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Total and Organic Hg in Fish from the Reservoir of a Chlor-alkali Plant in Tainan, Taiwan

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ABSTRACT

Total mercury (THg) and organic mercury (OHg) concentrations were determined by cold-vapor AAS in ten species of fish, which were caught in the reservoir of a chlor-alkali plant in Tainan, Taiwan that had been abandoned for 22 years. The fishes, including *Elops machnata*, *Pelates quadrilineatus*, *Gerres filamentosus*, *Leiognathus equulus*, *Thryssa hamiltonii*, *Orechromis* spp., *Nematalosa come*, *Liza macrolepis*, *Mugil cephalus* and *Chanos chanos*, were sampled from September to October 2003. THg and OHg concentrations (μg/g flesh wt.) in fish muscles were 0.43 ± 0.36 and 0.31 ± 0.21, respectively, similar to values reported from various chlor-alkali plants worldwide. Of the OHg values, 37% exceeded the food safety limit of 0.3 μg/g set by the World Health Organization. Moreover, 77% of the tested OHg values were over 0.17 μg/g, which had been suggested for seafood safety limit in Taiwan. Finally, 100 g of fish muscle is the maximum allowable weekly consumption amount for the adult residents to avoid health treat that arise from ingesting toxic OHg.

Key words: heavy metal, estuarine fishes, fish muscles, safety seafood consumption, health threat

INTRODUCTION

Elemental mercury (Hg) is emitted naturally into the environments through volcanic eruptions, forest fires, and biological emissions(1). Various human activities, such as mining, metallurgy, waste handling, chlor-alkali production, and coal burning, have caused severe Hg pollution(2-5), e.g. the "Minamata disease" from production of chlor-alkali. In 1953, Minamata disease was discovered in Japan and resulted in 121 severe poisoning cases(6). Moreover, the toxic effects on residents have persisted for three generations up to the present. For this reason, careful monitoring of the point sources of Hg from chlor-alkali waste is essential.

Traditional chlor-alkali industry introduced sea water as a raw material, and used the Hg-electrolysis method to produce chlorine. During production, the used Hg is released through drainage into the aquatic environment and then stored in the sediment. Therefore, the impacts on environment from chlor-alkali industry have been driven attentions by scientist worldwide(7-10). Previous studies showed that Hg levels in the air, water, and sediment have potential toxic effects on aquatic organisms. In fact, seafood is the main source of Hg for humans(11), and consumption of polluted fishes may result in irreversible damage to health.

Eight chlor-alkali plants existed in Taiwan from 1940 to 1988 before the ban on chlorine production by Hg-electrolysis. For every 900 kg of chlorine produced, one kg of Hg was discharged into the environment. About 1.26×10^5 tons of Hg-containing wastes were produced(12) during 48 years of operation, but research on chlor-alkali pollution has seldom been reported in Taiwan. Therefore, we investigated the total mercury (THg) and organic mercury (OHg) concentrations in fishes from a reservoir next to a chlor-alkali plant to determine its pollution status, in addition to giving recommendations for the amount of fish from the reservoir that can safely be consumed.

MATERIALS AND METHODS

I. *Samples*

From September to October 2003, sampling was

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conducted four times in the reservoir adjacent to a chlor-alkali factory which operated from 1938 to 1971 in Tainan City, Taiwan. The factory covered an area of 13.5 hectares. A total of 236 specimens of ten species of fish that commonly occur in estuaries were collected, including tenpounder (*Elops machnata*, Ema), fourlined terapon (*Pelates quadrilineatus*. Pq), whipfin silverbiddy (*Gerres filamentosus*, Gf), common ponyfish (*Leiognathus equulus*, Leq), Hamilton's thryssa (*Thryssa hamiltonii*, Th), tilapia (*Orechromis* spp., On), western Pacific gizzard shad (*Nematalosa come*, Nc), large-scaled mullet (*Liza macrolepis*, Lm), flathead mullet (*Mugil cephalus*, Mc), and milkfish (*Chanos chanos*, Cc)(Table 1). All fishes were sealed in polyethylene bags and kept on ice during transport to the laboratory, where they were stored at -20°C till further examination.

