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SPRING VIRAEMIA OF CARP DISEASE: EXPERIMENTAL INFECTION OF CULTURED COMMON CARP (CYPRINUS CARPIO L.) IN EGYPT

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SUMMARY

Experimental oral infection of common carp (*C. carpio L.*) fish was carried out via stomach tube with 2 local positive samples of Spring Viraemia of Carp Virus (*SVCV*) at water temperature 14± 2°C. Investigation of the pathogenesis of SVCV to common carp (*C. carpio* L.) revealed that virus was detected in gills at the 3rd day post infection, and in the internal organs one week post infection. Clinical and post mortem examination of experimentally infected fish were recorded. The established infection was confirmed using monoclonal antibodies against *SVCV* by dot ELISA technique and electron microscopy. Histopathological picture of the experimentally infected common carp (*C. carpio* L.) showed severe changes in gills, liver and intestine.

INTRODUCTION

Cyprinid fish are exotic breeds of fish that were introduced to Egypt with the expansion of the aquaculture projects (FAO, 2002). It is ideal for rearing under intensification conditions (Muir & Roberts; 1982), and widely used in aquatic research projects (ICLARM, 1986).

Rhabdoviruses constitute one of the most pathogenic viruses affecting carp cultures from which Spring Viremia of Carp Virus (*SVCV*) is a predominant member (Wolf, 1988, Ahne et al., 2002, and Siwicki et al., 2003). The virions are bullet shape approximately 100 nm long and 50 nm wide, contain lipid envelope remain stable for at least 14 days at temperatures ranging from -80 to 5°C and grew optimally at temperature 15°C in cultures of epithelioma papulosum cyprinid (EPC) cells (Mork et al., 2004).

The disease caused by the *SVCV* is termed the spring viraemia of carp. It is widely spread in European carp culture where it causes significant morbidity and mortality. The disease was designated a notifiable disease by the Office International Epizooties (OIE) (Ahne et al., 2002).

The ecopathology of the SVC infection is principally related to water quality, management characteristics, fish age, season and water temperature (Ortega, et al., 1995). Pesticides that may contaminate the water as result of the agriculture run off found to have a role in the replication of the SVCV in the aquatic environment (Cossarini & Hattenberger, 1988). Blood sucking insects as leeches and carp louse serve as mechanical vectors for the virus (Ahne et al., 2002). Overcrowdness, long transportation, rough handling and stress increase susceptibility to infection with SVCV (Ahne, et al., 2002). The young carp are more susceptible to infection than adult ones but the development of clinical picture appears mainly in carp above one year old (Ghittino et al., 1980, Ortega et al., 1993). Elevated levels of corticosteroids as a result of stress are considered the important factors render the fish more susceptible to SVC infection.

Water temperature and season are of major influence on disease occurrence. Water temperature within 10-20°C resulted in the development of neutralizing antibodies to *SVCV* in 2-8 weeks (Ahne, 1986). Sanders, et al., 2003, developed a pathogen model using zebra fish (Danio rerio) to study the effect of two different water temperature (20 - 24, and 15°C) on infection with SVCV. Mortalities reached more than 50% in fish reared at lower water temperatures and clinical signs became evident after 7 days post infection. Also in Europe, the disease exhibited its high mortalities and clinical signs at water temperature 10-17°C, typically in spring (Ahne et al., 2002).

The clinical signs recorded to such a disease are lethargy, reduced appetite, signs of skin irritation, ulceration; extensive skin hemorrhages leading to osmoregulation function failure and impairment in the skin functions and in late stages of the disease, the fish cease feeding and dead (Roberts, 1994, Way et al., 2003). *SVCV* was associated with asymptomatic cases were recorded in starry flounder (platichthys stellatus) collected during viral survey of fishes in USA (Mork et al., 2004). Post mortem picture of the disease manifested by increase amount of fluids in body cavities, inflammation in the swim bladder, generalized changes in all internal organs which ranges from inflammation to degeneration.

