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臺灣南部淡水魚塭之鱧魚 *Nocardia asteroides* 感染症之研究

An Epizootic in Formosa Snake-Head Fish (*Channa maculata* Lacepede), Caused by *Nocardia Asteroides* in Fresh Water Pond in Southern Taiwan

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Abstract

An epizootic in formosa snake-head fish (*Channa maculata* lacepede) of the fresh water pond was found to be caused by *Nocardia asteroides* in Pingtung Hsein, Taiwan, in September 1987. The accumulative mortality within two week was 20 % (6,000 out of total 30,000 fish) and the diseased fish were approximately 8-9 month-old with total length of 20-30 cm. Clinically, diseased fish swimmed slugishly at the surface of water or nearby the wall of pond. Fresh blood was found from oral and opercular cavity in moribund fish. Multiple, yellowish-white nodules, vary in size from 0.5-2.0 cm in diameter, were scattered throughout the muscle, gill, heart, liver, spleen, ovary and other internal organs. Many rods or filamentous bacteria were seen in the squashed preparation from these nodulous lesion. Histopathologically, the typical granulomatous lesion were found in the muscle, gill, liver, spleen and G-I tract, especially aboundant in spleen, kidney and liver. The microscopic morphology of the isolated bacterium from Brain Heart Infusion agar, blood agar and Ogawa's medium was similar to fresh smear from granulomatous lesion. Based on the growth character, morphological and biochemical properties, the isolated bacteria, was identified as *Nocardia asteroides*. This is the first report of *Nocardia asteroides* infection in formosa snake-head fish in Taiwan.

緒　　言

於 1963 年首先由 Valdez & Conroy 及 1964 年 Conroy 報告，從阿根廷的霓虹燈魚 (*Hyphessobrycon innesi*) 分離出 *Nocardia asteroides*, 1964 年 Snieszko 等也報告美國西維吉尼亞

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州立國家養鱒場於 1962-1963 年間首度爆發鱒魚苗亦感染本菌，病魚呈現腹部腫大，並由病灶區塗抹可見絲狀分枝桿菌。1965 年在德國亦發現七種魚類患有此病 (Ribelin & Miyaki, 1975)。1968 年加拿大境內河鱒 (Brooktrout) 也有病例報告 (Campell & Mackelvie, 1968)。日本也有黃尾魚 (*Seriola quinquiradiate* & *S. purpurascens*)，感染本病報告 (Kariyal *et al*, 1968)。本省 (Hsu *et al*) 曾發現 *Nocardia* 感染病例，但並未鑑定其種名。

材料與方法

臨床症狀及肉眼病變

1987 年 9 月屏東某淡水魚塭養殖鱧魚發生連續死亡，兩週內累積死亡率爲 20% (6,000 尾 /30,000 尾) 病魚爲 8-9 月齡，全身長 20-30 公分，體重爲 200-400 公克，臨床上病魚游至池邊，行動遲緩，食慾不振，腹部腫大等症狀，發病魚。

口腔及鰓腔有鮮血流出。肉眼病變，剖檢時腹部腫大，內有少量透明至黃色液體；肝、腎、脾、心、卵巢、肌肉等 (圖 -1, 2, 3, 4) 器官有 0.5-2 公分直徑之黃白色結節密發，切片可見黃白色濃汁流出。病灶區壓片檢查可見抗酸性及革蘭氏染色陽性菌。

病原菌之分離培養

細菌之分離培養可分爲兩組，即一組於 Brain Heart Infusion (BHI) Agar, (Difco), Blood Agar (以 Tryptic Soy Agar, BBL，爲基礎培養基含 5%山羊 RBC)，Ogawa medium (自配) 及 Sabouraund dextrose Agar (Difco), BHI broth (Difco) 等，另一組以 BHI Agar 及 BHI broth 中添加 3% NaCl，以無菌操作法取腹腔內之結節組織分別接種於上述培養基。細菌鑑定則測定 Casein、Xanthine、Tyrosine、Starch、Gelatin、Urea、Litmus milk 及 Nitrate 之分解利用及各種醣類分解、Indol、H_2S 產生等生化學性狀，另外測定各種不同溫度 (25°C, 37°C, 41°C 及 50°C) 下之生長或生存情形。

