Sains Malaysiana 40(10)(2011): 1049–1052

Histopathology of Marine and Freshwater Fish Lymphocystis Disease Virus (LCDV)

(Histopatologi Virus Penyakit Limfosistis (LCDV) pada Ikan Marin dan Ikan Air Tawar)

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ABSTRACT

Lymphocystis disease (LCD) in fishes is caused by the agent called lymphocystis disease virus (LCDV). LCDV is a chronic and benign virus. The disease affects 96 species of marine and fresh water fishes ranged among 34 families in the world. Affected fish with LCD has a typical external symptom with clusters consisted of enormously hypertrophied dermal cells on the skin and fins. The hypertrophied cells, generally named lymphocystis cells, have a thick hyaline capsule, an enlarged nucleus and prominent basophilic cytoplasmic inclusions. Among the four species of fishes, olive flounder Paralichthys olivaceus*, and rockfish* Sebastes schlegeli *were marine cultured fish, and gourami* Trichogaster leeri *and painted glassfish* Channa baculis *were freshwater ornamental fish. Although LCD causes low mortality, the disfigurement of infected fish can make them unsellable. Thus LCD has resulted in an important economic loss in the aquaculture industry. This study of histopathology may be adequate for a presumptive diagnosis of lymphocystis diseases both in marine and freshwater fish species.*

Keywords: Fish; histopathology; LCDV; lymphocystis disease; virus

ABSTRAK

Penyakit limfosistis (LCD) pada ikan disebabkan oleh satu agen yang disebut virus penyakit limfosistis (LDCV). LCDV merupakan satu virus kronik dan kurang bahaya. Penyakit ini memberi kesan kepada 96 spesies ikan marin dan ikan air tawar di dunia yang tergolong dalam 34 famili. Ikan yang terjangkit LCD mempunyai simptom luaran yang tipikal dengan kelompok sel dermis hipertrofi pada kulit dan sirip. Sel hipertrofi juga dikenali sebagai sel limfosistis yang mempunyai kapsul hialin tebal, nukleus yang besar dan sitoplasma basofilik. Antara empat spesies ikan kajian, Paralichthys olivaceus *dan* Sebastes schlegeli *adalah ikan marin kultur manakala* Trichogaster leeri *dan* Channa baculis *adalah ikan air tawar hiasan. Walaupun LCD membawa kepada mortaliti yang rendah, tetapi ikan terjangkit mempunyai bentuk cacat yang menyebabkan ia tidak dapat dijual. Oleh itu, LCD telah membawa kerugian ekonomi dalam industri akua-kultur. Kajian histopatologi ini mungkin sudah cukup untuk diagnosis presumptif ke atas penyakit limfosistis pada kedua-dua ikan marin dan air tawar.*

Kata kunci: Histopatologi; ikan; LCDV; limposistis; virus

INTRODUCTION

Iridoviruses have been implicated as the causes of severe disease, mortality and economic loss in farmed fish and ornamental fish in wild. The first iridoviral disease was described in fish as lymphocystis disease virus (LCDV) which infects a great variety of freshwater and marine species. To control the viral diseases, it is important to know the viral dynamics and ecological niches as well as host fish. Although great advances have been made in LCDV studies, the molecular mechanism, virus ecology, replication, spreading and pathogenesis are not clearly understood. A common method for determining whether a virus is present in the fish population is to attempt histological observation in fish tissues. Therefore, much interest is being focused to know the development of LCDV in the host fish species. During the last few decades, many attempts have been made to propagate LCDV *in vitro*, both in homologous and heterologous cells, and a complete replication of the virus has not been achieved for those cell lines (Midlege & Malsberger 1968; Perez-Prieto et al. 1999; Walker & Hill 1980; Wolf 1966; Zhang et al. 2003).

Previous studies have shown that the genera; *Ranavirus, Iridovirus* and *Lymphocystivirus* include structurally related viruses, all of them composed of similar protein units which contribute to the icosahedral outline structure (Heppel & Berthiaume 1992). LCDV is most frequently outbreaks in the skin and fins of fish and cause economic impacts to the farmers because diseased unsightly fish would not be sold. Histopathologically, LCD is characterized by cytomegaly of dermal fibroblasts cells and only rarely has systemic involvement (Oh et al. 2006; Wolf 1988; Zwillenberg & Wolf 1968). The diagnosis of LCDV has been mainly based on the observation of

symptoms. Therefore, the present research aims to study the LCDV in the marine and freshwater ornamental fish through histopathological investigation.