Characterization of the *SVCV* was performed using electron microscopy, serum neutralization test, immunoflurescence test, and nucleotide sequence analysis (Betts, et al., 2003). Identification of *SVCV* using combined RT-PCR and nested PCR is now added to the current confirmatory diagnostic methods (Koutna et al., 2003). ELISA and antigen capture ELISA found to be highly specific assay for diagnosis of *SVCV* (Mourton, et al., 1990).

In Egypt the prevalence of SVC disease in *C. carpio L.* fish was reported (Ibrahem, 2002). The present study focus on the pathogenesis of SVCV in experimental model.

MATERIALS AND METHODS

1- Fish used for experimental work:

Ninety apparently healthy common carp (*cyprinus carpio L.*) with body weight range from 50-70 gm were obtained from semi - intensive fish farm at Giza governorate. The fish divided in 6 glass aquaria supplied by dechlorinated tap water, and electric aerators to maintain a level of 6.5 ±0.2 mg/l, pH value of 7.1 ±0.1and a hardness of 150mg/l as calcium carbonate. The fish left for adaptation for 2 weeks before the start of the experiments. Water was changed twice a week during the time of the experiments. The fish were maintained on feeding rate of 3% of body weight / day using commercial diet containing 30% protein according to (Jauncy and Ross 1982). The first four aquariums contained 15 fish and were used for viral inoculation; the last 2 aquariums contained 15 fish each and were held as control. The temperature was adjusted at 14 ±2°C through out the experiment. Random samples from the original fish lot were examined using dot ELISA after the 2 weeks acclimatization, to check that fish were free from *SVCV* infection.

2- Source of SVCV:

Two captured antigens were supplied by Dr. Mai, D, Ibrahem, department of fish disease and management; and were detected from naturally infected *C. carpio* fish by Dot ELISA technique and were demonstrated by negative transmission Electron microscopy.

3- Monoclonal antibodies against SVCV:

Lyophilized SVC- perox for fish ELISA and IFA was obtained from cypress diagnostics (Leuven-Belgium) and was reconstituted with the buffer upon usage.

4. Clinical and post-mortem examination of experimentally infected fish:

The experimentally infected fish were examined daily, mortalities were recorded, clinical abnormalities such as external skin hemorrhages, tail or finrot, exophthalmia, ascitis or prolapsed vent. Behavioral and clinical abnormalities were documented. The fish were dissected under aseptic conditions; examined for any post mortem abnormalities such as swim bladder inflammation, enteritis, ascetic fluids or changes in liver, spleen and kidney according to (Noga, 1996). Infected fish were observed up to 20 days post infection.

5- Sample collection:

The samples were collected from one day post infection for 20 days as follows: Gills were collected separately in sterile cryo- tubes. the internal organs. namely, spleen, liver and kidney as will as brain were collectively collected in sterile cryo-tubes. The samples were divided to 2 parts, one part was kept in -70°C for the virological techniques, the second part was fixed in 10% buffered neutral formalin solution for histopathological examination.

6- Sample preparation for virological examination:

The preparation of samples was carried out according to Hetrick (1989) as follows: The tissue sample homogenate was prepared by grinding the samples with one ml of PBS 7.2 and a considerable amount of sterile sand, followed by three cycles of freezing and thawing for 30 minutes. The homogenate was then clarified by centrifugation at 3000 rpm for 15 min. and the supernatant was collected in sterile vial and incubated with penicillin, streptomycin 500µg / ml. + 25µg / ml. nystatin .then kept in - 70°C till used.

7- Dot ELISA Technique:

This assay was carried out as described by Way et al., (2003).

8- Electron microscopy:

It was performed according to Mork et al., (2004) on Dot ELISA positive samples.

9-Histopathological examination:

For histopathological examination tissue samples were taken from gills, liver, spleen, and intestine of common carp fish. The samples were fixed in 10% buffered neutral formalin solution, processed by standard paraffin methods, sectioned at 4-5 um and finally stained with Haematoxylin and Eosin stain (Bancroft et al., 1996).