組織病理學檢查

採病變臟器組織，以 10%中性福馬林液固定，石蠟包埋，切片以 Hematoxylin eosin 及 Acid fast 染色。

結　果

病原菌之分離培養

在 25°C 有氧的狀態下，經 4-5 日後，在 Blood Agar, BHI Agar 及 Ogawa medium 上出現細小，淡黃色圓形或摺疊樣砂粒狀性菌落。於 Sabouraund Dextrose Agar 上之形態爲黃色或橙色。在 BHI broth 中經過十日培養可見長絲狀分枝桿菌，本菌在 37°C 亦可發育，但生長較差，經革蘭氏及抗酸性染色均呈陽性反應 (圖 -11, 12)。分離菌呈現 Catalase 陽性，Oxidase 陰性；Casein, Xanthine, Tyrosin, Starch，及 Gelatin 均不水解，Indol, Motility, H_2S, Nitrate reduction, Litmus milk test 均呈陰性，Urease 呈陽性，本菌在 25°C 及 37°C 於 3% NaCl BHI broth 中發育良好。41°C 生長被抑制，於 50°C 作用 8 小時後，再放置於 25°C 培養亦能存活 (表 1)。

組織病理學變化

病理組織切片檢查，各臟器呈現多數大小不等之肉芽腫結節病變爲特徵，即該病灶有大量細菌及壞死組織及少量炎症細胞爲肉芽腫中心，並有明顯數層類上皮細胞包圍，其最外圈有一層結締組織當作被

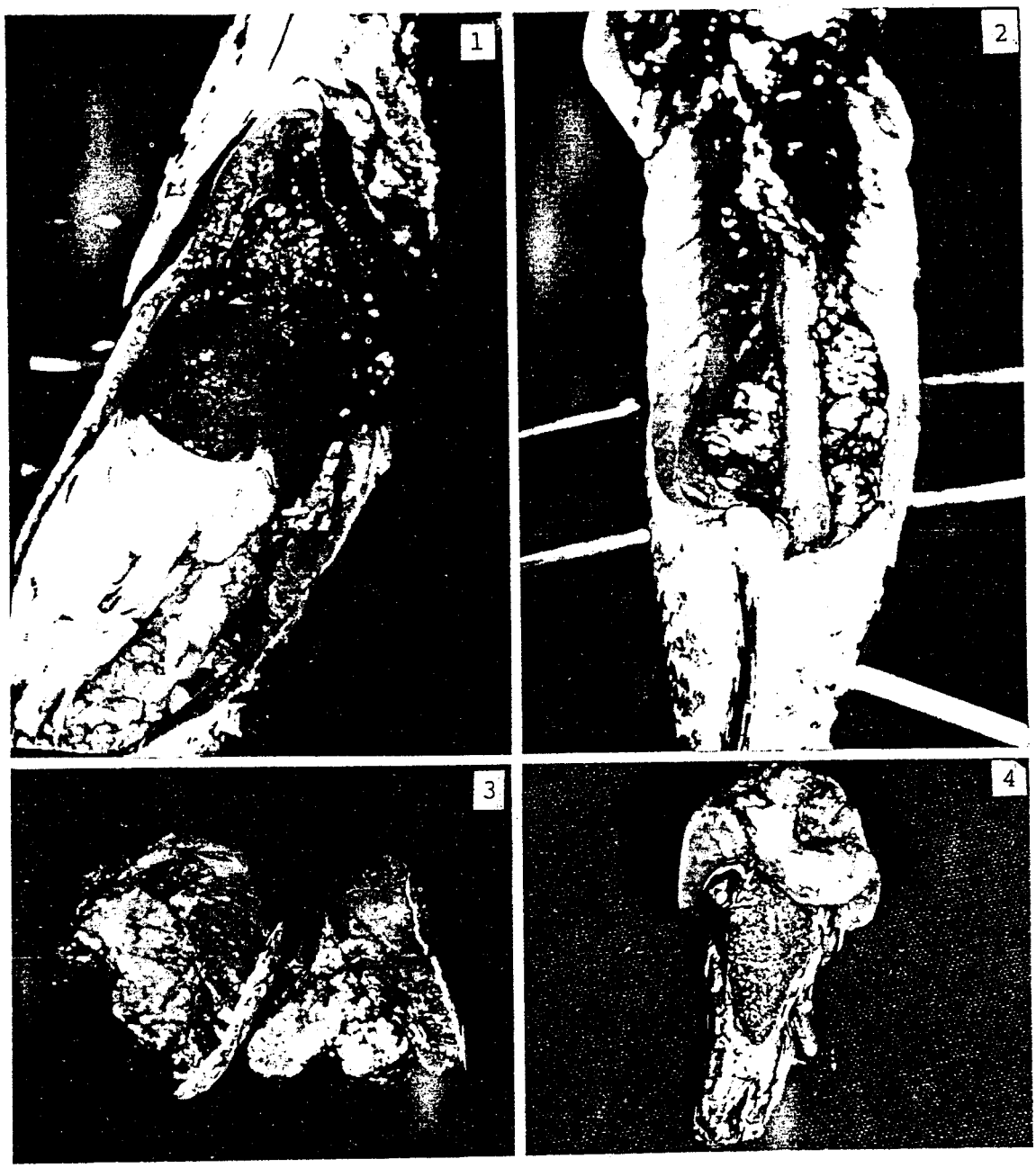

Fig. 1. Marked yellowish-white granuloma, vary in size, were found in gill, heart, liver of formosa snake-head fish.

