錦鯉疱疹病毒之免疫膠體金標定技術與 2015 年電顯室工作報告

生物研究組
吳介豪 助理研究員

摘要

錦鯉疱疹病毒(koi herpesvirus) 為感染錦鯉(*Cyprinus carpio koi*)及鯉魚(*Cyprinus carpio carpio*)造成急性死亡之高度傳染性疾病病原。我國於 2002 年首次爆發本病，由於錦鯉疱疹病毒傳播速度快、死亡率高，造成錦鯉養殖嚴重經濟損失。免疫膠體金標定技術是利用抗原抗體結合原理，具有高專一性，藉金粒子標定出特定抗原，可利於後續診斷及研究之進行。本研究以穿透式電子顯微鏡之負染色法，建立錦鯉疱疹病毒之免疫膠體金標定技術，將錦鯉疱疹病毒與初級抗體接合後，以免疫膠體金作標記，利用穿透式電子顯微鏡觀察膠體金標記之數量及結合型態；並比較抗原抗體不同結合時間之差異，結果顯示 3 至 6 小時之抗體作用時間較佳，相較於原實驗方法 4℃隔夜，可減少大量之試驗時間並觀察到更清晰之病毒型態；本試驗成功建立錦鯉疱疹病毒之免疫膠體金標定技術。

2015 年電顯室檢體總件數共 353 件，負染色檢體 346 件，包含禽類 78 件，草食動物 48 件，水產動物 144 件，豬 2 件，其他 74 件，超薄切片檢驗共 7 件，其中 136 件檢體有檢出特異性病原顆粒。

Establishment of an immune-gold labeling technique for visualization of Koi herpesvirus via electron microscopy and periodic report in 2015

Chieh-Hao Wu

Abstract

Koi herpesvirus (KHV) is an emerging virus which causes fatal disease in koi (*Cyprinus carpio koi*) and common carp (*Cyprinus carpio carpio*). Koi herpesvirus disease has been first reported in Taiwan in 2002. Because of its rapid spread and high mortality, this disease caused koi farms serious economic losses.

Immuno-gold labeling is a technique used to label specific antigens by employing colloidal gold-antibody conjugates. Based on the antigen-antibody binding method, the method is highly specific and is therefore useful to provide the following diagnosis and for further research.

The study aims to establish an immuno-gold labeling technique in koi herpesvirus with the negative staining of transmission electron microscopy (TEM). The specific antibody binds to koi herpesvirus and labelled with the colloidal gold particles. The number and morphology of labelled viruses were thus observed by TEM. Under the different binding time, it revealed that more clear and intact virus patterns were revealed in three to six hours incubation compared with the original method using a 4 ℃ overnight incubation. This study has established an immuno-gold labeling technique for the detection and visualization of koi herpesvirus.

A total of 353 samples were collected for electron microscopy in 2015. In addition to seven samples processed with the ultramicrotome, 346 samples including 78 samples from poultry, 48 samples from herbivores, 144 samples from aquatic animals, 2 samples from swine, and 74 samples were examined by negative staining for pathogen observation. One hundred and thirty-six of those specimens were positive for specific virons/bacteria.