Histopathological and Ultrastructural Study of Experimental Spring Viraemia of Carp (SVC) Infection of Common Carp with Comparison between Different Immunohistodignostic Techniques Efficacy

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Abstract: Spring viremia of carp (SVC) is an important disease affecting cyprinids, mainly common carp *Cyprinus carpio*. The disease is widespread in European carp culture, where it causes significant morbidity and mortality. This study describes some Histopathological, Immunohistological, and Ultra-microscopical characteristics of infection by this virus in experimentally infected target species; common carp. In this study the pathological changes in naturally and experimentally infected fish existed mainly in the hepatopancreas, kidney, spleen and gills. On the other hand, the changes were evoked to a lesser extent in the intestine and brain. The alterations were ranging between minor degenerative changes to severe necrotic picture. Immunostaining and immunoflourescence studies revealed the presence of antigen in SVCV infected tissue specimens. Transmission Electron Microscopy studies of liver, kidney and spleen samples revealed the presence of pullet-shaped electron-dense intra-cytoplasmic particles, with approximate length of 180-200 nm and approximate width of 90-100 nm. These particles resemble the characteristics of *Rhabdovirus* viral particles.

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1. Introduction

Spring Viraemia of Carp (SVC) is an acute, systemic, contagious disease caused by Rhabdovirus (Bootsma **and Ebregt, 1983; Wolf, 1988; Ahne *et al.*, 2002 and Saad 2005**). The term SVC and *Rhabdovirus carpio* (RVC) were firstly introduced by **Fijan *et al.* (1971)**. Tropism and replication of virus are confined to capillary endothelium, as well as in haemopoietic and excretory kidney tissues, causing an impaired salt–water balance, which is often lethal **(Fijan *et al.*, 1971)**. The systemic character of Spring Viraemia of Carp virus (SVCV) infection has been demonstrated by experimental infection of specific pathogen free (SPF) carp **(Ahne 1977, 1978)**. It affects primarily common carp (*Cyprinus carpio*), while other cyprinids and non-cyprinid species are also susceptible including Koi carp (*Cyprinus carpio koi*), Crucian carp and Goldfish (*Carassius auratus*), Bighead carp (*Aristichthys nobilis*), Grass carp (*Ctenopharyngodon idella*), Silver carp (*Hypophthalmichthys molitrix*), Sheatfish, (*Silurus glanis*), Orfe (*Leuciscus idus*), Tench (*Tinca tinca*) and Roach (*Rutilus rutilus*) as stated by **Haenen & Davidse (1993)**, And *Oreochromis niloticus* **(Abo Eisa 2008)**.

SVC disease is very important fish disease because of its world-wide distribution. The disease was firstly discovered in Yugoslavia 1969 **(Fijan *et al.*, 1971)**, then Czechoslovakia **(Tesarcik *et al.*, 1977)**, Scotland **(Richards and Buchanon, 1978)**, Malaysia **(Armstrong and Ferguson, 1989)**, Spain **(Lupiani *et al.*, 1989)**, Indonesia **(Glazebrook *et al.*, 1990)**, Russia **(Oreshkova *et al.*, 1995)**, Brazil **(Alexandrino *et al.*, 1998)**, Hawaii **(Johnson *et al.*, 1999)**, Northern Ireland **(Rowley *et al.*, 2001)**, United States in North Carolina and Virginia **(APHIS, 2003)**, China **(Liu *et al.*, 2004)**, Egypt **(Saad *et al.*, 2005)**, Canada **(Garver *et al.*, 2007)** and Iran **(Haghighi *et al.*, 2008)**

In European countries including Russia where the Carp is an important fish, SVCV causes high mortalities among carp farms with reduction of total returns from fish selling leading to severe economic

losses **(Richenbach-Klinke, 1973; Oreshkova *et al.*, 1995; Björklund *et al.*, 1996; Siwicki *et al.*, 2003)**. Details about the specific histopathologic lesion induced by virus infection can be used as a tool for viral infection diagnosis.

2. Material and Methods

Fish

In the present study, European common carp (*C. carpio carpio*) of the R3×R8 strain which are the offspring of a cross between fish of Hungarian origin (R8 strain) and of Polish origin (R3 strain) were used (**Irnazarow, 1995**). In this study, we refer to the European common carp subspecies as carp, unless stated otherwise. Carp were bred in the central fish facility of Wageningen University, The Netherlands, and raised at the Veterinary Research Institute (VRI), Brno, Czech Republic, in recirculating UV-treated water and fed pelleted dry food (Trouvit, Nutreco) daily.