Fig. 2. Marked enlargement of granuloma with yellowish-white cheese-like pus was seen in kidney of formosa snake-head fish.

Fig. 3. Massive granuloma was found in incision of kidney of formosa snake-head fish.

Fig. 4. Marked yellowish-white granuloma, vary in size, were showed in spleen of formosa snake-head fish.

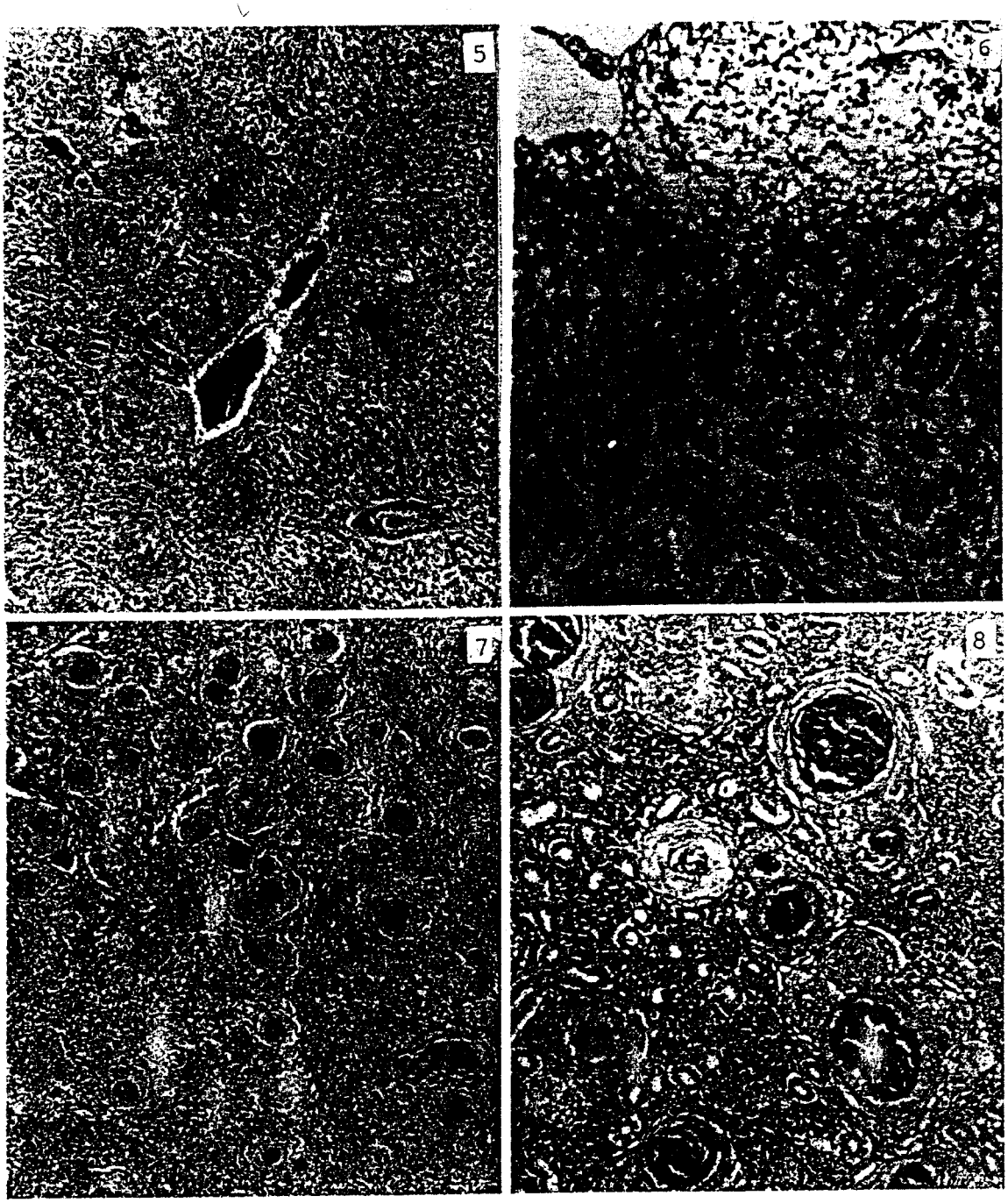

Fig. 5. Marked granuloma, inflammatory cells with cellular debris in the center and encapsulated by fibrous tissue & epitheliod cells, were found in liver of formosa snake-head fish. 40X, H&E.

Fig. 6. Marked granuloma were seen in epicardium of formosa snake-head fish. 200X, H&E.

Fig. 7. Marked granuloma, vary in size, necrotic foci in the center and surrounded by thick fibrous tissue, were found in spleen of formosa snake-head fish. 40X, H&E.

