DEVELOPMENT OF A METHOD FOR CONCENTRATION AND DETECTION OF ROTAVIRUS IN OYSTER

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การพัฒนาวิธีทำให้ไวรัสเข้มข้นและตรวจไวรัสโรตาในหอยนางรม (DEVELOPMENT OF A METHOD FOR CONCENTRATION AND DETECTION OF ROTAVIRUS IN OYSTER)

การศึกษาครั้งนี้เสนอแนวว่า การทำให้ไวรัสเข้มข้นด้วยวิธี acid adsorption-alkaline elution และตรวจด้วยวิธี RT-nested PCR นี้เป็นประสิทธิภาพในการตรวจไวรัสโรตาจากหอยนางรม สำหรับข้อมูลจีโนทัยของไวรัสโรตาที่ปนเปื้อนในหอยนางรมจะเป็นประโยชน์ต่อการศึกษาด้านวิทยาการระบาดและการประเมินความเสี่ยงทางจุลชีววิทยาของหอยนางรมต่อไป

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ABSTRACT

Viral foodborne diseases are significant public health problem. Outbreaks of viral gastroenteritis have been reported and associated with consumption of raw or slightly cooked oysters. Identification of viruses in outbreak-implicated shellfish have been difficult because of low levels of contamination, and natural inhibitors in shellfish tissues. In this study, a method for concentration of rotavirus was developed for detection of the virus from oysters. Three different concentration methods were compared: direct alkaline elution, acid adsorption-neutral elution, and acid adsorption-alkaline elution. Rotavirus was seeded into oyster meat, concentrated, extracted for RNA, and examined using reverse transcriptase-nested polymerase chain reaction (RT-nested PCR). The results showed that the acid adsorption-alkaline elution gave the highest sensitivity of rotavirus detection ($3.12 \times 10^3$ infectious forming units [IFU]/25 g or $125$ IFU/g) followed by the acid adsorption-neutral elution ($1.25 \times 10^4$ IFU/25 g), and direct alkaline elution ($2.5 \times 10^4$ IFU/25 g). The acid adsorption-alkaline elution process included acid adsorption at pH 4.8, elution with 2.9% tryptose phosphate broth containing 6% glycine pH 9.0, twice polyethylene glycol precipitation, chloroform extraction, and speedVac reconcentration. From August 2005 to February 2006, 120 oyster samples were collected from local markets in Bangkok (60 samples) and oyster farms in Surat Thani Province (60 samples). All raw oyster samples were concentrated using the acid adsorption-alkaline elution and determined for rotaviruses using RT-nested PCR. Rotaviruses were found in four oyster samples, one from the local market and three from the oyster farms. PCR products (346 basepairs) were sequenced and analysed for their phylogenetic tree. It was found that the oyster samples contained rotavirus sequences associated with human rotavirus genotypes G9 (two samples), G3 (one sample), and G1 (one sample).

This study recommends acid adsorption-alkaline elution as the virus concentration method and RT-nested PCR for detection of rotaviruses from oyster samples due to its high sensitivity. Data of rotavirus genotypes contaminated in oyster samples would be useful for epidemiological study and microbiological risk assessment.

KEY WORDS: ROTAVIRUS/ OYSTER/ VIRUS CONCENTRATION/ RT-NESTED PCR