrils were swollen, while other fibrils appeared thinner upon experimental infection.

-Immunohistochemistry

The results were recorded depending on the following positively degrees: -ve, +ve (weak), ++ve (moderate), +++ve (strong) and ++++ve (intense).

The presence and the strength of the brown granules indicated the severity of infection and concentration of the virus in the tissues. The virus was detected as brown granules in the infected organs.

Naturally infected *Oreochromis niloticus* liver revealed marked positive anti-SVCV in the pre-central vein (Fig. 11). While that injected with $1 \text{ ml} \times 10^{-2.66}$ of viral dilution ($10^{-2.66}$) viral dose showed weak positivity in few hepatocytes (Fig. 12).

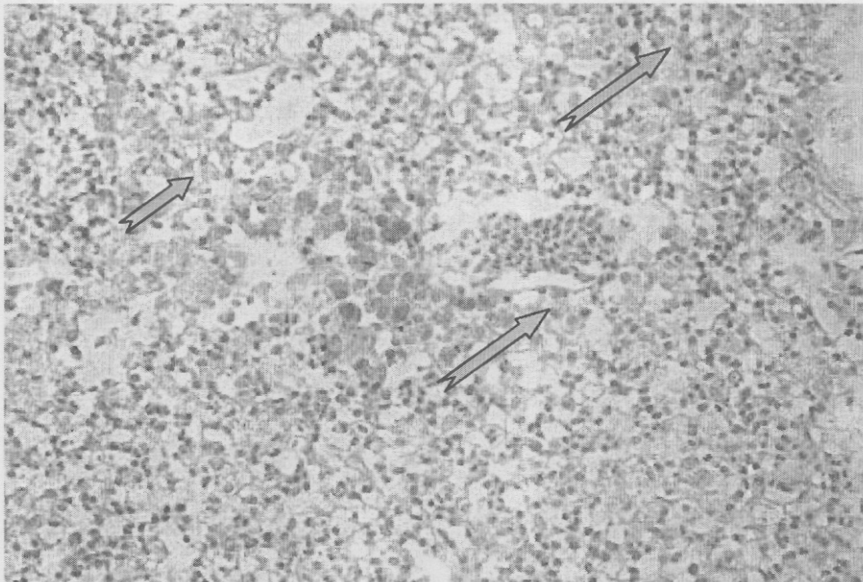


Fig. 11. Paraffin section of liver of naturally infected *Oreochromis niloticus* showing weak positive anti-SVCV in the pre-central vein (arrows) (DAB X 400).

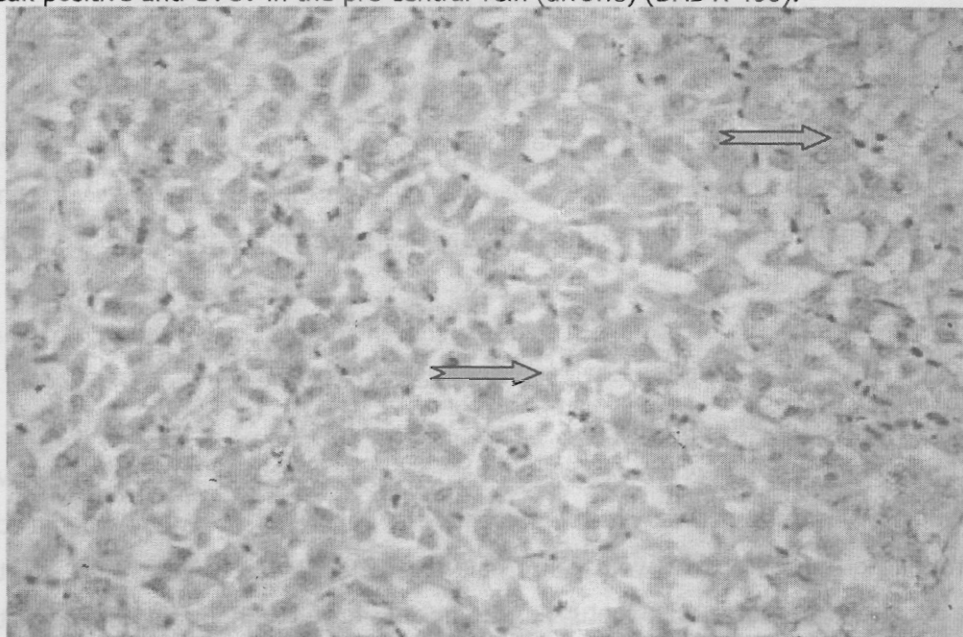


Fig. 12. Paraffin section of liver of experimentally infected *Oreochromis niloticus* 4 weeks post-infection with the virus showing weak positive anti-SVCV in few hepatocytes (arrows) (DAB X 400).