Before dissection, each fish was identified to species, and the fork length (cm) and body weight (g) measured. Then a piece of dorsal muscle was removed for analysis. For smaller species such as Ema, Gf, Leq, Th, On, Nc, and Cc, samples from 3 to 7 fish of similar size were combined to make a pooled sample. Fish of other species were analyzed individually. In total, 99 samples were analyzed (Table 1).

II. *THg and OHg Digestion*

Measurement of THg was based on the method established in previous studies[13-15]. Briefly, for each sample, 0.3 g of the homogenized muscle was weighed and placed into a 75-mL glass test tube (Pyrex). One milliliter of HNO_3 and 4 mL of H_2SO_4 were added to digest the sample with heat up to 75°C. Then, 15 mL of 5% $KMnO_4$ was added sequentially to complete the digestion. A final volume of 25 mL was made up by double distilled water.

For OHg analysis, the same method was used as

Table 1. Fish species, code, food items, fork length (FL), and body weight (mean ± S.D. and range) of fish used to determine mercury contents of muscle found in fishes caught in the reservoir adjacent to a chlor-alkali plant in Tainan City, Taiwan

Scientific name	Common name	Code	Food items	Total fish number	Sample number	FL (cm)	BW (g)
Elops machnata	Tenpounder	Ema	small fishes, crustaceans	20	10	23.7 ± 4.8 (15.1~31.3)	116.6 ± 72.5 (26.9~263.1)
Pelates quadrilineatus	Fourlined terapon	Pq	small fishes, invertebrates	2	2	11.8 ± 0.3* (11.6~12.0)	42.4 ± 5.2 (38.7~46.0)
Gerres filamentosus	Whipfin silver-biddy	Gf	detritus, small crustaceans, polychaetes, forams	5	3	10.6 ± 1.4 (9.3~12.9)	33.0 ± 14.7 (22.2~58.8)
Leiognathus equulus	Common ponyfish	Leq	detritus, polychaetes, small crustaceans, small fishes	24	3	9.6 ± 0.8 (8.3~11.9)	27.4 ± 7.4 (17.2~51.7)
Thryssa hamiltonii	Hamilton's thryssa	Th	prawns, zoobenthos, zooplanktons	3	1	9.5 ± 0.5 (9.0~9.5)	8.3 ± 1.5 (6.8~9.7)
Orechromis sp.	Tilapia	On	detritus, zoobenthos, zooplanktons, plants	76	38	15.2 ± 3.4* (8.7~22.6)	81.2 ± 50.9 (12.1~229.1)
Nematalosa come	Western Pacific gizzard shad	Nc	detritus, zoobenthos, zooplanktons, plants	74	20	10.5 ± 3.5 (6.2~18.7)	30.6 ± 29.7 (4.4~142.2)
Liza macrolepis	Large-scaled mullet	Lm	detritus, zoobenthos, plants	7	7	19.4 ± 1.2 (18.2~21.3)	118.5 ± 25.3 (899.7~164.4)
Mugil cephalus	Flathead mullet	Mc	detritus, zooplanktons, plants	5	5	32.6 ± 7.6 (23.5~40.7)	549.4 ± 330.3 (176.1~930.0)
Chanos chanos	Milkfish	Cc	plants, detritus, zoobenthos, zooplanktons	20	10	24.1 ± 3.1 (19.7~27.2)	228.9 ± 84.5 (114.1~326.3)
		Sum		**236**	**99**		

*Total length.

Chen *et al*. (2002)[15] adopted from Shum *et al*. (1979)[16]. First, acetone was added to 0.3 g of muscle in a 50-mL centrifuge tube to remove lipid, then 5 mL of 3 M KBr and 10 mL of 0.1 M $CuSO_4$ were added to extract the organic phase. The extract was transferred into another 50-mL centrifuge tube for further extraction by 5 mL of toluene twice. The upper layer was placed in a 10-mL centrifuge tube and extracted with 1 mL of 0.01 M $Na_2S_2O_3$ solution. Afterwards, 1 mL of 0.01 M $Na_2S_2O_3$ was placed in a 75-mL test tube and once again taken through the THg digestion procedure as previously described. All chemicals were from Merck, GR degree.