Viral infection of carp

Spring viraemia of carp virus (SVCV) strain CAPM V 539 (**Koutna *et al.*, 2003**) was propagated in EPC (Epithelioma Papulosum Cyprini, (**Fijan *et al.*, 1983**)) cells at 15 °C. Cells were grown in Eagle's Minimal Essential Medium (MEM) containing 2% fetal bovine serum (FBS) and standard concentration of antibiotics. The virus titers, given as tissue culture infective dose ($TCID_{50}$/ml), were calculated by the method of **Reed and Muench (1938)**.

Ten-month-old carp were raised at 15 °C to an average weight of 30–40 g. This temperature is optimal for SVCV infectivity (**Ahne *et al.*, 2002**). Fish (n = 110) were exposed, by immersion, to SVCV-infected tissue culture (10^3 $TCID_{50}$/ml) for 2 hours. Control fish (n = 62) were treated similarly and exposed to control cultures only. Infected fish were divided over two replicate tanks for subsequent representative tissue sampling at 2 weeks and 3 weeks postinfection, six fish were killed to collect tissue samples. Infection of carp with SVCV was performed at the central fish facility of the Veterinary Research Institute (VRI), Brno, Czech Republic.

Detection of SVCV

To confirm that all fish used in our experiment were infected by the SVC virus, virus-specific primers were used in a single tube reverse transcription (RT)-PCR and nested PCR reaction (**Koutna *et al.*, 2003**).

Histopathological examination

After complete necropsy of experimentally infected fish, fresh tissue specimens were collected from gills, liver, spleen, kidney, brain, gas bladder, musculature and intestine for histological examination. These specimens were rapidly fixed in Davidson's fixative for 24 hours then transferred to 70% ethanol till processing proceeds. The fixed specimens were processed through the conventional paraffin embedding technique (dehydration through ascending grades of ethanol, clearing in xylen and embedding in paraffin wax at 60^o C). Paraffin blocks were prepared and cutting 3 µm-thick tissue sections by using microtome (Leica 2155). Then 5 replicates from the same section were mounted on silane (Sigma Aldrich)-pretreated slides, and then the slides were divided between H&E staining, Immuno-histochemistry and Immuno-fluorescent techniques.

Immuno-histological examination

In all Immunohistodetection methods in this study heat-induced antigen retrieval was required for optimal staining with SVCV antibodies. So paraffin sections mounted on slides were heated to 60^o C for 30 minutes to retrieve the viral antigen inside the tissue. Anti-SVCV Rabbit antiserum was kindly provided by Prof. Dr. Peter Dixon (SVC OIE Reference Laboratory, Centre for Environment, Fisheries and Aquaculture Science CEFAS, Weymouth Laboratory, The Nothe, Weymouth, Dorset DT4 8UB, UK).

Immunohistochemistry

Immunohistochemical staining for SVC antigen was performed by two methods for comparing the efficacy.

The first method was by using peroxidase activity as antigen indicator. After deparaffinization, 3 µm-thick tissue sections were incubated in methanol with 3% hydrogen peroxide to block endogenous peroxidase activity. The sections were incubated overnight with primary SVC rabbit antiserum (1:100 dilution) at 4°C. For negative controls, normal rabbit serum was used at equivalent concentrations. Thereafter, endogenous nonspecific antigens were blocked by placing sections in 2.5% skimmed milk for 20 minutes. Then sections were incubated with secondary antibody-conjugated universal immuno-enzyme polymer; Histofine Simple Stain MAX-PO kit (Nichirei, Japan); for 30 minutes at room temperature. The bound antibody was visualized using 0.016% diaminobenzidine tetrahydrochloride (DAB) substrate, 0.24% H_20_2 in PBS (Wako, Japan) revealing brown precipitate. Sections were counterstained with Gill's hematoxylin and "Blued" with 0.1% ammonia water.

The second method was using avidin/biotin conjugate alkaline phosphatase (ABC) procedure. After deparaffinization, 3 µm-thick tissue sections

were incubated overnight with primary SVC rabbit antiserum (1:100 dilution) at 4°C. For negative controls, normal rabbit serum was used at equivalent concentrations. Thereafter, endogenous nonspecific antigens were blocked by placing sections in 2.5% skimmed milk for 20 minutes. Then sections were incubated with secondary antibody-Alkaline Phosphatase Conjugated Swine Anti-Rabbit polyclonal Immunoglobulin (Dako, Denmark) for 30 minutes at room temperature. Sections were developed in New Fuchsin chromogen/substrate solution (Dako, Denmark) within 15 minutes at room temperature revealing red precipitate. Sections were counterstained with Gill's hematoxylin and "Blued" with 0.1% ammonia water.