Naturally infected gills showed marked positive reaction of anti-SVCV concentrated on the oedematous epithelial cells of the gill lamellae upon experimental infection *Oreochromis niloticus* gills showed moderate positive (++) brown granulation of anti-SVCV in the cartilaginous border of the gill arch.

In the brain, immunohistochemical detection of SVCV antigen illustrated marked positive anti-SVCV in the dilated blood vessels and the nuclei of the vacuolated neurons with negative reaction in the nerve bundles in natural infection.

Experimentally infected *Oreochromis niloticus* gas bladder showed an intense positive anti-SVCV (4+ve) in tunica interna, tunica externa and the capillary complex (rete mirabile) (Fig. 13)

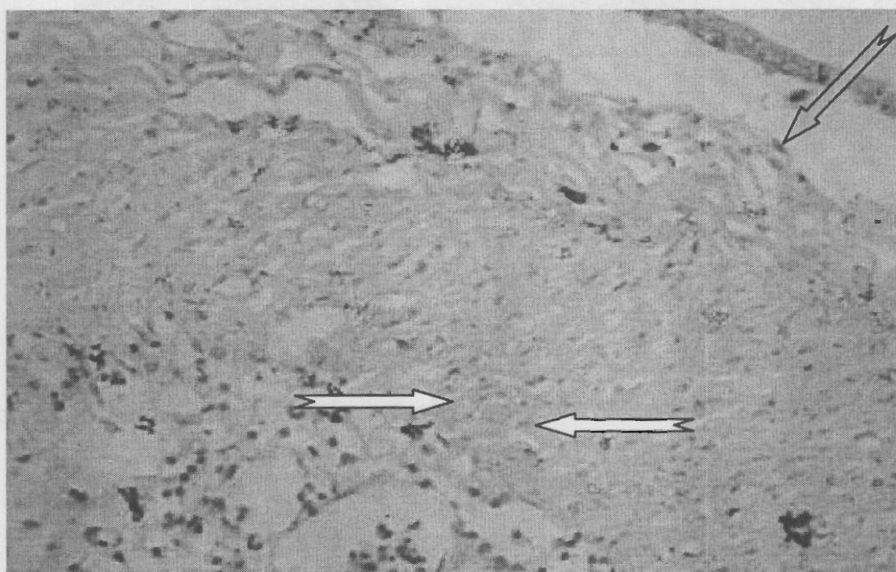


Fig. 13. Paraffin section of gas bladder of experimentally infected *Oreochromis niloticus* 4 weeks post-infection with virus showing intense positive anti-SVCV in tunica externa, (green arrow), tunica externa and the capillary complex (rete mirabile) (white arrows) (DAB X 400).

Moreover the degrees of positive results of different organs were found to be more strong intense in case of injection of virus and dexamethasone.

-Electron microscope results

Naturally infected *Oreochromis niloticus* liver cells showed large nuclei with intranuclear inclusion bodies with the presence of rhabdovirus structural viral particles composed of nucleocapsides with dense halo (Fig. 14). While experimentally infected one showed large nuclei with rupture of the nuclear envelope and aggregation of the virus particles at the cytoplasm.