Experiments and results

Experiment (1):Experimental infection of common carp (*C. carpio*) fish by the local isolates:

Sixty *C. carpio* fish were divided to 4 aquaria as 15 fish for each, they were kept with out feeding for 24 hrs, then experimentally infected with the 0.5 ml of the captured antigens of *SVCV* using a stomach tube (measuring 21) gage according to Mahmoud (1996). 2 aquaria each contain 15 fish were held as control. The water temperature was adjusted to 14±2°C through out the experiment.

The fish were visually inspected for any behavioral abnormalities; gross lesions and mortalities. Samples were collected daily for 20 days from fish by scarifying 2 fish from infected groups and one from the control group and examined externally and internally. organ samples were divided in to 2 parts: first for virological examination. The second part was for histopathological examination.

The results:

No mortalities were recorded in the first 2 days. in the 3rd day, 2 infected fish died without showing any specific clinical signs, some Behavioral abnormalities as nervous manifestation, speedy swimming, fighting and signs of respiratory distress were noticed. On the 7th day post infection, the infected carp showed petichaeal hemorrhages on the surface of the skin especially at the area of caudal peduncle (Fig.1a), in other examined fish, ulcers on the dorsal surface were common (Fig.1b). At the 15 day post infection, abdominal distention, skin ulcers, and increased areas of skin hemorrhages were observed. Internally, examination revealed hemorrhagic patches on the swim bladder as well as black discoloration on the internal abdominal muscle (Fig.1c). Mortalities reached 25 % (7 fish out of 28 fish) in the last 5 days from the observation time as shown in Table (1).

Table (1): Clinical, post-mortem, behavioral changes and mortalities of experimentally infected *C. carpio* fish with SVCV.

Time / Abnormalities	Ist week	2nd week	3rd week
mortalities	In the 3rd day death of 2% fish	————	25 % mortalities
Behavioral & clinical signs	Nervous manifestation as speedy in swimming and fighting, signs of respiratory distress.		
P.M. changes	No Lesions	Skin hemorrhages, tail and fin rot.	Abdominal distention, skin ulcers, and increased areas of skin hemorrhages and inflammation of the swim bladder.

Experiment (2): Virological examination of experimentally infected C. carpio fish with SVCV:

a- Dot ELISA test:

The samples collected from the experimentally infected and control fish were collected daily as follows: gills were collected separately, brain and internal organs were collected together, the samples were prepared for virological examination as mentioned before and the techniques of Dot ELISA was carried out.

b- Electron microscopy:

Positive samples by Dot ELISA test were subjected to negative transmission electron microscopy for further identification.

The results:

a- Results of Dot ELISA test:

The use of Dot ELISA technique revealed that, positive detection of *SVCV* antigens from gill tissue at 3rd day post infection while the brain and internal organs were negative.

At 7th day, till the end of the experiment, the antigens were detected in all examined organs (Table 2)

Table (2): Results of Dot ELISA test.

Organs \ Time	Ist week	2nd week	3rd week
Gills	Negative	Positive	Positive
Brain + internal organs	positive	positive	positive

b- Results of electron microscopy:

Bullet shape viral particles measuring 100 nm length and 50 nm width appeared by negative transmission electron microscopy (x 30000). (Fig.2a).

Experiment (3): Pathological examination of experimentally infected C. carpio fish:

Histopathological sections were taken from gills, liver, and intestine of experimentally infected *C. carpio* fish according to (Bancroft et al., 1996).

The Results:

The histopathological examination revealed that, the most common lesions were in gills, liver and intestine.

In branchial tissue, lamellar oedema and areas of hemorrhage were noticed between the secondary gill lamellae (Fig.2b). In other cases, the branchial blood vessels were severely congested while the lamellar tissue showed fusion of the lamellae by cellular inflammatory reaction (Fig. 2c).

In the liver, both hepatocytes and pancreatic acinar cells of hepatopancrease showed marked vacuolation. Some of the examined hepatocytes showed eosinophilic intracytoplasmic bodies (Fig.2d).