Immuno-flourescent Technique

The technique was done according to (Hayat, 2002). As after deparaffinization, 3 µm-thick tissue sections were incubated overnight with primary SVC rabbit antiserum (1:100 dilution) at 4°C. For negative controls, normal rabbit serum was used at equivalent concentrations. Thereafter, endogenous nonspecific antigens were blocked by placing sections in 2.5% skimmed milk for 20 minutes. Then sections were incubated with secondary antibody- FITC Conjugated Swine Anti-Rabbit polyclonal Immunoglobulin (Dako, Denmark) for 1 hour at 4°C in dark place. Sections were mounted by anti-fading water based mountant (glycerol-PVD 1:1) then cover-slipped. Positive antigenic signals appears as green fluoresce under FITC-specific UV wave length when viewed by UV microscope (Nikon Eclipse E200 + Hamamatsu CCD Camera).

Transmission Electron Microscopic (TEM) examination

At the beginning of sample processing we had lack of samples fixed specially for TEM examination by glutraldehyde, only Davidson's fixed paraffin embedded specimens were present. So retrieval of tissue from histological wax blocks for TEM is used despite the previous fixation method **(Kuo, 2007).** In some cases an area of interest, which may not be discovered by simply processing more tissue, can be retrieved from the wax block. The area of interest on the microscope slide was Identified by ringing it with a marker pen, and then matched against the specimen in the wax block and cut around with a razor blade. Carefully the piece of tissue was levered out. Ascending re-hydration of the specimen is done. Then the usual processing schedule for TEM specimens was continued with post fixation in osmium tetroxide, and epoxy resin embedding, then specimens were cut on ultra-microtome (Ultracut Reichert, Austria) into firstly nearly 1µm semi-thin sections by glass knifes, which is observed after toluodine blue staining to perform more trimming, to facilitate further ultrathin sectioning. After making nearly 0.1 µm ultrathin sectioning by diamond knife the grey floating sections were selected for better TEM results, which were caught over carbon-copper grids. After drying, the grids were subjected to negative staining by 2% uranyl acetate and lead citrate method **(Kuo, 2007)**. TEM observation is made by transmission electron microscope (JEOL 1200. Japan).

3. Results and Discussion

Table (1) summarizes the histopathological picture of examined fish tissue sections. Example pictures are shown in figure (1).

In the positive samples in immunohistological detection (immunohistochemistry and immunofluorescence) antigenic expression is noticed as seen in figure (1). While control negative samples, showed absence of antigenic expression.

The present study showed that the pathological changes in naturally and experimentally infected fish existed mainly in the hepatopancreas, kidney, spleen and gills. On the other hand, the changes were evoked to a lesser extent in the intestine and brain.

Microscopically, the hepatopancreas showed congestion, acute cellular swelling of the hepatocytes. Moreover, necrotic areas with corresponding alterations in nuclear and cytoplasmic staining characteristics indicating abnormal hepatocytes were also evident. These changes may be due to virus replication inside the cytoplasm of hepatocytes which lead to exhaustion of cellular resources leading to vacuolations progressed as necrosis and subsequent viral shedding, thus because of the hepatic multiple metabolic functions, such damage could have serious effects on all metabolic processes **(Roberts, 2001)**. The aforementioned alterations were partially similar to those described by **Negele (1977)** and **Osadcaja and Rudenko (1981)** who denoted that liver parenchyma showed multifocal necroses, adipose degeneration and hyperemia.

Bucke and Finlay (1979) described degenerative and necrotic changes in the hepatopancreas, with marked areas of karyorrhexis and hepatocyte cell-membrane breakdown, moreover **Saad (2005)**, **Abo Eisa (2007)** and **Soliman *et al.* (2008)** denoted hepatic lesions as pyknosis and necrosis, while **Haghighi *et al.* (2008)** found that the microarchitecture of the hepatic tissue was almost destroyed, with small parts of healthy tissue were visible in certain places, while the remaining tissue was atrophied due to pressure of the cellular infiltrate.

Table (1) The histopathological picture of examined fish tissue.