Materials and Methods

Fish sampling

Four different fish species isolates of LCDV; two from marine fish olive flounder (*Paralichthys olivaceus*) and rockfish (*Sebastes schlegeli*), and other two freshwater ornamental fish gourami (*Trichogaster leeri*) and painted glassfish (*Chanda baculis*) were collected from Yeosu, Korea. The sampled flounder and rock fish were preserved with ice, and on the other hand, the gourami and glassfish were brought live in the laboratory. The live fishes were anaesthetized with MS-222 (Sigma, St. Louis, USA) and subsequently killed by organ dissection. The infected fish organs were aseptically cut off for histological study and preserved at -80ºC until further use.

Histological sample preparation and ultrathin section

Lymphocystis tumor tissues were fixed with 10% buffered formalin solution for hematoxylin and eosin (H&E) and later fixed in 2.5% glutaraldehyde in phosphate buffered saline (PBS) (0.1 mol l–1, pH 7.4) for 2 h at 4°C for ultrathin section. Then tissues were post fixed in 1% osmium tetroxide in PBS following 1 h at 4°C. After fixation sample were dehydrated in an ascending ethyl alcohol grade series followed infiltration and embedded in post fixed Epon -812 epoxy resin according to standard procedure. The section samples were stained with 1% toludine and methylene blue and observed under ultra- microscopy.

Results and Discussion

In general the lymphocystivirus prefers to replicate in dermal fibroblasts, resulting in hypertrophied cells. Grossly, lesions affecting the skin and fins consists of masses of individual nodules.

In this study, we observed the lymphocystis cells were over folded and invaginated, multi-lobular state, inclusion body and hyaline capsules were predominant (Figure 1(a)). Fibroblasts that were infected with lymphocystivirus continually enlarged or hypertrophy but do not undergo mitosis. The cytoplasm also changes, developing basophilic, intracytoplasmic inclusion bodies that appear as dense vacuolated bodies. In addition, a thick hyaline capsule surrounds the hypertrophied fibroblast were observed in the cytoplasm, especially in the mature lymphocystis cells (Figure 1(b)). The cytoplasmic organelles were crowded in the cytoplasm (Figure 1(c)). The typical LCD cells were observed about 250 nm in diameter which may be budded from inclusion bodies and scattered throughout the cytoplasm (Figure 1(d), Figure 3(a) & (b)). The host fishes were frequently affected by lymphocystis disease that occurs in the fins and skin only (Figure 2). This result hypothesized that the lymphocystis disease virus is organ specific and multiply only in the fibroblast cells.

Histopathologically it was observed hypertrophied cells in the fins and skin tissues of fishes were supported by other researcher reports as LCDV specific to organ infections (Alanso et al. 2005; Perez-Prieto et al. 1999; Walker & Hill 1980). The LCD virus propagation in the cells has been detected by several methods like PCR, immunoblot or cytometry by other researcher (Cano et al. 2006; Iwamoto et al. 2002; Qin et al. 2006). According to Mosharrof et al. (2009) LCDV has two groups; marine and freshwater isolates depending on virus protein profiles which are expensive and it takes long time to detect LCDV. However skin or fin biopsies for histopathology provide a definitive diagnosis of lymphocytsis which is very easy.

LCDV is becoming important because the new group of fishes are affecting both marine and freshwater fishes. Although, the fish have different aquatic environment in the present study, the marine (Figure 1) and freshwater (Figure 3) fishes showed same patterns of viral infected hypertrophied cells in the infected fish. All the isolates showed a common cellular pattern of infection in the skin and fins which are fibroblastic in nature. Thus, the similarities among cellular patterns of different fish LCDV isolates from different hosts indicated that these profiles do not depend on the host species.

Histopathological and cytological study of lymphocystis infection described in the bluegill (Dunbar & Wolf 1966) are similar to this study. Although the duration of development and regression of the lymphocystis was different, certain stages like fibroblastic cells having basophilic cytoplasm and prominent nuclei was similar to the present studies. Lymphocystis was reported in Japan from *Sebastes schlegeli* by Tanaka et al. (1984) where they stated that lymphocystis has common characteristics which include cellular hypertrophy, cell enclosure by a hyaline capsule, enlarged nucleus and prominent inclusions bodies.