In the intestine, edema in the lamina propria was noticed together with mononuclear cells infiltration (Fig.2e). The subepithelial tissue of the intestinal mucosa showed mononuclear cells infiltration and hyperplastic proliferation of some epithelial cells (Fig. 2f).

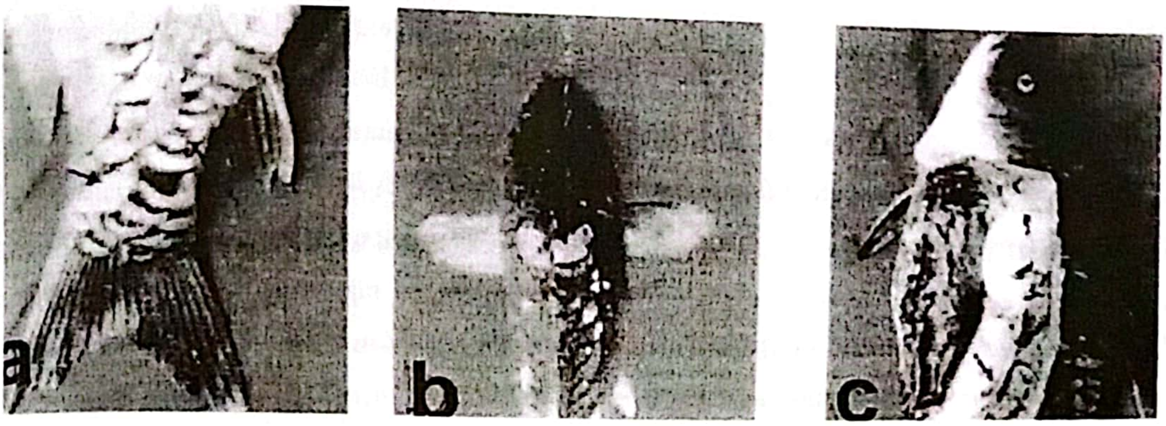

Fig.1: a- Common carp fish (*Cyprinus Carpio L.*) experimentally infected with SVC antigen showing. petichial hemor-rhages on the skin (arrow)

b- Common carp fish (*Cyprinus Carpio L.*) experimentally infected with SVC antigen showing dermal ulceration on the dorsal surface(arrow)

c- Common carp fish (*Cyprinus Carpio L.*) experimentally infected with SVC antigen showing hemorrhagic areas on swim bladder and black discolouration of the internal abdominal surface (arrow).

