GENE SEQUENCING FOR IDENTIFICATION OF PARAGONIMUS EGGS FROM A HUMAN CASE *

CHANG Zhen-shan1, WU Bu-d1, Blair D.2, ZHANG Yong-nian3, HU Ling4, 
CHEN Shao-hong5, CHEN Ming-gang5, FENG Zhen1, George M. Davis2

(1 Tropical Medicine Research Center and Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai 200025; 2 Department of Zoology and Tropical Ecology, James Cook University Townsville, Queensland 4811, Australia; 3 Academy of National Sciences of Philadelphia, USA)

Abstract [Objective] To identify the etiologic agent from a paragonimiasis patient using molecular techniques. [Method] The complete nuclear ribosomal DNA second internal transcribed spacer (ITS2) gene sequence of eggs in sputum from a paragonimiasis patient was obtained by directly auto-sequencing its PCR product. ITS2 genes from eggs of Paragonimus westermani and Paragonimus skrjabini (both from animal hosts) were also sequenced for comparison. In addition, morphological comparisons were made with eggs of the two species. [Results] The ITS2 gene from the human case was 100% identical with the sequence from the eggs of P. westermani from an experimentally infected dog but only 92% identical with the sequence from the eggs of P. skrjabini. Morphologically, the eggs from the human case more resembled those from P. westermani infected dog. [Conclusion] The patient was diagnosed to be suffered from paragonimiasis westermani by gene sequence analysis.

Key Words: paragonimiasis westermani, nuclear ribosomal DNA second internal transcribed spacer (ITS2) gene

The parasitological method of diagnosis of paragonimiasis westermani is microscopic morphological observation of the eggs in the sputum of patients. The recent rapid development of molecular biology techniques provides an alternative approach to identification of the etiologic agents of parasitic diseases. In this paper, the complete nuclear ribosomal DNA second internal transcribed spacer (ITS2) gene, which has been reported as a useful molecular tool for identification and taxonomy of Paragonimus species, was chosen for identifying Paragonimus eggs in the sputum of a human case.

MATERIAL AND METHODS

Eggs were collected in the sputum from a patient with paragonimiasis in Shanghai and at the same time eggs were isolated separately from the feces of two dogs which had been experimentally infected with Paragonimus westermani and Paragonimus skrjabini, respectively. All of these eggs were preserved in 90% ethanol until required for PCR and sequencing. Before being viewed under a microscope, the eggs were washed in 0.9% NS solution several times and put into 10 μl (3–5 eggs) digestion solution (50 mmol/L KCl, 1.5 mmol/L MgCl2, 10 mmol/L Tris-HCl pH 8.5, 0.01% gelatin, 0.45% NP-40, 0.45% Tween-20 and 400 mg/ml proteinase K), which was incubated at 55°C for 2–4 hours. Subsequently, the proteinase K was inactivated by heating to 95°C for 15 minutes. A 2 μl aliquot of the digestion solution, containing genomic DNA from the eggs, was used in a 50 μl polymerase chain reaction (PCR). The PCR reaction buffer contained 50 mmol/L KCl, 3.0 mmol/L MgCl2, 10 mmol/L Tris-HCl pH 9.0, 0.1% Triton X-100, 200 μmol/L dNTP, 200 μg/ml BSA, 0.8 mmol/L forward primer 3 S41, 0.8 mmol/L reverse primer A2841 and 0.5 μl 5 units/μl Tag DNA polymerase (Promega). An initial denaturation step (95°C for 1 min) was followed by 35 cycles at 95°C (50 s), 68°C (2 min) and extension of 68°C (5 min). All sequence data were determined directly from the PCR products. Cycle sequencing reactions were run on a Licor automated sequencer two times in both directions to confirm the sequence. Sequencing primers were fluorescent-labeled versions of the PCR primers. Sequence alignment was performed manually, aided by the sequence editor ESEE1.
We also undertook morphological observations of fresh eggs as soon as they had been isolated from the patient’s sputum. The fresh sputum sample was collected from the patient in the morning, and put into 3% NaOH solution for 60~90 min and centrifuged at 1200 g for 3 min. Eggs were present in the precipitate.

**RESULT AND DISCUSSION**

We obtained the complete ITS2 gene sequence data from eggs in sputum of a paragonimiasis patient and in the feces from dogs experimentally infected with *P. westermanni* and *P. skrjabini*, respectively (Fig. 1). The ITS2 alignment was 363 bases in length (ignoring a deletion of 2nt in the sequence of Ps-egg). The sequences of P-egg and Ps-egg were 100% identical, whereas P-egg and Ps-egg were only 92% identical (336nt/363nt). The molecular data therefore strongly supported the diagnosis of paragonimiasis westermanni.

Both morphological and molecular data supported a diagnosis of paragonimiasis westermanni. The range of egg dimensions further suggested that the patient might suffer from a mixed infection of *P. westermanni* diploid and triploid types. Average sizes of eggs for the two types of *P. westermanni* are respectively 72.7~77.0 × 44.0~44.4 μm and 93.6~97.6 × 48.3~51.2 μm

Unfortunately, ITS2 sequence data could not be distinguished between the two types. Ploidy states were best determined using chromosome-staining techniques.

