Viral detection in heart tissues has become a central issue for the diagnosis and exploration of the pathogenesis of idiopathic dilated cardiomyopathy (IDCM). In the present study, common cardiotropic viruses in 67 explanted heart samples of 31 IDCM adult patients were detected and semiquantified by using for the first time a new technology based on PCR assay coupled to electrospray ionization–time of flight mass spectrometry analysis (PCR-MS), with comparison to reference quantitative real-time PCR (RT-qPCR) assay. PCR-MS identified single or mixed enterovirus (EV) and parvovirus B19 (PVB19) infections in 27 (40.2%) of 67 samples, corresponding to 15 (48.3%) of the 31 patients, whereas RT-qPCR identified viral infections in 26 (38.8%) samples, corresponding to 16 (51.6%) of the patients. The PCR-MS results correlated well with EV and PVB19 detection by RT-qPCR (kappa = 0.85 [95% confidence interval {CI}, 0.72 to 1.00] and kappa = 0.82 [95% CI, 0.66 to 0.99], respectively). The levels of EV RNA (median, 550 [range, 178 to 3,200] copies/µg of total extracted nucleic acids) and of PVB19 DNA (median, 486 [range, 80 to 1,157] copies/µg of total extracted nucleic acids) were measured using PCR-MS and correlated with those obtained by RT-qPCR (r² = 0.57, P = 0.002 and r² = 0.64, P < 0.001 for EV and PVB19, respectively). No viruses other than EV and PVB19 strains were detected using the new PCR-MS technology, which is capable of simultaneously identifying 84 known human viruses in one assay. In conclusion, we identified single or mixed EV and PVB19 cardiac infections as potential causes of IDCM. The PCR-MS analysis appeared to be a valuable tool to rapidly detect and semiquantify common viruses in cardiac tissues and may be of major interest to better understand the role of viruses in unexplained cardiomyopathies.

In Europe and the United States, viral etiologies largely prevail over other causes of acute myocarditis (1, 2, 3). Moreover, 10 to 20% of viral acute myocarditis cases evolve toward a chronic active phase characterized by an inflammatory infiltrate of mononuclear cells with evidence of myocyte necrosis and fibrosis. In 9% of cases, this continues toward an end stage of dilated cardiomyopathy (DCM) that is a leading indication for cardiac transplantation (3, 4). Molecular analyses revealed that a broad panel of viruses commonly affecting human health could be detected, alone or in combination, in up to 70% of endomyocardial biopsy specimens from DCM patients, including 27% with multiple infections (1, 5). Cardiac viral infections could trigger or contribute as cofactors to the development of the disease in a large fraction of the DCM patients (6). However, the pathophysiological importance of common viruses detected in single and multiple cardiac infections in DCM patients remains to be explored (6). To gain insight into the pathogenesis of virus-associated DCM, the detection of a broad panel of virus specimens combined with the measurement of the viral load in heart tissue is required to assess the viral epidemiology and to distinguish active from chronic or persistent cardiac viral infections (7). Virological diagnosis of acute or chronic cardiomyopathies is currently based on combinations of quantitative real-time PCR (RT-qPCR) assays (1, 8). However, the wide range of viruses potentially responsible for cardiac infections, including both DNA and RNA viruses, with their highly various genetic characteristics, renders rapid and exhaustive virological diagnosis difficult using multiple monoplex RT-PCR assays (3). New molecular tools are needed for rapid and accurate multiple viral detection and quantitation in endomyocardial tissues of patients hospitalized for unexplained heart failure, including idiopathic DCM (IDCM) cases (9, 10). Such reliable detection systems might be of major interest not only to improve the clinical management of IDCM patients but also to assess the clinical consequences of several human viruses in DCM.