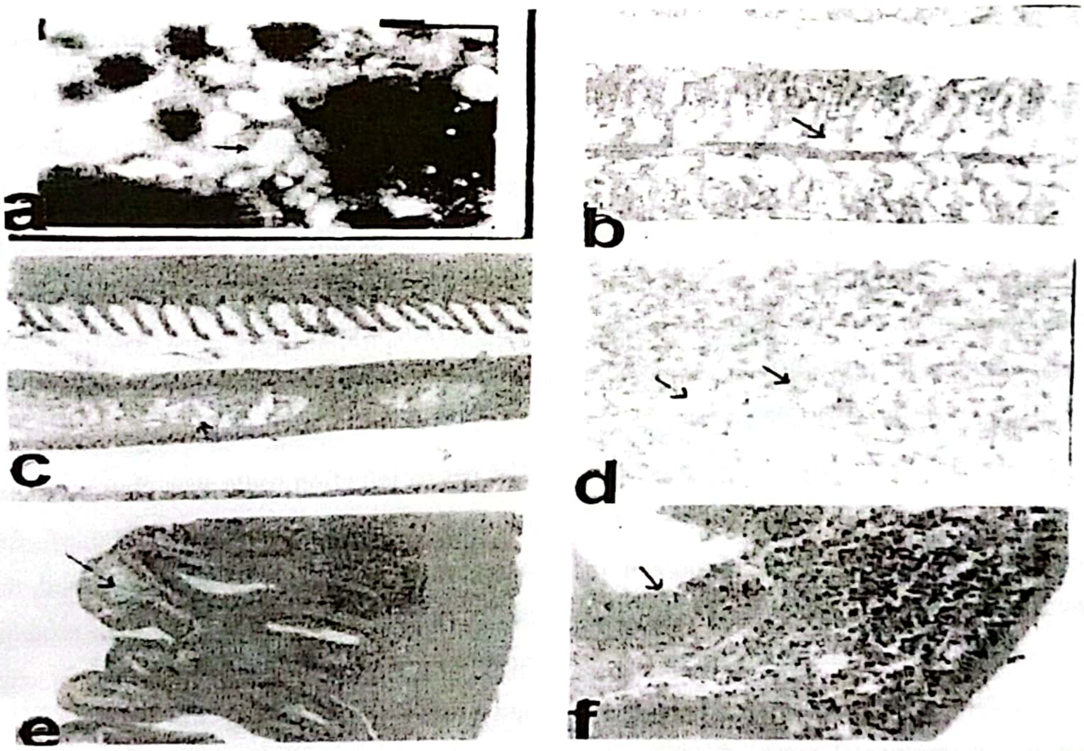

Fig.2: a- Negative staining technique of electron microscope showing bullet- shape SVC viral particles (arrow).x 30000.

b- Gills of Common carp fish (*Cyprinus Carpio L.*) experimentally infected with SVC antigen showing severe lamellar oedema (arrow).H&E stain x 400

c- Gills of Common carp fish (*Cyprinus Carpio L.*) experimentally infected with SVC antigen showing severe congestion of lamellar blood vessels and fusion of secondary gill lamellae (arrow).H&E stain x200

d- Liver of Common carp fish (*Cyprinus Carpio L.*) experimentally infected with SVC antigen showing vacuolar degeneration of both hepatocytes and pancreatic acinar cell and eosinophilic intracytoplasmic bodies (arrow). H&E stain x 400

e- Intestine of Common carp fish (*Cyprinus Carpio L.*) experimentally infected with SVC antigen showing oedema and mononuclear cells infiltration of lamina propria (arrow).H&E stain x200

f- Intestine of Common carp fish (*Cyprinus Carpio L.*) experimentally infected with SVC antigen showing hyperplasia of epithelial cells (arrow), and mononuclear cells infiltration. H&E stain x400.

DISCUSSION

Spring viraemia of carpvirus (*SVCV*) is from the specific fish viral agents the infect fishes specifically family Cyprinidiea.

SVCD was under study due to the following points: The rearing of susceptible host, the carp fish, the suitable environmental temperature and the spring season, various stress factors under intensification conditions and absence of vaccination programs against the Spring Viraemia of Carp disease (*SVCD*).

In the present study, experimental induction of *SVCD* was carried out. Common carp fish (Cyprinus Carpio L.) was chosen as a model fish for experimental work, like others who reported that carp fish is a principal host for Rhabdovirus carpio group (Baudouy et al., 1980., Dixon, 1997 .. Ahne et al., 2002 and Mork, et al., 2004).

Monitoring the water temperature to be 14 ± 2°C simulating the spring season during the course of the current experiment is in accordance by most of the researchers who induced the disease in the spring season as Ahne, (1986) where he observed influence of environmental temperature on the immune response of carp (Cyprinus Carpio L.), at 10°C *SVCV* persisted 12 weeks in blood of carp although antibodies were present between 10-20°C., neutralizing antibodies developed within 2-8 weeks. in addtion to Rodak et al., (1993) who noticed a marked effect of ambient temperature on *SVCV* infection, this was corresponded with the dynamics of natural outbreaks of the disease in which if the temperature spring is cold, the water temperature fluctuates over along period instead of rapidly rising to levels where antibody production is very rapid, and losses caused by *SVCD* are much higher than in years in which the rise to summer water temperatures occurs earlier. In High mortalities occur at water temperature of [illegible]-17°C typically in spring. At higher temperatures, infected carp develop humoral antibodies that can neutralize the spread of virus and such fish will develop solid immunity (Ahne, et al., (2002). The influence of environmental temperature on the course of *SVCD* was studied, (Essa et al, 2003), recoreded that gradual elevation of temperature from 14-18°C induced the overt *SVCD* higher (65%) and faster (18-23 days) in mortalities and development of clinical signs.