	2nd week		3rd week	
Hepatopancreas				
• Circulatory Disturbances	Blood vessels and sinusoidal congestion	++*	Blood vessels and sinusoidal congestion	++
• Degenerative Changes	Vacuolar degeneration	+	Multifocal vacuolar degeneration	++
• Proliferative Changes	-		-	
• Necrosis	-		Hepatocellular necrosis Nuclear pyknosis and karyolysis	++ +
• Infiltrations	-		-	
Spleen				
• Circulatory Disturbances	-		Hemorrhages	++
• Degenerative Changes	-		-	
• Proliferative Changes	-		-	
• Necrosis	Multifocal depletion of white pulp	+	Multifocal depletion of white pulp	++
• Infiltrations	-		-	+
Posterior kidney				
• Circulatory Disturbances	Edema infiltrating renal interstitium	++	Hemorrhages	+
• Degenerative Changes	Vacuolation of tubular epithelial cells Intracellular hyaline droplet formation	++ ++	Vacuolation of tubular epithelial cells	+++
• Proliferative Changes	Stimulation of interstitial hemopoietic tissue	+	-	
• Necrosis	Nuclear changes such as pyknosis	+	Tubular necrosis Interstitial necrosis	+ +
• Infiltrations	-		Diffuse mononuclear cell infiltration	+
Intestine				
• Circulatory Disturbances	-		-	
• Degenerative Changes	Villar vacuolation	+	Villar vacuolation	++
• Proliferative Changes	-		-	
• Necrosis	-		-	
• Infiltrations	-		-	
Gills				
• Circulatory Disturbances	Congestion	++	Congestion Edema	++ +
• Degenerative Changes	Intracytoplasmic vacuoles in-between the proliferated malpighian cells	++	Lamellar subepithelial spongiosis of secondary gill lamellae	+++
• Proliferative Changes	Diffuse lamellar fusion Hyperplasia of the epithelial lining at the base of secondary gill lamellae	++ ++	Diffuse lamellar fusion Hyperplasia of the epithelial lining at the base of secondary gill lamellae	++ +++
• Necrosis	-		-	
• Infiltrations	Eosinophilic Granular cells	+	Eosinophilic Granular cells	++

*Score value: - = none, + = mild, ++ = moderate, +++ = severe

Semiquantitative scoring: Histopathological alterations were assessed using a score ranging from – to + + + depending on the degree and extent of the alteration: (-) none, (+) mild occurrence, (+ +) moderate occurrence, (+ + +) severe occurrence.

