研究成果公開

研究報告書　厚生科学特別研究事業研究成果報告書　平成11年4月発刊　本書

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学会抄録

国外学会

第1回ヒトカリシウイルス国際ワークショップ（1st International Workshop for Human Caliciviruses; CDC, Atlanta, GA, USA, March 29 to 31, 1999)

[Title A]

Case Study of food poisoning: Detection of Human Calicivirus Genes from Feces of Patient, and Cook, and Food

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Abstract

A case of food poisoning involving 7 patients occurred in Nagoya city of Japan at 14:00 on January 30, 1998. The epidemiological survey revealed that the patients took common food in a certain restaurant around 17:30 on January 29. The bacteriological examination was all negative. Viral gastroenteritis was suspected from both the incubation time and specific symptoms such as nausea, vomiting, intense diarrhea and so on. Since we didn't detect A group, C group rotavirus, or Adenovirus type 40/type 41, human calicivirus infection seemed most likely. We tried to detect the human calicivirus genes by the amplification RT-PCR method. Although we failed to detect the genes in the 1st PCR with the primers, #35/#36, the nest PCR with the #81/#NW82/#SM82 mixture primers to the 1st PCR amplcons was successful and the human calicivirus genes were detected in the feces of 4 of 6 patients and the feces of one cook of the restaurant. Furthermore we detected the genes in the skinned squid without innards, which was the remains of the food served on that day and kept in a freezer in the restaurant. Then we determined the sequences of the genes. When compared with the database registered to DDBJ, EMBL, or Genbank, the genes turned out to be close to accession number L25114 and thus to belong to the genogroup 2. As the results of the alignment, the genes that derived from the food, and the feces of our patients and the cook were found to have the same sequence. From these information, this case of food poisoning by a cook of the restaurant, a carrier of human calicivirus.

[Title B]

Detection of Norwalk-like virus genes in the caecum contents of pigs

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Abstract

Norwalk-like virus (NLV) genes were detected by RT-PCR in th caecum contents of 6-months pigs that were brought in a slaughterhouse in Shizuoka prefectural in Japan. Positive PCR products were produced from five out of 1605(0.3%) samples by nested PCR using human SRSV primers. Nucleotide sequences between 4573 and 4864 region were determined. Between the Norwalk virus sequence and the sequences detected in pigs, there were 58.2% to 59.9% sequence homology. The swine sequences were located on genogroup 2 of human SRSVs, but forms a subgroup in the phylogenetic tree of caliciviruses.

The detection of NLV genes in pigs raises a possibility of the contribution of swine population to the resources of human SRSV genes.

第11回国際ウイルス学会(XI th International Conference of Virologists Societies; Sydney, Australia, 9-13 August 1999)

[Title A]

A nation-wide survey of food-borne viral gastroenteritis for human caliciviruses (HuCVs; small round structured viruses; SRSVs, Norwalk-like viruses; NLVs) in Japan

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Abstract

We conducted a nation-wide survey project and a network for viral gastroenteritis outbreaks from Okinawa (the most southern area) to Hokkaido (the most northern area) in Japan. SRSVs are usually prevailing as causative agents of food borne poisoning cases concentrated in winter and also sharing 90% or over among viral agents. We tentatively computed for data summations of which 902 viral specimens collected from 359 outbreaks in nine different areas were examined by electron microscope (EM) and/or reverse transcriptation polymerase chain reaction (RT-PCR) during the last 27 months. As the results, it became clear that SRSVs had a short prevailing period just before group A human rotavirus epidemics among infants. On the other hand, food-borne viral gastroenteritis seasonally distributed as 16% in spring, 9% in summer, 13% in autumn and 62% in