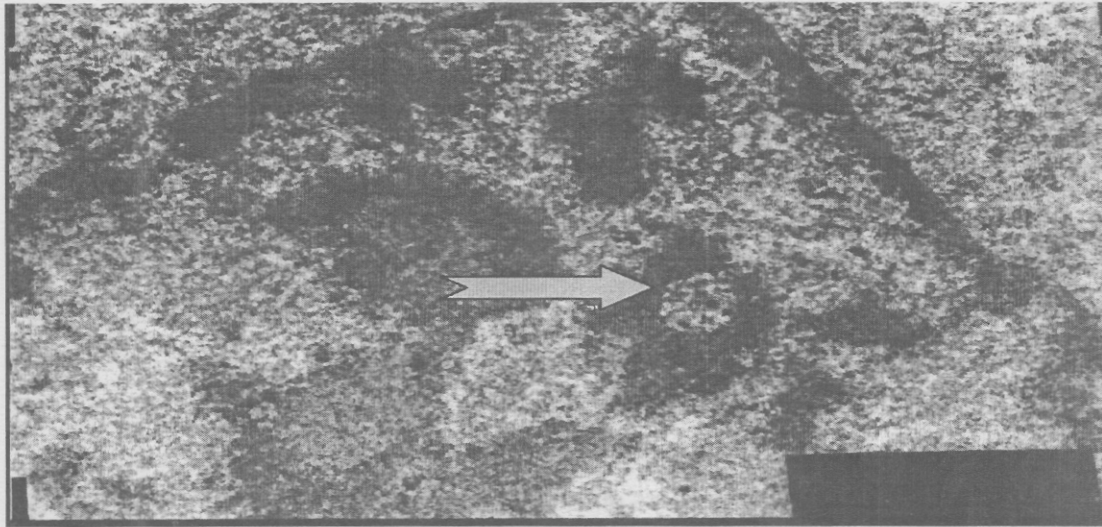


Fig. 14. EM photograph of naturally infected *Oreochromis niloticus* liver showing large nuclei with intranuclear inclusion bodies (arrow) (formalin + PTA X 14000).

Brain cells showed intranuclear inclusion bodies with dark particles of virion emerging from the nuclei into the cytoplasm upon experimental infection (Fig. 15).

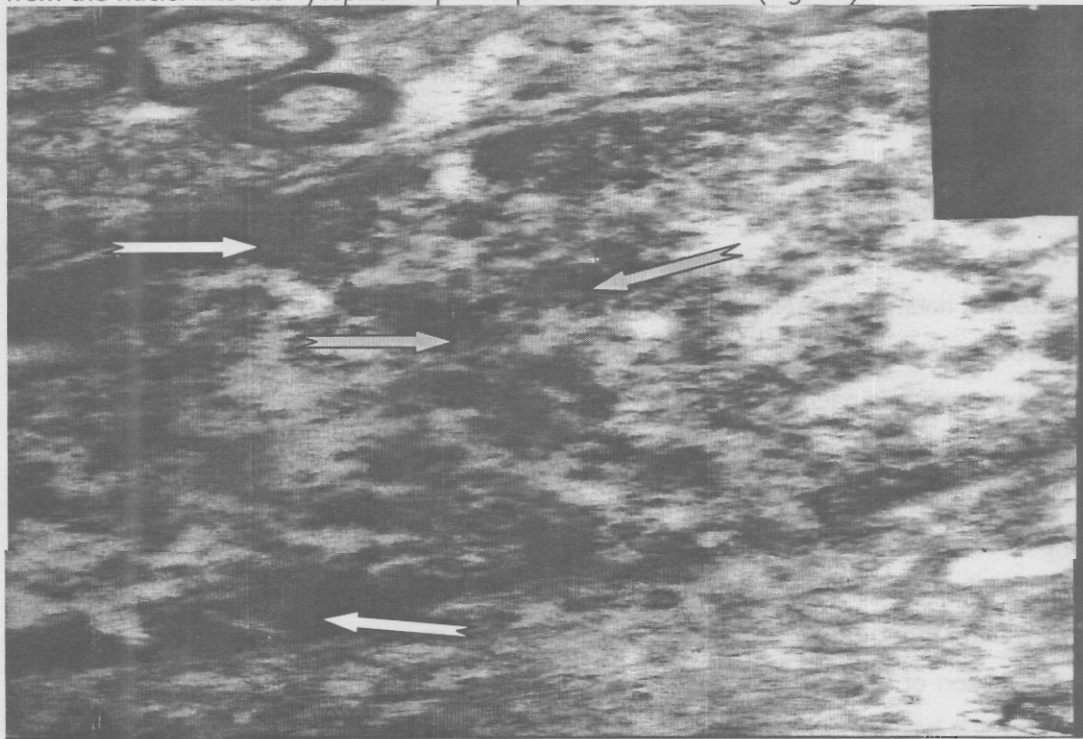


Fig. 15. EM photograph of brain of experimentally infected *Oreochromis niloticus* 4 weeks post infection with $1 \text{ ml} \times 10^{2.66}$ of viral dilution ($10^{-2.66}$) viral dose showing intranuclear inclusion bodies (green arrows) with dark particles of virion emerging from the nuclei into the cytoplasm (white arrows) (Formalin+ PTA X 14,000).

DISCUSSION

In the present study, the number of examined fish was 300 fish obtained from private fish farms and Nile cages. Only 3 samples found to be infected with *Aeromonas hydrophila*, while all samples gave negative results with mycological and parasitological examinations except mild infestation with monogenetic trematodes, so the clinical signs and PM lesions that appeared in the naturally infected fish seemed to be due to causes rather than bacterial or mycotic or parasitic agents, but at the same time, may be due to viral infection. (Bucke and Finlay, 1979).