The most important advantage of molecular genetic techniques over traditional parasitological methods is that only a small amount of parasite material is needed and it can be collected from any life-cycle stage of the parasite. Fragments of specimens, too damaged for morphological work, can yield gene sequences. In addition, although under ideal conditions we may distinguish the eggs of *P. westermanni* from those of *P. skrjabini* according to their shapes and sizes, the differences are very subtle. However, molecular genetic methods can easily distinguish between very similar organisms. We have successfully amplified genes not only from the adult and juvenile worms of *Paragonimus* species, but also from the single excysted metacercariae and even eggs (as in this study). DNA techniques will definitely provide alternative or complementary approaches for identifying the etiologic agent in clinical work and epidemiological research.

**ACKNOWLEDGMENTS**

We thank Ms. Yu JY for her kind-hearted help in raising experimental dogs.

**REFERENCES**

一例并殖吸虫病患者的虫卵 DNA 序列分析鉴定

常正山1, 吴波1, David Blair2, 张永平3, 卢玲3, 陈剑红3, 陈丽刚3, George M. Davis3, 冯正1

1 中国预防医学科学院寄生虫病研究所*, 热带医学研究中心, 上海 200025; 2 Department of Zoology and Tropical Ecology, James Cook University, Townsville, Queensland 4811, Australia; 3 The Academy of National Sciences of Philadelphia, 1 900 Benjamin Franklin Parkway, Philadelphia, PA 19103—1915, USA

摘要 [目的] 运用分子生物学技术分析虫卵基因序列鉴定并殖吸虫病类型。[方法] 先从并殖吸虫病患者粪中分离虫卵, 然后 PCR 扩增虫卵中的卡弗粒 DNA 第二个间隔区基因 (ITSS), 并直接用于测序从而获得该基因的核苷酸序列。同时, 亦可同法分别从动物宿主粪便中分离出的卫氏并殖吸虫和斯氏狸殖吸虫虫卵中获得 ITSS 基因序列作为 DNA 参照分析。此外, 本文还对从动物宿主粪便分离出的虫卵进行详细的形态学特征描述分析。[结果] 来自患者虫卵的 ITSS 基因序列与参照的卫氏并殖吸虫虫卵的基因序列 100%一致, 而与来自斯氏狸殖吸虫虫卵的基因序列有 92% 核苷酸相同。此外, 从形态学上讲, 来自患者的虫卵形态特征更与卫氏并殖吸虫虫卵相似。[结论] 通过基因序列分析, 可明确患者患的是卫氏并殖吸虫病。

关键词: 卫氏并殖吸虫病, 粪便 DNA 第二间隔区基因 (ITSS)

*本研究为美国国立卫生院 (NHLBI, USA) 资助课题 (No. 1RO1 AI 5946 1)

* 化学预防与病原、抗寄生虫病和抗虫病合作中心, 上海市寄生虫病医院与医学中心

文章编号: 1000-7423(2000)-04-0215-01

吉林市 61 例带绦虫患者感染方式的分析

(吉林市卫生防疫站, 吉林 132011) 王焕仁, 李振祥

(吉林市职工医大实习生, 吉林 132011) 王英伦

中图分类号: R383.32

文章编号: 1000-7423(2000)-04-0215-01

为了进一步加强带绦虫病与囊虫病的防治工作, 坚决切断带绦虫病的感染途径, 我们从 1993-1999 年的门诊患者病历资料中发现, 城市带绦虫病患者感染途径又出现了一种新方式。7 年来, 该门诊部共收治吉林省内带绦虫病患者 62 例, 对 61 例进行了流行病学调查, 其中食烤肉串者和食囊虫病患者分别为 35 例 (占发病人数的 57%) 和 4 例 (占发病人数的 6.5%), 其余有现场接触史和原因不详者 11 例 (分别占发病人数的 18%)。从流行病学调查的结果看, 城市带绦虫病患者的主要感染途径是食烤肉串之一类 (烤肉串、烤肉串、烤牛肉串等) 占总发病比例的 46.2%。从患者年龄上看, 分别为儿童和青年 46 例 (占 75.4%), 壮年和老年只有 15 例 (占 24.6%)。

经 t 检验, 二者差异显著 (t = 2.78; P < 0.05) 二者间差异显著。我国带绦虫病和囊虫病在政府协调下的大面积防治工作已经取得了显著成效。但根据目前情况看, 还远没有达到根治目的。从 80 年代以来, 由于控制本病流行因素中的若干问题, 如食品检验, 在市场开放初期, 由于检查人员少, 素质差, 管理混乱, 使市场上出现了病害肉, 表本病继续传播流行。这项工作过去是各级屠宰厂集中屠宰并检疫, 检疫工作比较好, 而市场开放以来, 形成分散个体屠宰经营, 这就为少数违法行为户带来了私利而愈演愈烈。肉检提供了条件, 一进市场中不少人千方百计将病害肉利用各种渠道销售给个体食品加工户, 携带或出售给食品加工小摊贩, 以街头摊贩为小摊贩。他们以烤肉串的方式卖给市民, 串串上洒上芝麻盐, 使人生吃容易沾上病害肉。这是我们在门诊流行病学调查中发现, 比较患者中发现 57% 的感染途径为食烤肉串和烤肉串的, 以这种方式传播的。因此, 建议行政部门有关单位切实加强生猪肉定点屠宰检疫工作的整顿。进一步加强对生猪肉的检疫工作, 严格禁止病肉从暗中流入市民。另一方面要加强对食品市场食品卫生监督管理工作。同时取缔城市街头烤肉串摊贩, 不但保证了食品卫生, 还可以防止城市空气污染。此外, 还要加强人民群众的健康教育工作, 改变群众的不良饮食卫生习惯和不良的生活方式。增强人们的自我保护意识。总之, 只要通过上述各种渠道综合治理, 一定可控使该病流行。