In ultra-microtome photographs we observed about 250 nm in diameter of LCD cells within the perinuclear cisterna and membrane-enveloped inclusions scattered in the cytoplasm but not in the nucleus (Figure 1(c) & 1(d)). Hyatt et al. (2000) reported that freshwater gourami showed hypertrophy in the infected cells which developed endothelial cells, hypertrophy and hemorrhagic dropsy in gourami and swordtails. However, in the present study there was no such a hemorrhage in rockfish, gourami and glassfish except olive flounder *Paralichthys olivaceus*. Although Sheng and Zhan (2006) found typical hypertrophic lymphocystis cells in the gill, sub-mucosa of head kidney, mesenteries of liver, and intestine in Japanese flounder *P. olivaceus*. in the present study, lymphocystis nodules were only in the fins and connective tissue beneath the epidermis of skin and not in the other tissues.

In conclusion, this study of histopathology may be adequate for a presumptive diagnosis of lymphocystis

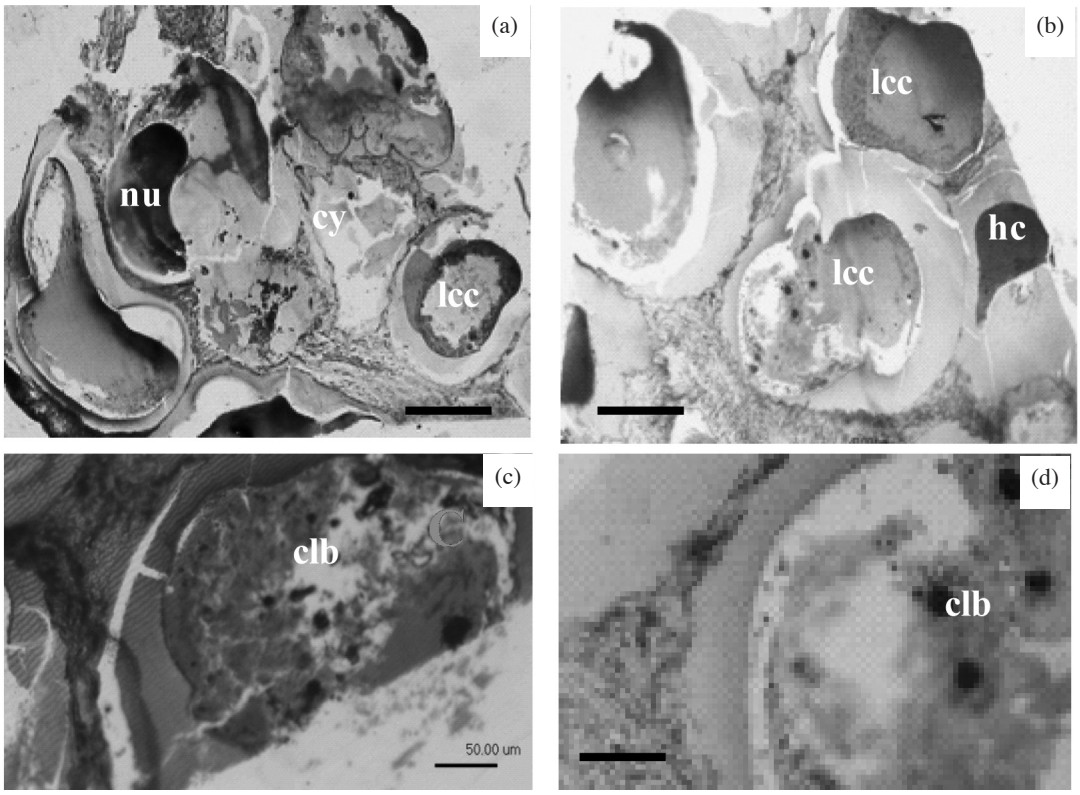

FIGURE 1. Histopathological features of lymphocystis disease virus of olive flounder and rockfish. (a) Ultrathin section showing cytoplasm (cy), nucleus (nu), lymphocystis cells (lcc); (b) Ultrathin section micrographs hyaline capsule (hc) and big LCD-cells (lcc); (c) Ultrathin section showing collapse lymphocystis cells with cytoplasmic inclusion bodies (clb); (d) Ultrathin section showing many cytoplasmic inclusion bodies (clb). (a&c, olive flounder; b&d, rockfish. a&c, methylene blue stained, b&d, toludine blue stained.)
(Scale bar: a, b, c, d, 50 μm, respectively.)