The main clinical signs appeared in the form of respiratory manifestation as the SVCV affect the gills causing paleness and destruction of the gill lamellae congestion in the operculum, erosion and redness of the tail, pectoral fins. There opaqueness on the eye with clear exophthalmia, inflammation of the anal opening and congestion of gill (Post, 1984).

SVCV inhibit protein and DNA synthesis and destroy the cells of internal organs which lead to exophthalmia, inflammation of the anal opening, fins rot and ascitis (Bjorklund et al, 1997). The darkening of the fish skin due to infection with SVCV attributed to that the SVCV stimulating the spleen melanocytes and melano macrophage centers causing increase in melanin pigment secretions which deposited under the skin leading to darkening of the body surface. (Post, 1987).

Congestion of internal organs may be due to that, the SVCV is intracellular microorganism and mainly affecting the tunica intima of blood vessels leading to oozing of blood out side the blood vessels causing haemorrhagic appearance and petechial haemorrhage. Moreover, the distance between the cells of blood vessels was increased leading to congestion, ascitis, exophthalmia and haemorrhage in all internal organs especially haemopoietic organs. (Post, 1984).

The liver lesions were being attributed to its effects on macromolecular synthesis and cell proliferation of hepatocytes (Fink-Germmels et al., 1995).

Moreover, Oreshkova et al. (1995), Hoffmann et al. (2002) and Saad, (2005), reported that the clinical signs of the virus infection are external and internal hemorrhages, peritonitis and ascitis. They also stated that affected fish with SVCV showed destruction of tissues in the kidney, spleen and liver, leading to hemorrhage, loss of water-salt balance and impairment of immune response.

-Immunohistochemistry findings

The main site for demonstration and in situ detection of the anti-SVCV was the hepato-pancreas and brain which gave the common features of the viral infection. The hepatocytes gave strong positively of anti-SVCV as a ring shape bounding the nuclear envelope in both natural and experimental infections.

Our results agreed to those stated by *Saad, (2005) and Abo eisa(2007)* who reported that, an intense brown granules in hepato-pancreatic cells in both Common and Silver carp, and these results confirmed the results of the present work and indicated that the SVCV is an RNA virus as the infection attached to the nucleus and nuclear membrane.

The gas bladder showed tinny granules of anti-SVCV in the dispersed epithelial cells and muscular layer. The tunica interna and tunica externa of the gas bladder showed an intense positive (4+ve) anti-SVCV. The intense positively observed also in the rete mirabile. This may be due to the destructive effect of the virus on the cells of the gas bladder tissue.

The present work agreed with the studies of *(Clerx et al., 1978)* who showed that the brown labeling granules was depended mainly on the level of antigen antibody reaction (SVCV antigen react with the SVCV antibodies), and may be due to the destructive action of virus , that represented a new diagnostic method of virus detection by immunohistochemical technique. The present work results attributed to the mono clonal antigen of SVCV used as a diagnostic test. Moreover, these results confirmed the isolation and identification of SVCV from *O.n.*

So, our results indicated that, the SVCV antigens have no a straight forward relationship with either host or geographical origin. This is in contrast to the relatively clear geographical distribution of genogroups in other fish viruses *(Snow et al., 1999)*.

-Electron microscope observations confirmed the results of the immunohistochemistry for the presence of SVCV in infected tissue. *(El-Tarabili et al., 2000)*.

Based on the results of CPE on cell line, immunohistochemistry by using specific monoclonal antibodies, E.M we can confirm the positive isolation of SVCV for the first time in Egypt from the *Oreochromis niloticus*, however the examined fish not considered one of the susceptible fish to SVCV.

To the best of the author knowledge, this is the first study to examine, in details the pathogenesis and anti-viral response associated with the SVCV infection in the *Oreochromis niloticus*. However, in the experimental infection with the SVCV, the immunohistochemistry of anti-SVCV in the *Oreochromis niloticus* gave weak to marked activity in brain, liver, gas bladder and gills.

Histopathological findings

In naturally and experimentally infected fish liver showed necrosis and degeneration of bile ducts with RBCs infiltration, pyknosis with atrophy of hepatic cells, also pyknotic nuclei. Moreover, there were red color nuclei cells (eosinophilic nuclei) which indicated the main characters of cells with viral infection. The changes in the

liver which observed in the infected fish may be attributed to the destructive effects of the virus. Degenerative and necrotic changes were also evident in the hepatopancreas and hepatocyte cell-membrane breakdown. There was an increase in fibrous connective tissue surrounding the vascular elements, and mononuclear cell infiltration. The exocrine tissue showed degenerative changes in the hepatopancreas and in free pancreatic tissue there was moderate-to-marked necrosis of the acini. The above mentioned lesions may be due to the destructive effect of SVCV on the tissue cells.