The per os infection route was chosen to conduct the experimental infection because of, first, it mimic natural mode of infection. Second, the tissue homogenate used contain foreign protein particles that by injection can cause tissue reaction and lead to misjudgment for the developed of clinical picture. The per os route was previously used by Ahne (1986), to study the influence of environmental temperature and infection route on the immune response of carp (*Cyprinus Carpio L.*) to *SVCV*. Also Dixon, 1997 immunized carp fish orally against *SVCV* by live attenuated vaccineand the results were convenient and practical more than the injection route. Water born route considered the predominant mode of transmission of rhabdoviridae beside the blood sucking insects (Ahne et al, 2002 and Pinto et al, 1993).

Characteristic gross lesions namely, skin hemorrhages (Fig.1a) ascitis and skin ulcers (Fig.1 b) and pale gills were the predominant clinical signs detected in the present study. These results were also observed by Baudoy et al; (1980 a), Baudoy et al; (1980 b), Ahne et al; (2002) and Way et al; (2003).

A characteristic nervous manifestation and swimming abnormalities were clearly found like those reported by Oreshkova et al; (1996) and Oreshkova et al; (1999) who detected the virus most reliably in fish brain and gills than in abdominal organs which could explain the clear behavioral abnormalities seen in this study.

Therapeutic and preventive measures against *SVCV* should based on the knowledge of the actual epizootiological situation, and consequently, require a specific and sensitive diagnostic method. Earlier methods for diagnosis of SVC infection were based on virus isolation and propagation in cell culture and the detection of specific antibodies by VN test, application of aforementioned methods have difficulties including: time consuming; requirement of laboratory experience and equipments; not suitable for examination of large numbers of samples and fish sera and organ homogenates have a toxic effect on cell culture, especially in low dilutions. Therefore, in the present study dot ELISA and electron microscopy were used as recommended by many researchers due to its sensitivity and accuracy for virus detection (Rodak et al; (1986), Mourton et al., (1990), Roberts (1994), Rowley et al., (2001), Ahne et al; (2002) and Koutna et al; (2003) . ELISA and EM are potential methods for rapid diagnosis of *SVCV*; however, recently recombined RT-PCR and nested PCR are included in the current confirmatory diagnostic methods.

Pathological effect induced in the current experimental model revealed marked pathological lesions in different organs. The gills showed oedema, congestion of blood vessels and fusion between gill lamellae. Such findings were previously seen by Ahne, (1978) who studied the pathogenesis of the virus and reported that *SVCV* enters through the gills after infection and then to other internal organs as liver, spleen and kidneys. This finding also explained the early detection of the viral antigen from gill tissue using dot ELISA.

Other histopathological findings including, petichial hemorrhages on the skin, vacuolation of the hepatocytes and enteritis were most common findings in the recent study. Although these findings are not pathognomonic for specific infective agents, it could be reliable to confirm the diagnosis of the viral antigen either by dot ELISA or electron microscopy. In this concern, Roberts, 1989 mentioned that the usual internal signs of SVCD are peritonitis, enteritis, oedema of the internal organs and swim bladder inflammation. In conclusion, the study reports the pathogenesis of *SVCV* and its clinical and pathological pictures.

REFERENCE

Ahne W. (1978): Uptake and multiplication of spring viraemia of carp virus in carp, cyprinus carpio L. J.fish.Dis., 1, 265-268.

Ahne W. (1986): The influence of environmental temperature and infection route on the immune response of carp (Cyprinus carpio) to spring viraemia of carp virus (SVCV). Vet Immunol. Immunopath. Jun 12 (1-4):383-6.