Fig. 8. Marked granuloma, vary in size, cellular debris in the center and ecapsulated by thick fibrous tissue, were found in kidney of formosa snake-head fish. 40X, H&E.

Table 1. Growth[a] and Biochemcial properties of *Nocardia asteroides*

Weakly acid fast staining reaction		+[b]	Catalase	+
Gram-postive rodes, cocci and filaments		+	Oxidase	−[e]
Blood agar		+	Motility	−
BHI agar[c]		+	H_2S	−
Sabouraud dextrose agar		+	Nitrate reduction	−
Ogawa medium	25°C	+	Litmus milk	−
	37°C	+	Urease	+
	41°C	−	Casein hydrolysis	−
BHI agar	50°C 2 hr	+[d]	Xanthine hydrolysis	−
	4 hr	+	Tyrosine hydrolysis	−
	8 hr	+	Starch hydrolysis	−
			Gelatin hydrolysis	−

a/ All the growth characterics were read after 14 days incubation at 25°C.
b/ Growth or positive.
c/ Brain heart infusion.
d/ BHI broth culture was treated in 50°C water bath for 2 hr, 4 hr or 8 hr, then was streak on BHI agar.
e/ No growth or negative.

膜，這類病灶廣泛分布於肝，心，腎，脾，腦，腸及卵巢等組織內（圖 -5, 6, 7, 8, 9）經抗酸染色呈現弱陽性反應（圖 -10）。

討　　論

革蘭氏陽性引起魚類慢性肉芽病變，包括棒狀桿菌（*Corynebacterium* Sp.），分枝桿菌（*Mycobacterium*），葡萄球菌（*Staphylococcus* Sp.）及 *Nocardia* Sp. (Rebelin & Miyaki, 1975)。本病例新鮮臟器塗抹標本及分離細菌可見具弱抗酸性及革蘭氏陽性球狀，桿狀或絲狀分枝桿菌，由以上性狀及細菌培養之菌落，及生長生化特性可以鑑別本菌爲 *Nocardia* Sp.。病魚臨床呈現腹部腫大，剖檢可見腹腔積水及內臟器官呈現肉芽腫樣病灶與 Snieszko 等在鱒魚報告相似。

由組織病理學各臟器呈現肉芽腫病變，經由 Ziehl-Neelsen 抗酸性染色呈現弱陽性及病灶區分離細菌於 Blood Agar 及 BHI Agar 上均呈現細小、淡黃色或折疊樣乾性顆粒樣菌落與 Robert 1983 所著魚類病理學所記載相同。本病例之新鮮塗抹標本，分離細菌及病理組織切片均呈弱抗酸性陽性與本省 (Hsu 1977) 所報告之 *Nocardia* 有所不同。

生菌生化學性狀除 41°C 不發育外，其他如對 Casein, Xanthine, Tyrosine, Starch 及 Gelatin 均不水解及 25°C, 37°C 均能發育及 50°C 下作用 8 小時尚能存活等性狀與江草 (1983) 及 Cowans (1974) 所記載之 *Nocardia asteroides* 相符合，因此本病診斷爲 *Nocardia asteroides* 感染鱧魚之 Nocardiosis。

摘　　要

1987 年 9 月，在屏東某淡水魚塭養殖鱧魚場發生 *Nocardia asteroides* 感染症。其兩週內累積死亡率達 20% (6,000 尾/30,000 尾)，病魚全長爲 20-30 公分，約 8-9 月齡。臨床上，病魚蹣跚浮游於水面或靠池邊。鮮血由口腔及鰓蓋流出。肉眼可見多發大小不同黃白色結節約 0.5-2 公分直徑，分

9

10

11

12

布於肌肉，鰓，心，肝，脾，卵巢和其他內臟器官。結節之壓片標本可見桿狀和絲狀分枝細菌。

組織病理學呈現典型肉芽腫病變，可見於肌肉，鰓，肝，脾和胃腸等，特別是在肝，腎及脾臟。由病灶區分離之細菌之形態和新鮮壓片檢查結果相同。由以上細菌之生長特性，形態及生化之性狀及組織病理學診斷爲臺灣首次發生之鱧魚之 *Nocardia asteroides* 感染症。

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Explanation for Figures

Fig. 9. Marked necrotizing area, long filamentous bacteria with cellular debris, were found in kidney. 40X, H&E.

Fig. 10. Marked granuloma with long rod bacterium were seen in liver of formosa snake-head fish. 400X, Acid fast stain.

Fig. 11. Long, filamentous bacteria were found from brain heart infusion broth at 10 days after cultured. 1,000X, Gram stain.

Fig. 12. Long, filamentous bacteria were found from brain heart infusion agar at 7 days after cultured. 1,000X, Acid fast stain.