These results agreed with those of *Negele, (1977); Bucke and Finlay, (1979)* they found that the histopathology of Carp tissue, which results from experimental infection with SVCV, were extensive. There were necrotic areas and extensive congestion in the liver and hepato pancreas.

Jiang and Ahne (1989) studied the virus infection in some marine fish species and reported 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 **brain** showed eosinophilic nuclei (red nuclei cells), congestion of RBCs in wide blood vessels, pyrenocytes with vacuolated nucleus and cytoplasm. Eosinophilic inclusions were evident in a few glial cells in the forebrain. The changes in brain may be due to the multiplication of the virus in these cells causing severe destruction (*Saad, 2005 and Abo eisa, 2007*).

The **gas bladder** showed degenerated nuclei, the posterior chamber showing multilobular gas glands, dilatation and congestion of blood vessels, with vacuolated epithelial cells of tunica interna and eosinophilic nuclei of epithelial cells indicating the viral infection. The changes in the gas bladder may be due to the multiplication of the virus in these cells causing severe destruction (*Saad, 2005 and Abo eisa, 2007*).

The **intestine** showed inflammatory reaction in sub mucosa, with necrosis of eosinophilic granular cells, red nuclei cells in epithelial cells due to intranuclear inclusion bodies. These results similar to those investigated by *Hoffmann (2005) and Jiang and Ahne (1989)*, who observed in case of virus infection, shortening and fusion of the villi with intestinal epithelial damage.

Clearly that, cell death due to Rhabdovirus infection has been considered to be resulted from necrosis following cell membrane damage caused by the budding virions.

The histopathological changes indicated that the virus was able to invade and spread through out the vasculature and adjacent tissues. (*Tolskaya et al. 1995*)

From the previous results of this study, we can say that, the *Oreochromis niloticus* is more resistant to the SVCV than the susceptible hosts (Family *Cyprinidae*), but due to the several stress conditions which are facing the cultured fish in our culture pond systems, which lead to immunosuppression (the same role played by cortisone injected to the examined fish during the experimental infection), the *Oreochromis niloticus* can catch the infection with the virus, with little or, in some times, no specific clinical signs or post mortem lesions and may serve as a carrier to the virus. So, by improving the surrounding environmental conditions in our culture pond systems, we can avoid *Oreochromis niloticus* to be infected with the virus to avoid it to play its role as a possible carrier to the SVCV.

Moreover, the isolation and identification of SVCV from *Oreochromis niloticus* which know that, the virus is only specific for the carp species put a question mark about the role of *Oreochromis niloticus* as a carrier. However, it demonstrated a mild signs of infection but under stress it gave the same signs of SVCV in its specific hosts. More research is badly needed to clarify these phenomena.

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أول تسجيل لعزل و تصنيف فيروس حمى المبروك الربيعى من البلطى النيلى فى مصر

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فى هذه الدراسة تم تجميع ٣٠٠ عينة من أسماك البلطى النيلى من محافظات البحيرة و الاسكندرية و كفر الشيخ، و تم عمل الاختبارات المعملية و التى أثبتت عزل و تصنيف ميكروب الايرومونات هيدروفيليا من ٣ عينات. بينما أظهرت نتائج العزل الفطرى و الطفيلى خلو الأسماك من الاصابة بهما عدا بعض الاصابات الخفيفة بالديدان وحيدة العائل.

من ٣٠ عينة، وجد أن ١٣ عينة مصابة بفيروس حمى المبروك الربيعى عند اختبارها باختبار ال Immunohistochemistry باستخدام الأجسام المناعية المضادة الخاصة بهذا الفيروس.

و تم تأكيد العزل بدراسة ال Cytopathic Effect (CPE) على الزرع النسيجى لخلايا من مبايض المبروك العادى و كذلك باستخدام الميكروسكوب الالكترونى.

تم دراسة الأعراض الاكلينيكية و الصفة التشريحية و التغيرات الباثولوجية للأسماك المصابة طبيعيا و كذلك الأسماك المحقونة تجريبيا.

و طبقا للنتائج، تعتبر هذه أول مرة يتم فيها عزل و تصنيف فيروس حمى المبروك الربيعى من أسماك البلطى النيلى لأول مرة فى مصر و منطقة الشرق الأوسط.