

## Serologic Prevalence of Coxsackievirus Group B in Greece

M.D. MAVROULI,<sup>1</sup> N. SPANAKIS,<sup>1</sup> S. LEVIDIOTOU,<sup>2</sup> C. POLITI,<sup>3</sup> S. ALEXIOU,<sup>4</sup>  
P. TSELIU,<sup>5</sup> M. HATZITAKI,<sup>6</sup> K. FOUNDOULI,<sup>7</sup> A. TSAKRIS,<sup>1</sup> N.J. LEGAKIS,<sup>1</sup>  
and J.G. ROUTSIAS<sup>1</sup>

### ABSTRACT

Coxsackieviruses are human enteroviruses, which have been associated with myocarditis/pericarditis and sudden death. In one investigation (Spanakis N, Manolis EN, Tsakris A, Tsiodras S, Panagiotopoulos T, Saroglou G, and Legakis NJ: *J Clin Pathol* 2005;58:357–360), a cluster of cases of fatal myocarditis in Greece was linked to coxsackievirus B3. The information from this investigation prompted us to study serologically the prevalence of coxsackieviruses B throughout Greece. Sera were obtained from 506 healthy blood donors from various transfusion centers, covering the entire country. All sera were tested for the presence of IgG and IgM antibodies, using ELISAs with various antigenic specificities: (1) heat-denatured coxsackievirus type B1 and B5 virions, (2) a synthetic peptide from the N terminus of the VP1 protein of coxsackievirus B3, and (3) a synthetic peptide from the N terminus of the VP1 protein of coxsackievirus B4. Sera positive for IgG antibodies against coxsackieviruses B1/B5, B3, and B4 were detected in 6.7 to 21.6% of the individuals tested in the various regions of Greece. Statistical analysis revealed that the highest prevalence of IgG antibodies against coxsackieviruses B1/B5 was found in blood donors from Crete ( $p = 0.025$ ), whereas the highest prevalence against coxsackievirus B4 was detected in blood donors from Athens ( $p = 0.01$ ). IgM antibodies against coxsackievirus B were detected at low percentage, less than 5%, with no significant viral preference for particular geographic regions. The preference of anti-coxsackievirus IgG antibodies for particular geographic regions could be potentially related to the previously reported clustering of cases of insulin-dependent diabetes mellitus and myocarditis in Athens and Crete, respectively.

### INTRODUCTION

COXSACKIEVIRUSES ARE HUMAN ENTEROVIRUSES belonging to the family *Picornaviridae*. They are further subdivided into two serogroups, A and B, which

comprise 23 (1–22 and 24) and 6 (1–6) serotypes, respectively. Coxsackieviruses are transmitted primarily via the fecal–oral route and as respiratory aerosols. They replicate in the oropharyngeal and intestinal epithelium and, from there, they can be carried by the blood stream

<sup>1</sup>Department of Microbiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece.

<sup>2</sup>Department of Microbiology, Medical School, University of Ioannina, Dourouti University Campus, Ioannina, Greece.

<sup>3</sup>Blood Transfusion Center, G. Genimatas General Hospital, Athens, Greece.

<sup>4</sup>Department of Microbiology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece.

<sup>5</sup>Blood Transfusion Center, Saint Andrews General Hospital, Patras, Greece.

<sup>6</sup>Blood Transfusion Center, General Hospital of Larisa, Larisa, Greece.

<sup>7</sup>Blood Transfusion Center, University General Hospital of Iraklio, Crete, Greece.

to the cells of the reticuloendothelial system and certain target tissues or organs such as the pancreas, liver, myocardium, meninges, and skin. More than 90% of these infections progress subclinically and they rarely cause life-threatening illness (26,27). Coxsackieviruses A have been associated with mild clinical syndromes such as mild flu-like illness (e.g., summer flu) as well as with conditions such as outbreaks of aseptic meningitis, whereas coxsackieviruses B are responsible for pancreatitis, hepatitis, aseptic meningitis, and myocarditis/pericarditis (5,14,25,27).

Viral infections of the heart are important causes of morbidity and mortality in all age groups without sex predominance. Coxsackieviruses B, especially CVB3 and CVB5 (15,17,19), are the most common causes of viral myocarditis and may be detected in more than 25% of sporadic cases of acute onset of dilated cardiopathy (8,12,18), whereas coxsackievirus B1 is mentioned as a rare cause (16). In addition, coxsackievirus B5 is frequently associated with sporadic cases of neurological diseases and epidemics of meningitis (21). Coxsackievirus B4 has long been implicated in the development of insulin-dependent diabetes mellitus (IDDM) (11,22), but there are studies indicating that several other serotypes of coxsackieviruses B, such as B1, B2, B3, and B5, might play a role in the pathogenesis of this chronic disease (2,22).

Among others, climate appears to be an important factor in the circulation and prevalence of enteroviruses in temperate regions. Enteroviruses are generally present at low levels in the winter and spring, but are isolated far more commonly during the summer and fall. Even in the United States, healthy children in the southern cities harbor a greater abundance of enteroviruses than do those of comparable age in the northern cities (7).

In one report, a nationwide investigation of a cluster of cases of acute respiratory tract syndrome associated with myocarditis and/or pericarditis took place in Greece, from January to April 2002. This study indicated that there was a link between enteroviruses, especially coxsackievirus B3, and direct tissue damage in three fatal myocarditis cases in Crete (24).

The information in this report prompted us to study serologically the prevalence of coxsackieviruses B throughout Greece, to determine whether there is a viral preference for particular geographic regions.

## MATERIALS AND METHODS

### *Sera*

Sera were obtained from 506 healthy blood donors, aged between 25 and 35 yr, from different collection sites throughout Greece. Collection sites were selected to

cover the entire country, including northern (Thessaloniki), southern (Iraklio/Crete), western (Ioannina and Patra), and eastern (Larisa and Athens) Greece (Fig. 1). The samples were collected during the summer of 2005 (from June 1 to August 31) and the ratio of male to female was about 1:1. All sera were tested for the presence of IgG and IgM antibodies by enzyme-linked immunosorbent assay (ELISA) with different antigenic specificity: (a) heat-denatured coxsackie type B1 and B5 virions, (b) a synthetic peptide representing the N terminus of the VP1 protein of coxsackievirus B3 (peptide CVB3), and (c) a synthetic peptide representing the N terminus of the VP1 protein of coxsackievirus B4 (peptide CVB4).

### *Synthetic peptides*

Amino acid sequences of the major VP1 antigen of coxsackieviruses were compared between different serotypes of coxsackievirus B species. Differences were observed mainly in the amino-terminal region of the VP1 antigen, a region previously defined to hold B cell antigenic determinants, as determined by epitope-mapping experiments. Therefore, two peptides spanning amino-terminal region 1–15 of coxsackievirus B3 (GPVEDAITAAIGRVA) and coxsackievirus B4 (GPTEESVERAMGRVA) were synthesized by solid-phase peptide synthesis (Bio-Synthesis, Lewisville, TX). The peptides were purified by high-pres-



**FIG. 1.** Sera were obtained from 506 healthy blood donors from various hospitals of northern (Thessaloniki), southern (Iraklio/Crete), western (Ioannina and Patra), and eastern (Larisa and Athens) Greece.