III. *Analysis*

Determinations of Hg were conducted by use of cold vapour atomic absorption spectrophotometer (AAS, HITACHI Zeeman-8200, Tokyo, Japan) connected to a hydride formation system (HFS-2, HITACHI, Tokyo, Japan) with a T-type joint sampling device installed at the inlet[13] to improve the reaction efficiency of the sample and the reductant.

IV. *QA and QC*

In every batch of 32 samples measured, two sample blanks were inserted to detect alien contaminant. Certified reference materials (DORM-2, muscle of dogfish and DOLT-2, liver of dogfish), bought from the Research Council of Canada, were analyzed in duplicate to ensure reliability and consistency. Comparison of our results for DORM-2 [THg = 4.49 ± 0.25 (n = 10) and OHg = 3.86 ± 0.27 (n = 10) μg/g dry wt.], and DOLT-2 [THg = 2.14 ± 0.12 (n = 10) and OHg = 0.741 ± 0.050 (n = 10) μg/g dry wt.] with the certified values of DORM-2 (THg = 4.64 ± 0.26 and OHg = 4.47 ± 0.32 μg/g dry wt.) and DOLT-2 (THg = 2.14 ± 0.10 and OHg = 0.693 ± 0.053 μg/g dry wt.) showed that the recovery percentage was within 100 ± 10% except for OHg of DORM-2, for which recovery was 86%.

V. *Detection Limit*

Reagent blanks were inserted into every 10th sample measurement. Detection limits (DL) were calculated as the mean added 3-fold of the standard deviation of the blanks. The DLs of the equipment and method were 0.5 μg/L and 0.042 μg/g flesh wt., respectively. THg and OHg concentrations in fish muscles were all expressed as μg/g flesh wt.

VI. *Statistics*

Since the THg and OHg concentrations in the muscles of each species did not exhibit significant size difference[17], the difference of muscle THg and OHg among species was tested by one-way ANOVA (One-way Analysis of Variance) followed by Duncan's multiple range test ($p < 0.05$)[18].

RESULTS AND DISCUSSION

I. *THg and OHg in Fish Muscles*

The THg concentrations in the fish muscles ranged from 0.08 to 2.10 μg/g flesh wt., with the lowest in *M. cephalus* and the highest in *E. machnata*. THg concentrations showed significant species differences ($p < 0.05$), where *E. machnata* (1.20 ± 0.61) = *P. quadrilineatus* (1.05 ± 0.03) > *G. filamentosus* (0.45 ± 0.18) ≧ *L. equulus* (0.39 ± 0.07) = *Orechromis* spp. (0.38 ± 0.15) = *N. come* (0.32 ± 0.10) = *C. chanos* (0.26 ± 0.10) = *L. macrolepis* (0.21 ± 0.03) ≧ *M. cephalus* (0.11 ± 0.02) ($p < 0.05$). Muscle concentration of THg in *T. hamiltonii* was 0.61 (Figure 1).

The OHg concentrations ranged from 0.05-1.10 μg/g flesh wt., with the lowest in *M. cephalus* and the highest in *P. quadrilineatus*. Muscle OHg concentrations were significantly different among species ($p < 0.05$), showing a similar trend to that of THg, with *P. quadrilineatus* (0.74 ± 0.07) = *E. machnata* (0.61 ± 0.29) > *G. filamentosus* (0.36 ± 0.15) = *Orechromis* spp. (0.31 ± 0.18) ≧ *L. equulus* (0.28 ± 0.04) = *N. come* (0.25 ± 0.08) = *C. chanos*