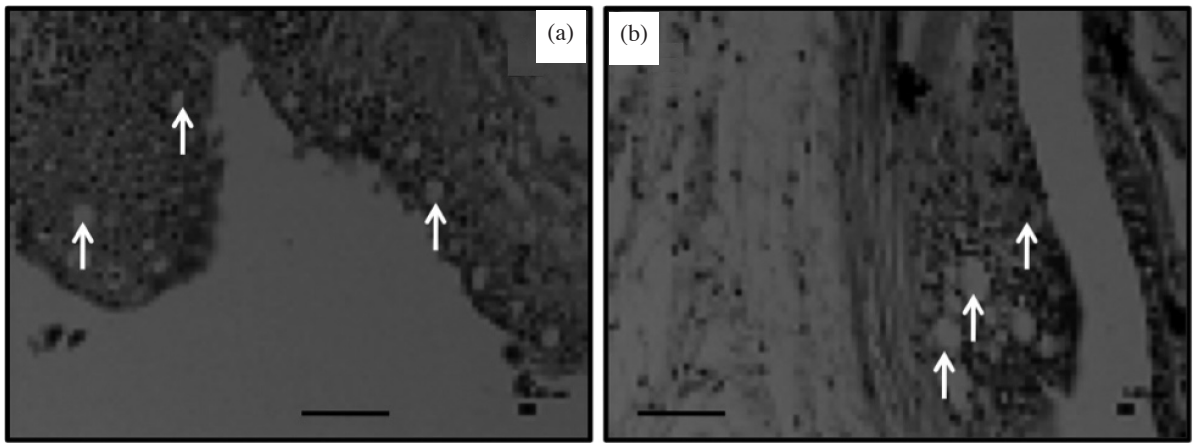

FIGURE 2. Arrows showing hypertrophied fibroblast cells in the LCDV infected fish.
(a) Skin and (b) Fin. (Scale bar-50 μm.)

diseases both in marine and freshwater fish species. The detection of asymptomatic carriers by histology using skin and fin sampling, which does not imply animal killing, could be an important tool to control epizootics caused by LCDV.

ACKNOLEDGEMENTS

The authors are grateful to Prof. Dr. Jung S.J. Division of Food Science and Aqualife Medicine, Chonnam National University, Korea for providing facilities to carry out the research works in her laboratory and useful suggestions.

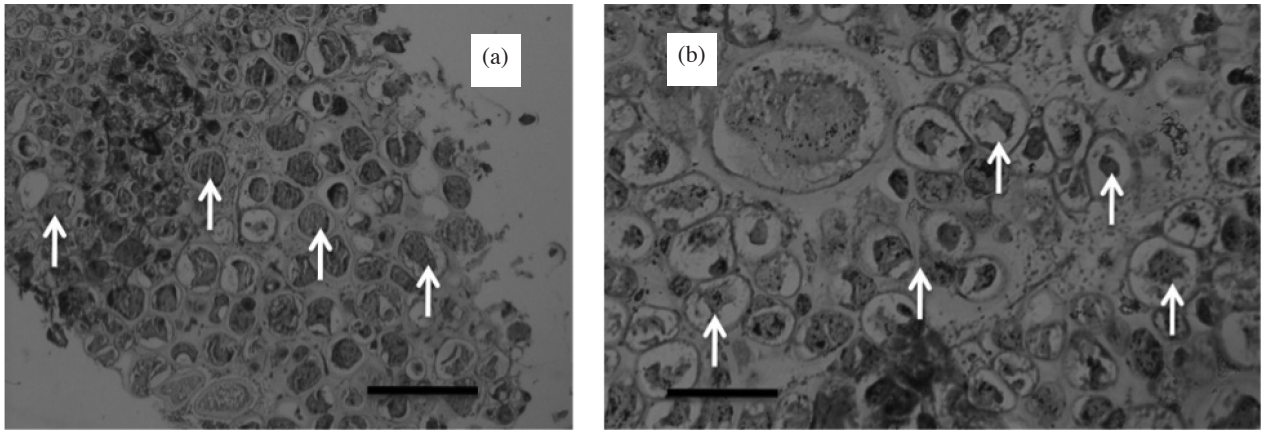

FIGURE 3. Arrows showing cross section of enlarged LCD infected tumor cells (H-E stain).
(a) Gourami isolate and (b) Painted glassfish isolate. (Scale bar-100 μm.)