Ahne W., Bjorklund HV., , Essbauer S., Fijan N, Kurath G. and Winton JR.,(2002): Spring viraemia of carp (SVC) Dis. Aquat. organ. Dec 10; 52 (3) 261-72.

Bancroft, D.; Stevens, A. and Turner, R. (1996): Theory and Practic of Histological Techniques, 4th ed. Churchill Livingstone, Edinburgh, London, Melbourne.

Baudouy AM, Danton M and Merle G. (1980): SVCV infection of Carp. Ann Rech vet 11 (3) 245-249.

Baudouy AM, Danton M and Merle G. (1980a): Virrmie printaniere de la carpe: Etude expermentales de l' infection evoluant a differentes temperatures. Annales de virologie (paris) 13 IE, 479-488.

Baudouy AM., Danton M. and Merle G. (1980b): Experimental infection of Susceptible carp fingerlings with *SVCV* under wintering environmental condition in: Ahne W (ed) Fish diseases. Third COPRAQ session, Spirnger- Verlag, Berlin, pp. 23-27.

Betts AM. Stone DM., Way K,Tory C., Chilmonczyk S., Benmansour A and de Kenkelin P. (2003): Emerging vesiculo- type virus infectious of freshwater fishes in Europe. Dis Aquat organ. 29: 57 (3): 201-12.

Cossarini -Dunier M., and Hattenberger AM. (1988): Effect of pesticides (atrazine and Lindane) on the replication of spring viraemia of carp virus in vitro. Ann Rech vet 1988; 19 (3): 209-11.

Dixon P. (1997):Immunization with viral antigens: viral diseases of carp and catfish . Dev Biol stand 90:221-32.

Essa, A.A Manal; Tamam, S.M. and Madbouly, H.M. (2003): Some studies on SVC in common carp (Cyprinus carpio) in Egypt. Bani-Suef Vet.Med. J. vol. XIII. No (1) Oct., (301-314).

FAO, (2002): Fisheries department, Food Agriculture Organization, 2nd edition Rome Italy.

Gershy -Damet GM, Sangare. GA. Cisse A, Ouattara SA, Kone PI, Dosso M, Faye H and Kouakou K. (1987): Detection of the hepatitis A virus in fish at of fish farm. Bull soc Pathol Exot filiales 80 (5) :737-40.

Ghittino P.; Fijan N. and De Kinkelin, P. (1980): control methods for major viral diseases of fish in Europe. Bulletin de l' office international des epizooties 92, 967 - 978.

Hetrick, R.P. (1989): Fish viruses in method for microbiological examination of fish and shellfish text book by Austin and Austen, Ellis Horwood limited.

Ibrahem,M.D. (2002): Ph.D. Thesis , The role of some ecological factors on infection of fish with viral infection in Egypt. Fish Diseases and Management. Cairo univercity.

ICLARM (1986): Culture of common carp in floating net cages B.A. Costa-Pierce, Rusydi, A. Safari and G.W. Atmadja. 1989. ICLARM Educ. Ser. 7, 42 p.

Jauncy, K. and Ross, B. (1982): A guide to tilapia feeds and feeding institute of Aquaculture, University of Stirling UK ISBN.

Koutna M, Vesely T.Psikal I and Hulova J.(2003): Identification of spring viraemia of carp virus (SVCV) using combined RT-PCR and nested PCR. Dis Aquat organ. 4; 55 (3): 229-35.

Mahmoud, A.M. (1996): Studies of pathological changes in tilapia species due to motile aeromonad infection. Pathology, M.V. Sc Thesis, Cairo Univ.

Mourton C., Bear Zotti M, Piechaczyk M., Paolucci .F., Pau B., Bastide JM and de dinklin. P. (1990): Antigen captures ELISA for viral haemorrhagic septicaemia virus serotype1. J Virol Methods Sep; 29 (3): 325-33.

Mork C., Hershberger P., Kocan R., Batts W. and Winton J. (2004): Isolation and characterization of a Rhabdovirus from starry flounder (Platichthys stellatus) collected from the northern portion of Puget sound, Washington, USA. J. Gen viol. Feb; 85 (Pt 2):495-505.