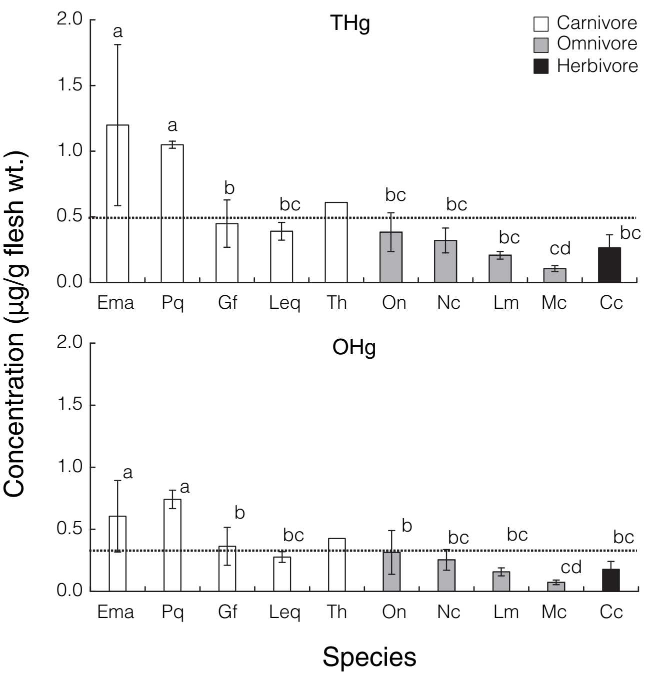

Figure 1. Total Hg (THg) and organic mercury (OHg) concentrations in muscles of 10 fish species from the reservoir adjacent to a chlor-alkali plant in Tainan City, Taiwan. The food habits (as in Table 1) of the different species are indicated by shading. The dotted line indicates seafood THg < 0.5 and OHg < 0.3 μg/g flesh wt. set by WHO. Abbreviations of species are in Table 1. No significant difference was observed between species if the same letter (a, b, or c) is marked above the histogram bars.

(0.18 ± 0.06) = *L. macrolepis* (0.16 ± 0.03) ≧ *M. cephalus* (0.07 ± 0.02) ($p < 0.05$). The only OHg data for *T. hamiltonii* was 0.43 (Figure 1).

The carnivorous fishes (*E. machnata* and *P. quadrilineatus*) were higher in Hg concentrations than those of omnivorous (*Orechromis* spp., *N. come*, *L. macrolepis* and *M. cephalus*), and herbivorous fishes (*C. chanos*), which were similar to various previous studies[19,20], representing a biomagnification effect. However, carnivorous *G. filamentosus* and *L. equulus* accumulated similar muscle THg and OHg to the omnivorous fishes, possibly due to their smaller sizes with shorter exposure times, and diets including small crustaceans and polychaetes[21] from a lower trophic than the prey of other carnivorous species.

Overall, 22% of the THg and 37% of the OHg measurements in fish from the reservoir exceeded the WHO safety levels of 0.5 and 0.3 μg/g, respectively[22].

II. *Comparison with Common Fishes from an Unpolluted Lagoon in Taiwan*

The THg levels of the fishes in the reservoir were extremely high in comparison with the baseline found in Chi-ku Lagoon of Taiwan[14]. The THg levels of *E. machnata*, *P. quadrilineatus*, *G. filamentosus*, *L. equulus*, *N. come*, *L. macrolepis*, *M. cephalus*, and *C. chanos* (THg = 0.11-1.05 μg/g flesh wt.) were 5-81 times higher than that of the same species from Chi-ku Lagoon (THg = <0.025-0.068 μg/g flesh wt.). Moreover, because the sizes of fish in the two waters were similar, the high Hg concentrations measured in this study were much more likely to be caused by Hg pollution from the chlor-akali plant, rather than being caused by longer mercury accumulation times for larger fish[23,24].

III. *Comparison with Chlor-alkali Plants Worldwide*

High THg levels in fish muscles found in this reservoir were similar to those found around chlor-alkali plants worldwide. The highest THg we measured (2.10 μg/g flesh wt. in *E. machnata*) was lower than that measured for *Bagre marinus* in south Florida estuaries (2.22)[25], *Aspius anguilla* in Skalka Reservoir of Czech Republic (3.40)[26], and various fish species (15.00) in Minamata Bay of Japan in 1959[26]. However, the THg values in our study were still higher than value of 1.74 found in unidentified species in Minamata Bay after clean up in 1989[27], *Diplodus sargus* (1.66) in Haifa Bay of Israel[28], *Stizostedion vitreum* (1.41) in the Great Lakes of Canada[29], *Anguilla anguilla* (1.23) in the Cecina river basin of Italy[30], *Dicentrarchus labrax* (1.1) in Ria de Aveiro Lagoon of Portugal[31], *Epinephelus coiodes* (0.98) in Kuwait Bay[32] and *Odontesthes microlepidotus* (0.38) in the upper Negro River of Argentina[33]. As noted above, such high levels of Hg in fishes showed typical chlor-alkali pollution that contained markedly elevated Hg concentrations.