winter, respectively. An occurrence index of the outbreak was calculated as 0.48 (average), 1.45 (Tokyo) and 1.52 (Osaka) cases/100,000 persons/year, respectively. A detection rate was 55% by EM and 58% by RT-PCR, respectively. Of RT-PCR positives, southern hibridizations using of P1a, P1b, P2a, and P2b proves previously reported showed an enforcement rate in 44% as genogroup typing. The typing rate of genogroup (G) among domestic SRSVs was 0.5%/G1, 57%/G2 and 42.5%/not type, respectively. However, it was still remained not typing PCR products of 30% by RT-PCR (#35/36NLV, MR3/4, #81/82NLV/82SM, and Yri22F/R). Therefore, it had to be developed to the most critical genodiagnostics for domestic SRSVs in Japan. Finally, we propose a revision RT-PCR using new primer set designed from analysis among domestic 171 genomic collections sequenced by us from 1989 to 1998. Our nation-wide survey project is supporting by national grant of health science special research, and composed by Japanese virologists of 18 local Institute of Health (Hokkaido, Aomori, Akita, Miyagi, Nigata, Tokyo, Yokohama city, Shizuoka, Aichi, Nagoya city, Gifu, Fukui, Osaka, Osaka city, Hiroshima city, Ehime, Fukuoka and Okinawa), National Institute of Infectious Diseases and National Institute of Public Health.

[Title B]

Sequence diversity of human caliciviruses (HuCVs) from samples in non-bacterial gastroenteritis outbreaks in Japan, 1989 to 1998

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Abstract

We determined the nucleotide sequences of RT-PCR products amplified from the RNA polymerase region of the HuCV genomes in fecal, vomit and oyster samples from the non-bacterial gastroenteritis outbreaks in Japan, 1989 to 1988. The sequences of different strains revealed great heterogenicity, with a range of 60 to 100% homology among strain. The sequences were compared with the sequence of reference viruses for the genetic characterization of viruses prevailing in Japan. Of the 171 different HuCVs found, approximately 90% of the genes could be classified into Snow Mountain like viruses (genogroup type 2; G2) and other strains as Norwalk like viruses (genogroup type1; G1) based on genotyping with homology analysis. Furthermore, the strains belonging to the G2 could be classified into 4 additional subgroups or over, which we tentatively designed subgroups, 2A to 2D or alone 2E, with more than 93% homology in amino acid among strains. We believe that two subgroups of 2C to 2D or alone 2E should make it possible to be new specific subgroups (JPN-1, 2) in the G2 in Japan from the phylogenetic and pairwise comparison studies. No strains belonged to human Sapporo-like virus (genogroup 3; G3). Simultaneous infection with different genogroups was found in the same patients or the same outbreaks and was likely to be due to contact with contaminated oysters or other food or due to person-to-person spread. The results obtained in this study should not only provide information on the diversity of strains causing outbreaks in Japan, but also we could propose new universal primer set designed on the basis the alignments of 171 nucleotide sequences for facilitating the most critical detection of almost HuCVs prevailing in Japan.

国内学会

第40回日本臨床ウイルス学会（大阪市）平成11年5月

ウイルス性食中毒遺伝子検出検査指針確立と行政対応に関する研究

川本尋義、沢田春美・大山徹、斎藤博之、三上稔之、秋山和夫、篠川旦、関根大正、野口有三、杉枝正明、柴田伸一郎、山下照夫、松本和男、春木孝祐、山崎謙治・大石功、池田義文、大瀬戸光明、大津隆一、大野　惇、宇田川悦子、西尾治（厚生科学全国ウイルス性食中毒研究班長・岐阜県生物産業技術研究所、各地方衛生研究所；北海道、秋田県、青森県、宮城県、新潟県、東京都、横浜市、静岡県、名古屋市、愛知県、福井県、大阪市、大阪府、広島市、愛媛県、福岡県、沖縄県及び国立感染症研究所、国立公衆衛生院）

目的　全国地方で発生した食中毒等事例中、ウイルス性もしくは原因物質不明食中毒事件、有症苦情、施設内集団発生の分類事例検証と対策を検討すべく、本班は平成7年末にウイルス性食中毒国内実態を計量把握し公表した。その成果は広く活用され平成9年5月に食品衛生法が改正され法史上初めて小型球形ウイルス等ウイルスが登場し法的認知も得るに至った。これまで原因究明と対策のための遺伝子検査指針（初版）策定後、厚生省へ提案し採択され現在に至っているが迅速効率的ウイルス診断検査法確立と安全食品食材供給のあり方、ウイルス汚染による健康被害低減さらには阻止をめざし行政対応等整備に関する総合的な調査研究開発を展開中。今後は環境ウイルス生態学調査とその制御の基礎研究を計画。