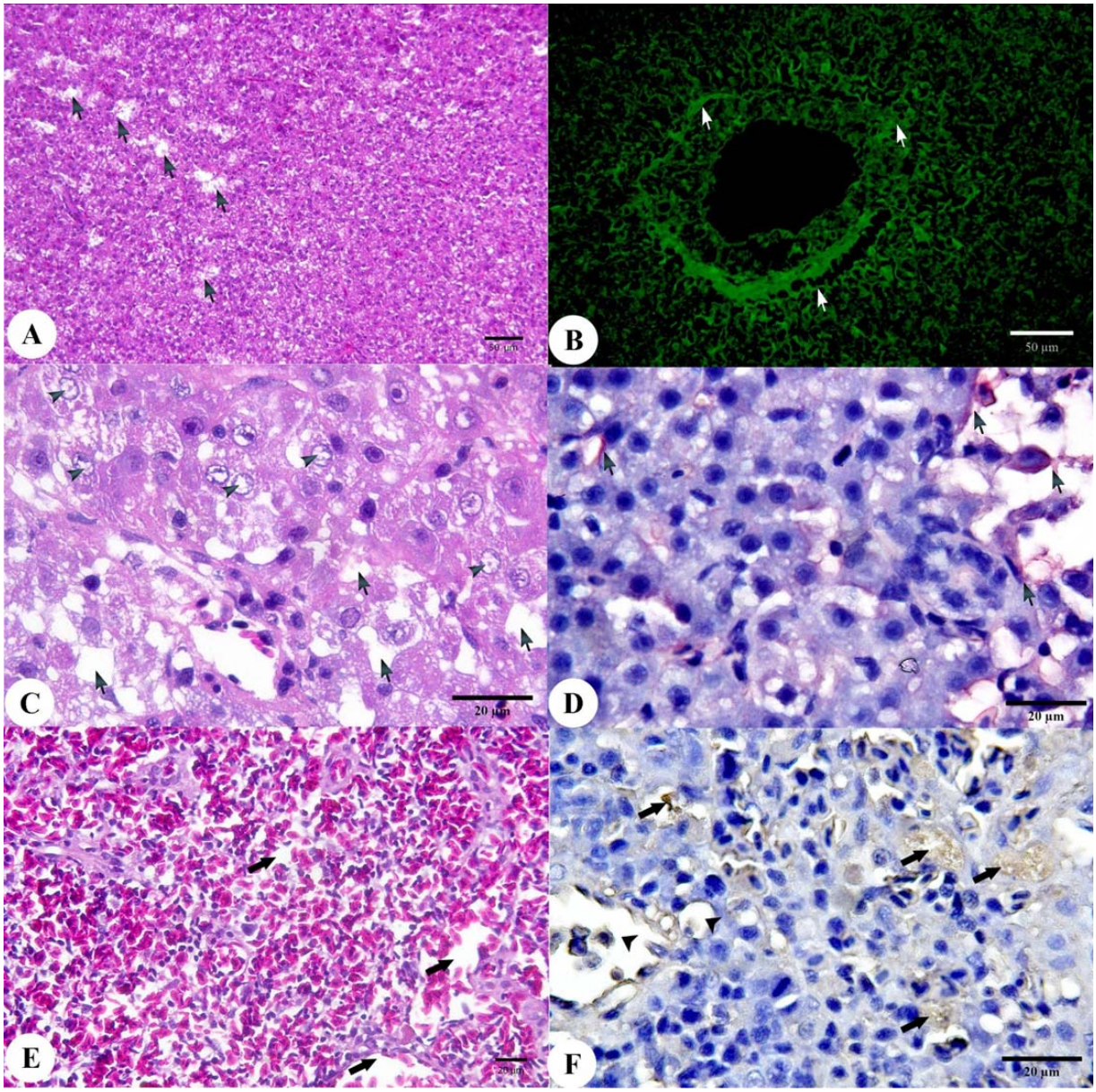

Figure (1): (A) - Common carp after 2 weeks of experimental SVCV infection showing ascitis (arrowhead) with haemorrhagic prolapsed anal opening (arrow). (B) - Hepatopancreas of common carp after 2 weeks of experimental SVCV infection showing green fluorescence (arrows) concentrated at peripancreatic acinar area and surrounding vacuolated hepatic tissue. Immuno- Fluorescence Technique. (C) - Hepatopancreas of common carp after 3 weeks of experimental SVCV infection showing hepatic necrosis represented by nuclear karyolysis (arrowheads) and vacuolations (arrows). H&E. (D) - Hepatopancreas of common carp after 2 weeks of experimental SVC infection showing positive antigenic red staining concentrated at necrotic areas. Immuno-Alkaline Phosphatase, New Fuchsin substrate – Gill's Hematoxylin counterstain. (E) - Spleen of common carp after 3 weeks of experimental SVCV infection showing multifocal depletion of white pulp and hemorrhages (arrows). H&E. (F) - Spleen of common carp after 3 weeks of experimental SVCV infection showing brown staining (arrows) within splenocyes. Immuno-Peroxidase, DAB substrate – Gill's Hematoxylin counterstain.