REFERENCES

Alonso, M.C., Cano, I., Garcia-Rosado, E., Castro, D., Lamas, J., Barja, J.L. & Borrego, J.J. 2005. Isolation of lymphocystis disease virus (LCDV) from sole (*Solea senegalensis*) and black spot sea bream (*Pagellus bogaraveo*). *J. Fish Dis*. 28: 221-228.

Cano, I., Alonso, M.C., Garcia-Rosado, E., Rodriguez Saint-Jean, S., Castro, D. & Borrego, J.J. 2006. Detection of lymphocystis disease virus (LCDV) in asymptomatic cultured gilt-head sea bream (*Sparus aurata* L.) using an immunoblot technique. *Vet. Microbiol*. 113: 137-141.

Dunbar, C.E. & Wolf, K. 1966. The cytological course of experimental lymphocystis in the bluegill. *J. Infec. Dis*. 116: 466-472.

Heppel, J. & Berthiaume, L. 1992. Ultra-structure of lymphocystis disease virus (LCDV) as compared to frog virus 3(FV3) and chilo iridescent virus (CIV): effects of enzymatic digestions and detergent degradations. *Arch. Virol*. 125: 215-226.

Hyatt, A.D., Gould, A.R., Zupanovic, Z., Cunnigham, A.A., Hengstberger, S., Whittngton, R.J., Kattenbelt, J. & Coupar, B.E.H. 2000. Comparative studies of piscine and amphibian iridoviruses. *Arch. Virol*. 145: 301-331.

Iwamoto, R., Hasegawa, O., LaPatra, S.E. & Yosumizu, M. 2002. Isolation and characterization of the Japanese flounder (*Paralichthys olivaceus*) lymphocystis disease virus. *J. Aquat. Animal Health,* 14: 114-123.

Midlege, F.H. & Malsberger, R.C. 1968. In vitro morphology and maturation of lymphocystis virus. *J. Virol*. 2: 830-835.

Mosharrof Hossain, Jung, S.J., Kim, W.S., Kim, S.R. & Oh, M.J. 2009. Comparison of lymphocystis disease virus proteins between marine and freshwater fish. *J. Fish Pathol*. 22(2): 49-53.

Oh, M.J., Kim, W.S., Kitamura, S.I., Lee, H., Son, B.W., Jung, T.S. & Jung, S.J. 2006. Change of pathogenecity on olive flounder, *Paralichthys olivaceus* by co-infection of *Vibrio harveyi*, *Edwardsiella tarda* and marine birnavirus. *Aquaculture*, 257: 156-160.

Perez-Prieto, S.I., Rodriguez-Saint-Jean, S., Garsia-Rosado, E., Castro, D., Alvarez, M.C. & Borrego, J.J. 1999. Virus susceptibility of the fish cell line SAF-1 derived from gilt-head seabream. *Dis. Aquat. Org*. 35: 149-153.

Qin, W.Q., Gin, H.Y.H., Lee, L.Y., Gedaria, A.I. & Zhang, S. 2006. Development of cytometry based method for rapid and sensitive detection of a novel marine fish iridovirus in cell culture. *J. Virol. Method*. 125: 49-54.

Sheng, X.Z. & Zhan, W.B. 2006. Histopathological studies on the target organs of lymphocystis disease virus of fish. *J. Ocean Univ. China* 36: 749-753.

Tanaka, M., Yoshimizu, M., Kusakari, M. & Kimura, T. 1984. Lymphocystis disease in kurosoi S*ebastes schlegeli* and hirami *Paralichthys olivaceus* in Hokkaido, *Japan. Bull. Japanese Soc. Sci. Fish*. 50: 37-42.

Walker, D.P. & Hill, B.J. 1980. Studies on the culture assay of infectivity and some *in vitro* properties of lymphocystis virus. *J. General Virol*. 51: 385-395.

Wolf, K. 1966. The fish viruses. *Adv. Virus Res*. 12: 36-101.

Wolf, K. 1988. Lymphocystis disease. *In Fish Viruses and Fish Viral Diseases*, edited by Wolf K., Ithaca: Cornell University Press.

Zhang, Q.Y., Ruan, H.M., Li, Z.Q., Yuan, X.P. & Gui, J.F. 2003. Infection and propagation of lymphocystis virus isolated from the cultured flounder *Paralichthys olivaceus* in grass carp cell lines. *Dis. Aquat. Org*. 57: 27-34.

Zwillenberg, L.D. & Wolf, K. 1968. Ultrastructure of lymphocystis virus. *J. Virol*. 2: 393-399.

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Received: 7 September 2010
Accepted: 31 January 2011