In the present study, we used for the first time a new technology allowing broad viral detection in clinical samples that couples broad-range PCR amplification to electrospray ionization–time of flight mass spectrometry analysis (PCR-MS) (11). The method was initially developed for the identification of viruses, bacteria, and fungi, including previously unknown or uncultivable agents, in samples where multiple microorganisms may be present (11). Using this new technology, viruses were detected and semiquantified in explanted heart tissues from IDCM patients. The qualitative and semiquantitative results obtained using PCR-MS were compared with those obtained by reference RT-qPCR assays.
**TABLE 1** Primer pairs used for viral detection in cardiac tissues from idiopathic dilated cardiomyopathy adult patients<sup>a</sup>

<table>
<thead>
<tr>
<th>Primer</th>
<th>Target&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus 1 943</td>
<td>HEX</td>
<td>TTGCAGAGTGGGCCACCCCATCAGAT</td>
</tr>
<tr>
<td>Adenovirus 2 769</td>
<td>HEX</td>
<td>TCCACCAACCTGTAACGTCCGAGT</td>
</tr>
<tr>
<td>Alphavirus 1 966</td>
<td>NC</td>
<td>TCCATGTACATGCTAGGTTTGGCCA</td>
</tr>
<tr>
<td>Alphavirus 2 2499</td>
<td>NC</td>
<td>TGCACGCAIACTGIGAICAIATGAC</td>
</tr>
<tr>
<td>Enterovirus 1 3760</td>
<td>5’ UTR</td>
<td>TGCGTCTGTTGGCGGCC</td>
</tr>
<tr>
<td>Enterovirus 2 3758</td>
<td>5’ UTR</td>
<td>TCCAGGGCCCTGTTAAGTG</td>
</tr>
<tr>
<td>Flavivirus 1 2215</td>
<td>NC</td>
<td>TACGACCGGACATCCTGATGATG</td>
</tr>
<tr>
<td>Flavivirus 2 2217</td>
<td>NC</td>
<td>TGGTCTGTAACATGATGGAAAAAGAGA</td>
</tr>
<tr>
<td>Alphaherpesvirus 1 3398</td>
<td>DNA-Pol</td>
<td>TCTGGAGTTTGACAGTGAATTGG</td>
</tr>
<tr>
<td>Alphaherpesvirus 2 3399</td>
<td>DNA-Pol</td>
<td>TCGCGCGGCGTGCTTCATGAGT</td>
</tr>
<tr>
<td>Betaherpesvirus 1 3379</td>
<td>DNA-Pol</td>
<td>TCGGCGCGGCTGTTACGTACG</td>
</tr>
<tr>
<td>Betaherpesvirus 2 3377</td>
<td>DNA-Pol</td>
<td>TGGACAAAGGACGCTGCGGCATCAA</td>
</tr>
<tr>
<td>Gammaherpesvirus 1 3407</td>
<td>DNA-Pol</td>
<td>TCGTCCCCATCGACATGTAC</td>
</tr>
<tr>
<td>Gammaherpesvirus 2 3405</td>
<td>DNA-Pol</td>
<td>TGGACAAAGGACGCTGCGGCATCAA</td>
</tr>
<tr>
<td>Parvovirus 1 3110</td>
<td>NS</td>
<td>TGGGCGCGGCAAGACTGAAAACC</td>
</tr>
<tr>
<td>Parvovirus 2 3118</td>
<td>VP1</td>
<td>TTCACAAAGGCTGGCGGGAACATG</td>
</tr>
</tbody>
</table>

<sup>a</sup> PCR assays were followed by electrospray ionization and mass spectrometry analysis.
<sup>b</sup> UTR, untranslated region; Pol, polymerase.

**MATERIALS AND METHODS**

**Patients.** Explanted heart tissue samples (n = 67) were obtained from 31 adult patients (23 male, 8 female; mean age [years], 44.3 [standard deviation] 12.2; range, 19 to 67) with idiopathic dilated cardiomyopathy (IDCM) according to the classification of cardiomyopathies by the Heart Failure Association of the European Society of Cardiology (12). For each patient, a mean number of 2.1 (SD = 1.3; range, 1 to 7) large heart tissue samples were fixed, within 2 h of heart transplantation, in 10% neutral buffered formalin and paraffin embedded for classical histopathological analyses (13). All of these 67 heart tissue samples demonstrated histopathological findings compatible with DCM (14).