Muir J., and Roberts R. (1982): Recent advances in aquaculture. Croomhelm, London, UK. Pp 266-355.

Noga, E. J.; (1996): Fish Disease: diagnosis and treatment. Moshy-Year book, Inc. Naples, Tokyo, New York .pp. 294.

Oreshkova SF, Takunova NV, Shchelkunov IS, Shchelkunova TI and Il'chev A. A. (1996): Detection of the spring viraemia of carp virus by hybridization with non radioactive DNA probes. Mol Gen Mikrobiol Virusol Apr-Jun; (2): 22-5.

Oreshkova SF, Shchelkunov IS, Takunova NV, Shchelkunova TI, Puzyerev AT, and Il'chev A. A. (1999): Detection of the spring viraemia of carp virus isolates by hybridization with non radioactive DNA probes and amplification by polymerase chain reaction.Virus Res.Sep; 63 (1-2): 3-10.

Ortega, C; Planas, E.; Docando, J.; Muzquiz, J. L.; ALQ nos J. L. and Simon, M. C. (1993): Epidemiological risk factors affecting presentation of viral agents in fresh water aquaculture in north eastern Spain. .Bulletin of the European association of fish pathologists 13 (5) 154-156.

Ortega C, Muzquiz, J. L.; Docando J, Planas E, Alonso JL, Simon MC. (1995): Ecopathology in aquaculture: risk factors in infectious disease outbreaks.Vet Res 26 (1): 57-62.

Pinto,R.M.; Jofre, J.; Abad, F.X.; Gonzalez-Dankaart, J.F. and Bosch, A.(1993): Concentration of fish enveloped viruses from large volumes of water. J Virol Methods; 43 (1): 31-40.

Roberts , R. J. (1989): Fish Pathology. 2nd ed.Bailliere tindall , London , England.

Roberts, A. M. (1994): Spring viraemia of carp.Veterinary Record 134 (24) 636.

Rodak L, Pospisil Z, Tomanek J., Vesely T. and Hampl, J (1986):Enzyme linked immunosorbent assay (ELISA) diagnosis of spring viraemia in carp [B].Veterinarni Medicina 31 (8):513-518.

Rodak L., Pospisil Z, Tomanek J, Vesely T.OBR and Valicek L. (1993): Enzyme linked immunosorbant assay (ELISA) for detection of spring viraemia in carp virus (SVCV)in tissue hemogenates of carp, (Cyprinus carpio l) J.fish.Dis., 16, 101-111.

Rowley H., Graham DA, Camphell S., Way K., Stone DM., Curran WI and Bryson DG. (2001): Isolation and characterization of rhabdovirus from wild common bream Abramis brama, roach Rutilus rutilus, farmed brown trout Salmo trutta and rainbow trout Oncorhynchus mykiss in north Ireland. Dis Aquat organ. Dec 20, 48 (1): 7-15.

Sanders GE, Batts WN andWinton JR., (2003): Susceptibility of zebrafish (Danio rerio) to a model pathogen, spring viraemia of carp virus. Comp Med. Oct; 53: 514-21.

Siwicki AK, Pozet F, morand M, Kazun B, Trapkowaska S. and Malaczewska J. (2003): influence of methisoprinol on the replication of Rhabdoviruses isolated from carp (Cyprinus carpio) and cat fish (Ictalurus melas):in vetro study. Pol J Vet Sci.6 (1):47-50.

Way K., Bark SJ., Longshaw CB., Denham KL, Dixon PF., Feist SW, Gardiner R, Gubbins MJ, Le Deuff RM, Martim PD, Stone DM and Taylor GR. (2003): isolation of rhabdovirus during outbreaks of disease in cyprinid fish species at fishery sites in England. Dis Aquat organ. Dec 3, 57 (1-2): 43-50.

Wolf, K. (1988): fish viruses and viral diseases Ithaca, Cornell University Press. Pp 250-252.