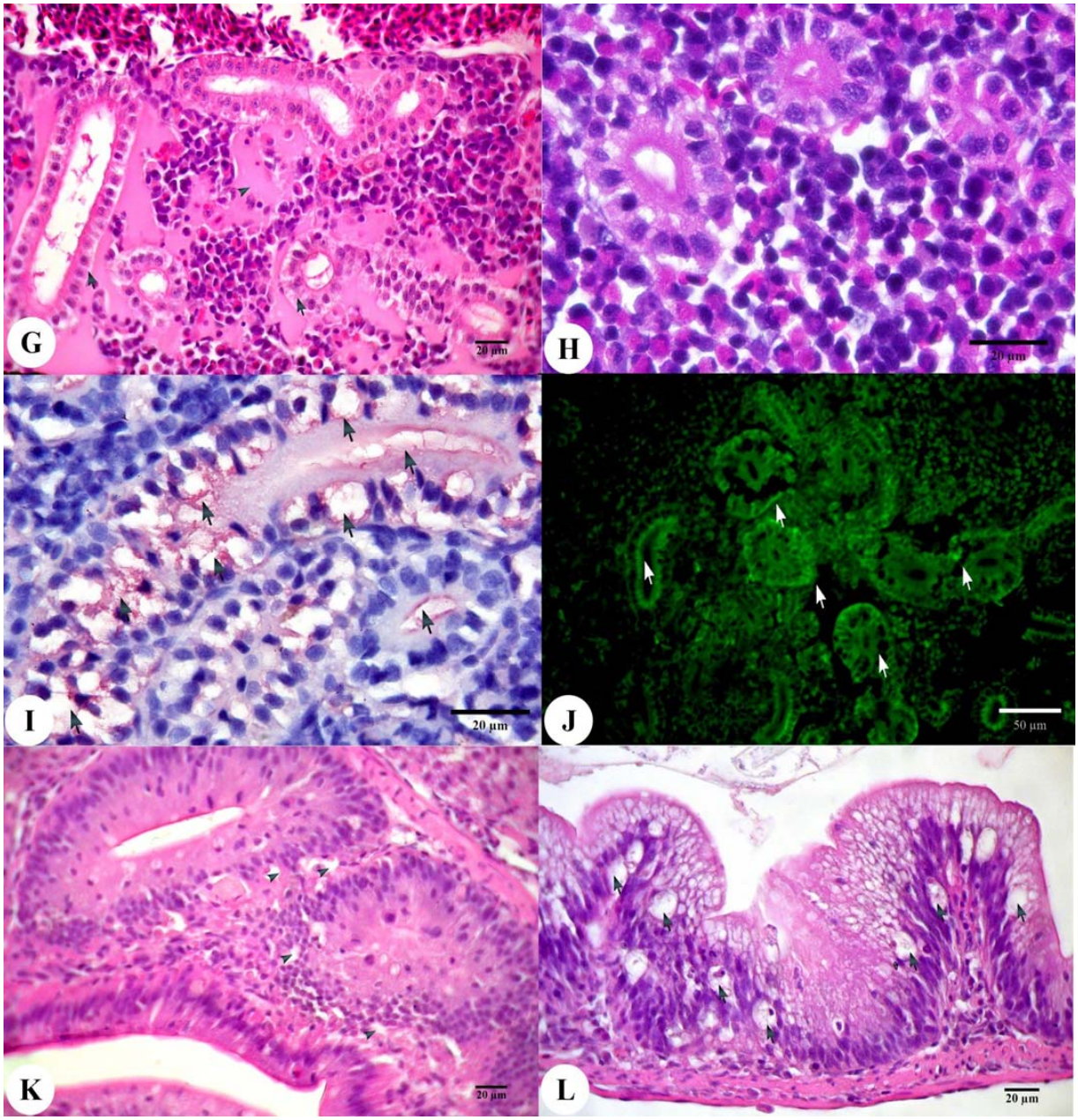

Figure (1) continued: (G) - Posterior kidney of common carp after 2 weeks of experimental SVCV infection showing diffuse amorphous oesinopilic material (arrowhead) infiltrating renal interstitium with tubular necrosis (arrows). H&E. (H) - Posterior kidney of common carp after 3 weeks of experimental SVC infection showing diffuse interstitial infiltration of mononuclear cells with some vacuolation inside the cytoplasm of tubular epithelium. H&E. (I) - Higher magnification of Fig (36) showing posterior kidney of common carp after 2 weeks of experimental SVCV infection showing red staining concentrated in vacuolated tubular epithelial cells and inside tubular lumina. Immuno-Alkaline Phosphatase, New Fuchsin substrate – Gill's Hematoxylin counterstain. (J) - Posterior kidney of common carp after 2 weeks of experimental SVCV infection showing green fluorescence (arrows) in the tubular epithelial cells and from the interstitial tissue and also inside the tubular lumina. Immuno-Fluorescence Technique. (K) - Intestine of common carp after 2 weeks of experimental SVCV infection showing mild villar vacuolation (arrowheads). H&E. (L) - Intestine of common carp after 3 weeks of experimental SVCV infection showing villar vacuolation (arrows). H&E.