All of the 31 study IDCM patients were from the Paris or Ile de France area and were clinically followed up at the Georges Pompidou European Hospital (HEPG) (Paris, France) where their cardiac tissues were sampled between 2002 and 2009. The Hospital Ethics Committee (HEGP, Paris, France) approved the study, and informed consent was obtained from the patients or subjects’ families at the time of heart transplantation. Our investigations conformed to the principles outlined in the Declaration of Helsinki for use of human tissue or subjects.**

**Histopathological analysis.** For each heart sample, four transverse paraffin sections were performed at 3 levels and were examined by classical histopathology to analyze the presence of active or borderline myocardial inflammation according to the Dallas classification, to confirm the clinical findings of end-stage DCM (14). Two pathologists (P.B. and P.F.) separately examined the cardiac tissue sections.

**Viral strains.** Reference cosxaccaviirus B1 and B3 to B6 (CVB) strains (ATCC numbers VR-1032, VR-1034, VR-1035, VR-1036, and VR-1037) were used as positive controls for PCR-MS genotyping identification.

**DNA and RNA extraction from explanted heart tissues.** After deoxy-axing, 5-ml amounts of cardiac tissue samples were subjected to proteinase K (250 µg/ml) (Ambion; Life Technologies, Saint-Aubin, France) digestion in extraction buffer containing 20 mM Tris-HCl (pH 8.3) (Sigma-Aldrich, St. Louis, MO) and 0.5% SDS (Sigma-Aldrich) in a water bath at 56°C for 2 h. Total nucleic acid (DNA and RNA) extraction from tissue sample lysates was performed using a NucliSens easyMAG instrument (bioMérieux, Marcy l’Etoile, France) according to the manufacturer’s instructions (15). Briefly, 50 µl of a lysate sample was centrifuged (6,000 × g for 1 min), and then the supernatant was added to 2 ml of lysis buffer in a plastic vessel and incubated for 10 min at room temperature. One hundred forty microliters of silica was added to the mixture. This was followed by an automatic magnetic separation phase (16). Nucleic acids were recovered in 60 µl of elution buffer and stored at −80°C until use. Total extracted DNA and RNA were quantified by the optical density at 260/280 nm using a Picodrop microtiter spectrophotometer (Picodrop, Saffron Walden, United Kingdom). A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RT-PCR assay performed using DNase-treated samples demonstrated the ability to amplify at least a 600-bp amplicon of the RNA extracted from formalin-fixed, paraffin-embedded archival cardiac tissues (17).

**Quantitative viral detection by RT-qPCR assays.** For each sample, 80 ng of total nucleic acid extracts was subjected to RT-qPCR using either commercial (Argene EBV R-gene and HSV1 HSV2 VZV [herpes simplex virus 1 and 2 and varicella-zoster virus] R-gene assays; bioMérieux, Verniolle, France) or in-house techniques for the detection of cytomegalovirus (CMV), parvovirus B19 (PVB19), enterovirus (EV), and adenovirus (ADV) (8, 18–23). In positive samples, the viral load was expressed as the number of genomic DNA or RNA copies per µg of total extracted nucleic acids (DNA and RNA) (6).

**PLEX-ID analysis.** PCR and high-resolution mass spectrometry (MS) analyses were carried out on total nucleic acid extracts using an Ibis T5000 biosensor (Ibis Biosciences, Carlsbad, CA, USA) at the Athogen laboratory (Irvine, CA, USA). The viral assay used for analysis was the Ibis sterile fluids viral surveillance kit (Ibis Biosciences), consisting of primer pairs targeted to ADV6 alphaherpesviruses, herpesviruses (with the notable exception of human herpesvirus 6), PVB19, flaviviruses (dengue viruses 1 to 4, West Nile virus, Japanese encephalitis virus, and St. Louis encephalitis virus), and EVs (Table 1). The assay-specific database contains more than 80 viral species. Sealed 96-well assay plates (Ibis T5000 sterile fluids viral surveillance kit; Ibis Biosciences) containing 40 µl of a PCR master mix without enzymes were thawed at room temperature. Plates were centrifuged briefly, followed by the addition of 5 µl of enzyme mixture per well (3.0 U AmpliTaq Gold Taq polymerase; Applied Biosystems, Foster City, CA, USA), 2.0 U SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA), and 0.08 to 0.8 µg of T4 gene 32 protein (Roche, Indianapolis, IN) per reaction mixture volume. For each clinical sample, an amount of 80 ng of total extracted nucleic acids was added to each of the 16 reaction wells. Cycling was done according to the manufacturer’s instructions. Desalting and MS analysis using the Ibis T5000 biosensor (Ibis Biosciences) have been described previously (11, 24). In sterile water, viral primers reliably detected as few as 10 to 15 copies of genomes per PCR well (not shown). The molecular weight of the amplicons was determined by an electrospray ionization-time of flight mass spectrometer (Ibis T5000 biosensor).
FIG 1 Viral findings obtained in 67 heart samples taken from 31 patients with dilated cardiomyopathy using classical quantitative real-time PCR (RT-qPCR) and PCR followed by electrospray ionization-mass spectrometry analysis (PCR-MS). (A) Number of samples positive (+) or negative (−) by indicated assay. (B) Number and percentage of cases positive for virus(es) by indicated assay. EV, enteroviruses; PVB19, parvovirus B19; *, P > 0.05 according to Fischer’s exact test.