調査・方法　最近の１８都道府県内のウイルス性食中毒、感染症サーベイ、陸水環境等のＳＲＳＶ遺伝子検出と検査法改良を進めつつＳＲＳＶ実態動向と検出ウイルス遺伝子配列決定など解析研究を実施中。配列データ集積は現在１５０株、今後増加予定。

結果・考察　過去5年間のウイルス性食中毒国内実態調査と食衛法改正後の調査成果を対比し疫学解析を実施中。国内ＳＲＳＶ遺伝子群型はＧ２が圧倒。しかし、現用検出プライマー対（＃３５・＃３６、＃８１・＃８２ＮＷ／ＳＭ、ＹＲ２２Ｒ・２２Ｆ）でも検出困難傾向をみ、Ｇ１の台頭が示唆される。汎用迅速検査法確立に向け班内意見統一を図りつつある。ＲＴ－ＰＣＲ改法を広く用て頂くには、無損傷ＲＮＡの効率的抽出とｃＤＮＡ転写効率の向上、試料中の他種生物由来遺伝子汚染も効果的防止し手技等も簡便なことが重要改正点である。核酸抽出に積極的に効率の良いキットも導入し、ＰＣＲ術式も簡素化（ＲＴ逆転写とＰＣＲ遺伝子連鎖増幅も同一管内反応系とするなど）を図る予定。なお、本研究は厚生省厚生科学特別研究平成9、１０年度事業として支援され、現在平成11年度も継続中。

編集後記

本研究事業は北海道、秋田県、青森県、宮城県、新潟県、東京都、横浜市、静岡県、岐阜県、名古屋市、愛知県、福井県、大阪市、大阪府、広島市、愛媛県、福岡県、沖縄県の地方衛生研究所、国立感染症研究所、国立公衆衛生院による全国縦断広域共同研究プロジェクトであり、各関係機関ならびに行政部局のご理解とご支援に感謝します。平成11年度研究では、ウイルス環境生態学調査と平成10年度に設計したＳＲＳＶ遺伝子増幅新ユニバーサルプライマーの検出効果の評価も併せ行いたいと存じます。従って、本書はこれまでに研究開発し確立した検査法の纏めをいたしました。本書の発刊にあたり、班分担研究者・協力研究者各位の精力的なご尽力に感謝し、また本書がウイルス性胃腸炎研究に広く活用されることを期待します。

付記

私たちはこれまで小型球形ウイルス（ＳＲＳＶ）とウイルス性食中毒を厚生省と食品衛生法に認めて頂くため平成3 年度以来研究成果を積み上げて参りましたが、ＳＲＳＶのウイルス名も最近では新たな国際分類名に統一する方向で国際分類命名委員会にて検討が進められております。命名委員会決定は平成 11 年度中と推察されます。今後、学術用語としてＳＲＳＶ標記はカリシウイルス属ヒトカリシウイルスなどとなり、その内に遺伝子群型別で例えば Norwalkie など (Genogroup Ⅰ) など、が提唱されています。日本のＳＲＳＶの特徴は、 Snow Mountain like の Genogroup Ⅱ に属するものが多くあり、更に群内亜型（ Subgroup; JPN-1,2:JAPONICA ）に属すものが多く出現する傾向が認められました。研究班では学術的にも今後はＳＲＳＶ標記を改め、国際ウイルス分類委員会の決定作業に提案を行いつつヒトカリシウイルス（ＨｕＣＶ）と称することを奨めたいので広くご理解とご支持を願いたく存じます。

ウイルス性食中毒原因の遺伝子検査標準法
確立と全国行政対応整備に関する研究

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