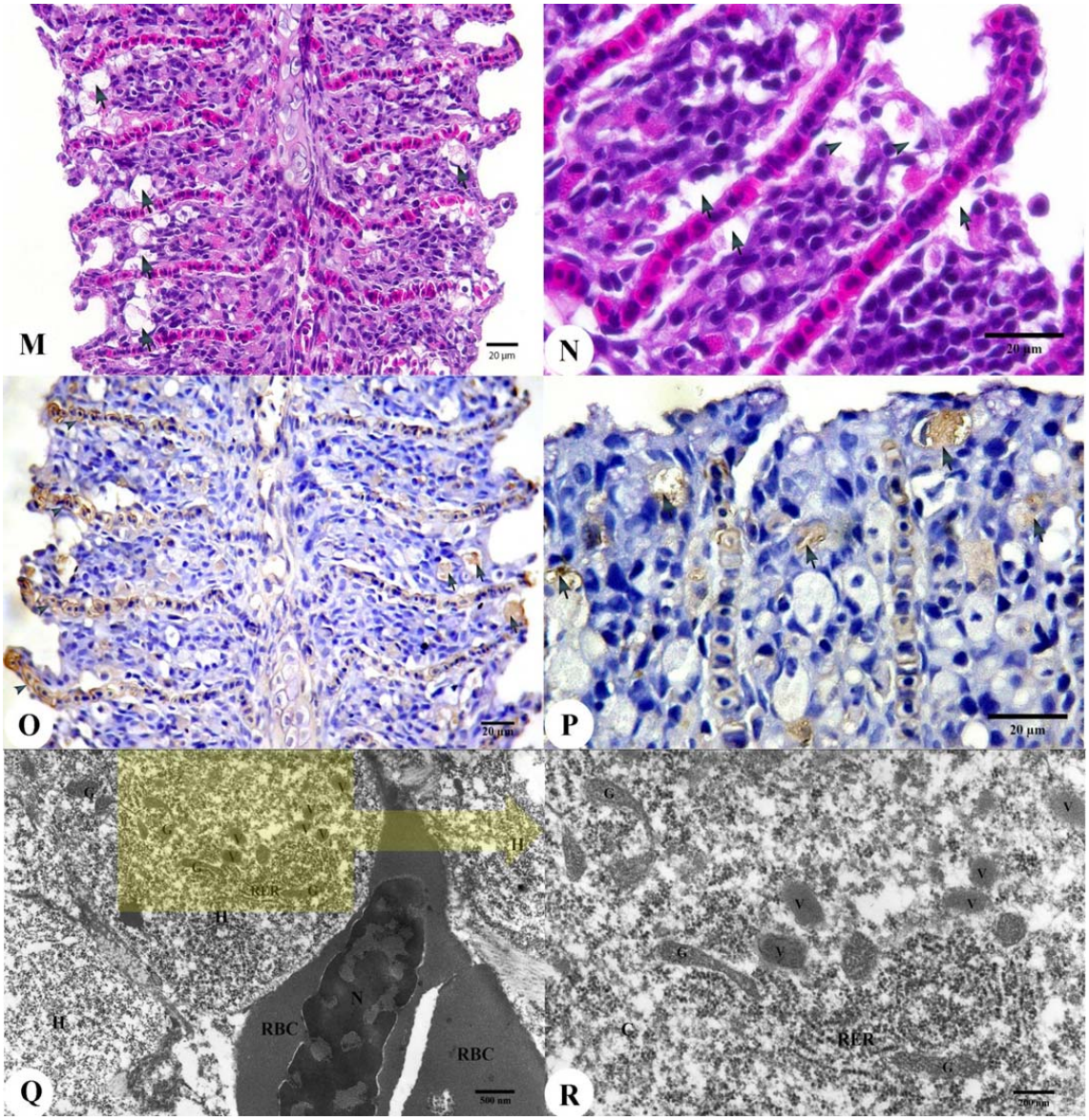

Figure (1) continued: (M) - Gills of common carp after 2 weeks of experimental SVCV infection showing diffuse lamellar fusion due to hyperplasia of the epithelial lining at the base of secondary gill lamellae with development of vacuoles (arrows) in the proliferated malpighean cells. H&E. (N) - Gills of common carp after 3 weeks of experimental SVCV infection showing degenerative changes of epithelial lining of secondary gill lamellae; subepithelial spongiosis; (arrowheads) with development of focal necrotic areas of the proliferated malpighean cells (arrows). H&E. (O) - Gills of common carp after 2 weeks of experimental SVCV infection showing brown staining demarcating the lamellar blood capillaries and inside vacuolated malpighean cells (arrows). Immuno-Peroxidase, DAB substrate – Gill's Hematoxylin counterstain. (P) - Higher magnification of Fig (43) showing gills of common carp after 2 weeks of experimental SVCV infection showing brown staining demarcating the lamellar blood capillaries and inside vacuolated malpighean cells (arrows). Immuno-Peroxidase, DAB substrate – Gill's Hematoxylin counterstain. (Q) - Electron micrograph of SVCV infected hepatocytes of common carp 2 weeks post-infection showing intra-cytoplasmic presence of SVCV as clear pullet shaped electron-dense particles (V) in the vicinity of Golgi apparatus. C= cytoplasm, G= Golgi apparatus & RER= rough endoplasmic reticulum. (R) - Electron micrograph of SVCV infected hepatocytes of common carp 3 weeks post-infection. Note the numerous virions (V) scattered in the cytoplasm at the vicinity of the nucleus. N= nucleus, C= cytoplasm, NM= nuclear membrane & RER= rough endoplasmic reticulum.

In pancreatic acini necrotic changes which were concurrent with hepatic changes were greatly similar to those observed by **Bucke and Finlay (1979)** as the exocrine tissue showed degenerative changes in the hepatopancreas and in free pancreatic tissue, there was moderate to marked necrosis of the acini. The Microscopical picture of posterior kidney showed proteineous dystrophy in the form of vacuolar degeneration of the tubular epithelium. Furthermore, tubular necrosis with lymphocytic infiltration was noticed. These results may be a sequelle of virus replication. Hyaline droplet in the tubular epithelium and eosinophilic detritus in their lumina were noticed, with evident mononuclear cell infiltration. These results may be due to virus replication in glomerular capillary tuft, which became more permeable to plasma protein including albumin. The marked depletion in haemopoietic elements; which was evident in spleen and kidney; was probably caused by virus cytopathic effect. The aforementioned alterations were seemingly similar to those described by **Negele (1977)** and **Osadcaja and Rudenko (1981)** who stated that the tubules of the kidney were clogged by the tube casts, vacuolation and hyaline degeneration. **Bucke and Finlay (1979)** denoted moderate to marked glomerulonephritis were evident in kidney. Meanwhile **Sulimanovic *et al.* (1986)** described trunk kidney to have diffuse necrosis of haematopoietic tissue as almost all cells of haematopoietic tissue were necrotic, with a varying degree of cell degeneration and some necrosis in tubules, also focal infiltration in haematopoietic tissue and peritubular oedema with apparent partial degeneration and necrosis in tubules were present.