IV. *Consumption Risk*

Based on the provisional tolerable weekly intake (PTWI) of methylmercury (MeHg) of 1.6 μg/kg/week as set by WHO[34], Taiwanese males and females weighing 65 and 55 kg, would have a PTWI of 104 and 88 μg/week, respectively. The weekly amount of fish products consumed by Taiwanese were estimated to be 482 g for male and 319 g for female adults[35]. We assumed that the OHg contents were all MeHg, therefore, the MeHg limit of the fish meats should be below 0.24 μg/g in order to meet the safety requirement under the general diet habits of Taiwanese. Accordingly, seven species (*E. machnata*, *P. quadrilineatus*, *G. filamentosus*, *T. hamiltonii*, *L. equulus*, *Orechromis* spp. and *N. come*) and 45% of the examined data exceeded this limit (Figure 1). Furthermore, if all seafood were major Hg source to humans[36] and the weekly consumption of seafood was 678 g for males and 519 g for females in Taiwan[35], Hg concentration in the seafood should be reduced to 0.17 μg/g to avoid the risk of Hg poison from seafood[36]. Seventy-seven percent of our measured values exceeded this requirement. In this study, we chose the minimum Hg concentration (0.07 in *M. cephalus*), the mean of all fish species sampled (0.31), and the maximum (0.74 in *E. machnata*) as values of OHg to evaluate the risk arising from the amount of fish consumed weekly (Figure 2). The meats from fish such as *C. chanos* and *M. cephalus* with the lowest mean OHg would allow consumption of more than 1200 g/week. However, if the likelihood of consuming each fish species was equal, their total mean Hg concentration, which was similar to the mean value of *Orechromis* spp. meat, would allow consumption of 300 g of fish per week. Finally, if fish containing the highest mean Hg value was eaten, the

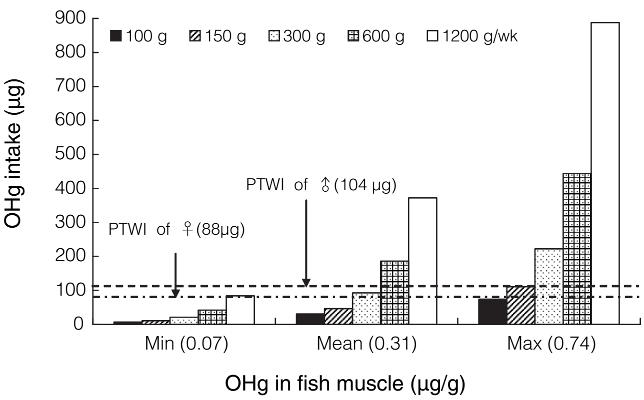

Figure 2. Assesment of human intake of OHg according to the amount of fish muscle consumed (g/week) that contained minimum, mean, and maximum OHg levels found in fishes caught in the reservoir next to a chlor-alkali plant in Tainan City, Taiwan. PTWI = provisional tolerable weekly intake.

allowable amount would be only 100 g per week, which was far less than the average consumption of fish products by Taiwanese (319-482 g fish/week). Conclusively, in order to avoid a health threat from excess Hg intake from eating fish caught in the reservoir, less than 100 g of fish meat can be consumed weekly.

CONCLUSIONS

Although the Tainan chlor-alkali plant has been closed for 22 years, fishes living in the adjacent reservoir still have accumulated Hg concentrations as much as 81 times higher than the baseline of Taiwan. The high Hg concentrations in the fish meats were likely to be harmful to human. Therefore, government organizations should purge the waste as soon as possible and educate the local inhabitants to avoid eating fishes caught in the reservoir.