The Microscopical picture of spleen revealed various degrees of degenerative and necrotic changes as lymphocytic (white pulp) depletion which appeared as focal areas of empty spaces in splenic parynchyma, this may be attributed to cytopathic effect of SVCV on splenic tissue and/or relocation of lymphocytes into other viral-infected organs. The detectable lesions in spleen were in agreement with those reported by **Negele (1977)** who stated also that spleen was hyperemic and showed a considerable hyperplasia of the reticuloendothelium, with siderocytes and cells with increased lipofuscin storage were present. Also **Osadcaja and Rudenko (1981)** and **Way *et al.* (2003)** described haemorrhage and inflammation in the spleen. The activation of melanomacrophage centers in spleen, was commonly noticed. It is quite known as an unusual sequel to infection or irritation in fish and is related to fish immune response as reported by **Robert (2001)**.

Various degrees of pathological harm in gills due to SVCV infection were evident. The remarkable lesions were severe hyperplasia of basal malpighian cells of primary gill lamellae, progression of this migration lead to lamellar fusion, with presence of malpighian cell vacuolations (spongiosis) which might be attributed to the drastic cytopathic effects of the SVCV. After that separation of the proliferated cells from the capillary bed supervened leaving subepithelial vacuolation, which might be attributed to viral replication in capillary wall. As a result of epithelial hyperplasia, inter-lamellar spaces decreased interfering with gaseous exchange. Moreover, as gills are important also for osmoregulation and excretion of toxic waste products as mentioned by **Robert (2001)**, thus any harm in the gills leads to impairment of such vital functions revealing respiratory distress, impaired osmoregulation and retention of toxic wastes. The detectable lesions in gills were reported by **Bucke and Finlay (1979)** as a moderate hyperplasia of the lamellar epithelium with associated macrophage cells at the tips of the lamellae in the gill tissue were observed. **Sanders *et al.* (2003)** described multifocal branchial necrosis and melanomacrophage proliferation in gills. Furthermore **Haghighi *et al.* (2008)** described fusion in secondary gill lamellae with necrotic foci due to SVCV infection in fish.

The Microscopical picture of gasbladder revealed degenerated gas gland cells with the loss of connective tissue symmetry due to heavy infection with virus and its destructive effects during multiplication. **Negele (1977)** denoted that lamina epithelia of the gasbladder changed into a discontinuous multilayer and in the submucosa dilated vessels and hemorrhages were evident. While **Wunner and Peters (1991)** described petechial haemorrhages occurred in the internal wall of gasbladder. Also **Haghighi *et al.* (2008)** described oedema of all internal organs as well as of the wall of gasbladder

The Microscopical picture of intestine revealed villar vacuolation which agreed with **Negele (1977)** who observed perivascular inflammation and desquamation of epithelium with a subsequent atrophy of the intestinal villi. Also with some extent with **Bucke and Finlay (1979)** who recorded marked degenerative and necrotic changes, with desquamation of the epithelial linings with complete destruction of the mucosal rugae in the posterior intestine, also **Osadcaja and Rudenko (1981)** denoted acute catarrhal enteritis, with necrosis and desquamation of the epithelium, as well as **Sulimanovic *et al.* (1986)** described necrosis and sloughing off of the epithelial layer of the intestine, oedema and necrosis in submucosa and oedema between internal and external muscular layer and in visceral peritoneum.

By using immunohistochemistry and Immunoflourescence the localaization of viral antigen was concentrated intra-cellular in hepatocytes, pancreatic acinar cells, renal tubular epithelium, gas gland cells and gill basal epithelium of primary filaments (malpighian cells), neuronal bodies. While intercellular distribution was localized in spleen, renal in

In conclusion, we report that there are similarity in accuracy between various methods of both immuno-staining techniques (Immuno-peroxidase and Immuno-alkaline phosphatase) and Immuno-flourescence technique in demonstration of SVCV in histological tissue sections with the preference of authors for immuno-staining techniques despite it takes more time in processing than Immuno-flourescence, as it doesn't need sophisticate equipments such as fluorescent microscope and it produces permanent slides can be used for scientific demonstration, this statement is agreed by **Faisal and Ahne (1984).**

In Transmission Electron Microscopy, rehydration of paraffin blocks and then re-processing for TEM observation produced satisfactory results and revealed the viral particles in SVCV infected fish specimens.

In the present study, electron microscopy of SVCV infected fish tissue revealed the presence of virus particles inside the cytoplasm especially nearby Golgi apparatus, this finding agreed with **Granzow *et al.* (1997)** who stated that SVCV particles adsorb to the plasma membrane and enter the host cell by receptor mediated endocytosis, and the first sign of viral replication is the formation of inclusion bodies in the cytoplasm, then virus budding at the plasma membrane and at membranes of dilated Golgi vesicles followed by maturation and release of virus from the cells. And the finding disagreed with **Saad (2005)** and **Abo Eisa (2007)** who referred to the presence of intra-nuclear viral inclusion bodies.

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