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碳氮源對浸漬培養金頂側耳胞外多醣之生成及碳水化合物組成之影響

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摘　　要

本研究探討不同碳、氮源對浸漬培養金頂側耳胞外多醣之生成影響及胞外多醣碳水化合物組成之變化。實驗結果顯示，生成金頂側耳菌絲體與胞外多醣的最佳碳、氮源爲果糖與酵母蛋腖白粉末。胞外多醣經水解後以氣相層析儀分析，結果顯示爲異質多醣或多醣混合物，其碳水化合物組成爲甘露糖、半乳糖、葡萄糖、木糖與岩藻糖，以甘露糖爲主要組成。在不同培養條件下，金頂側耳生成的胞外多醣之碳水化合物組成百分比亦有所改變。

關鍵詞：碳水化合物組成，金頂側耳，浸漬培養，胞外多醣

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不同季節裡海膽（*Paracentrotus lividus*）卵之產率、脂肪酸、胺基酸及一般組成

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摘　　要

海膽（*Paracentrotus lividus*）卵對人類而言是一份頗受歡迎的佳餚。本研究中，不同季節裡海膽卵之產率、脂肪酸、胺基酸及一般組成被探討。平均產率爲 5.45 ± 2.21%。蛋白質、粗脂肪、水分、灰分及碳水化合物含量分別爲 12.03 ± 1.26%、3.05 ± 0.50%、79.87 ± 1.43%、2.25 ± 0.24% 及 2.80 ± 2.41%。其中，C16:0、C20:5n3 及 C22:2n6 脂肪酸爲重要脂肪酸；而主要胺基酸爲麩胺酸（非必須，NE）、甘胺酸（NE）、離胺酸（必須，E）、天門冬胺酸（E）及精胺酸（NE）。必須 / 非必須胺基酸之比例爲 0.58 ± 0.01；脂肪酸方面則富含多元不飽和脂肪酸。本論文斷定，海膽卵是不飽和脂肪酸、蛋白質及胺基酸等人類必要營養組成的豐富來源。

關鍵詞：海膽卵，化學組成，胺基酸，脂肪酸

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台南鹼氯工廠海水貯存池中魚類肌肉之總汞及有機汞濃度

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摘　　要

本研究利用冷蒸氣原子吸收光譜測定來自台南鹼氯工廠旁海水貯存池中的十種魚類（海鰱、四線雞魚、曳絲鑽嘴魚、短棘鯿、漢式稜鯷、吳郭魚、環球海鰶、大鱗鮻、烏魚及虱目魚），魚肉中的總汞及有機汞濃度。結果顯示十種魚類的肌肉平均總汞及有機汞濃度分別爲 0.43 ± 0.36 及 0.31 ± 0.21 μg/g 鮮重，與其他國家的鹼氯廠址測值相似。其中有 37% 的有機汞測值超過世界衛生組織所訂水產品甲基汞安全限值（0.3 μg/g 鮮重）；同時，77% 的有機汞測值超過臺灣地區海鮮建議安全食用限值（甲基汞應低於 0.17 μg/g 鮮重）。當地居民每週食用該蓄水池中的魚類應不得超過 100 克，以避免汞中毒的健康危害。

關鍵詞：重金屬，河口魚類，魚肉，水產品安全食用量，健康危害

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應用多套式 PCR（Multiplex PCR）快速檢測病原性大腸桿菌及沙門氏菌

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摘　　要

本研究目的主要發展快速且具特異性之多套式 PCR，可同時檢測食品中熱不穩定腸毒性大腸桿菌（LT ETEC）及沙門氏菌（*Salmonella* sp.），多套式 PCR 使用二組引子應用於熱不穩定腸毒性大腸桿菌及沙門氏菌檢測，皆可分別得到 425 bp 及 163 bp 大小之 PCR 產物。SCLB（selenite cystine-lactose broth）培養基應用於食品及糞便的預培養，對 *Salmonella* 及 LT ETEC 其靈敏度爲 10^1-10^2 cells/g。進一步以多套式 PCR 檢測 160 種天然樣品中之熱不穩定腸毒性大腸桿菌及沙門氏菌。由結果顯示，多套式 PCR 方法於家禽肉及糞便檢出熱不穩定腸毒性大腸桿菌，而傳統方法（BAM）則於糞便檢出熱不穩定腸毒性大腸桿菌。

關鍵詞：多套式 PCR，熱不穩定腸毒性大腸桿菌，沙門氏菌