FIG 2 Identification of mixed viral cardiac infections. A mixed infection was defined as the presence of both EV and PVB19 in a same sample or the presence of an EV-positive sample and a PVB19-positive sample taken from the same patient. Statistical analyses. Spearman’s rank correlation was used to evaluate linear associations between viral load values obtained with RT-qPCR and the PCR-MS system. Fischer’s, Mann-Whitney, and kappa tests were carried out with SAS software, version 8.2 (SAS Institute, Cary, NC, USA). Results were considered statistically significant for two-sided P values of <0.05.

RESULTS

Prevalence of viral detection in heart tissue samples from IDC patients. PCR-MS identified single or mixed enterovirus (EV) and parvovirus B19 (PVB19) infections in 27 (40.2%) of 67 samples, whereas RT-qPCR identified viral infections in 26 (38.8%) samples. Mixed EV-PVB19 infections were evidenced by PCR-MS and RT-qPCR in 4 and 2 samples, respectively (Fig. 1A). No viral species other than PVB19 and EV was identified using the new PCR-MS technology in the samples analyzed. The PCR-MS results correlated well with EV and PVB19 detection by RT-qPCR (kappa = 0.85 [95% confidence interval {CI}, 0.72 to 1.00] and kappa = 0.82 [95% CI, 0.66 to 0.99], respectively). Using PCR-MS, viral genomes were detected in heart tissues of 15 (48.3%) of the 31 IDC patients (EV, n = 4 [12.9%]; PVB19, n = 5 [16.1%]; and EV-PVB19, n = 6 [19.3%]), whereas RT-qPCR assays identified viral infections in 16 (51.6%) (EV, n = 7 [22.6%]; PVB19, n = 4 [12.9%]; and EV-PVB19, n = 5 [16.1%]) of the study patients (Fig. 1B).

Conventional histological analyses detected borderline myocarditis in only 10 (35.7%) of the 28 heart samples identified as virus positive by the classical or the new molecular technique, corresponding to 4 (25%) of the 16 virus-positive patients (not shown). PVB19 and EV genomes were detected alone or in association in 8 of the 10 heart tissue samples with borderline myocarditis (not shown).

Comparison of the viral semiquantitative detection results obtained by PCR-MS and RT-qPCR. As depicted in Figure 2A and B, the estimated EV and PVB19 viral loads were not significantly different between the two molecular techniques (median range), 550 [178 to 3,200] versus 385 [50 to 2,108] EV copies/μg, P = 0.26, and 486 [80 to 1,157] versus 504 [186 to 2,730] PVB19 copies/μg, P = 0.34). These moderate median viral loads obtained for EV and PVB19 were compatible with persistent cardiac infections in IDC patients. In cases of positive detection by the two molecular methods, the levels of EV genomic RNA and PVB19 DNA measured by the new method correlated significantly with those obtained by classical PCR assay (r = 0.57, P = 0.002, and r = 0.64, P < 0.001, respectively) (Fig. 2C and D). Concerning EV results that were discrepant between the traditional RT-qPCR and the PCR-MS detection technique (Fig. 1A), the only PCR-MS-positive/RT-qPCR-negative sample corresponded to an untyped EV strain (see discussion of genotyping below) with a viral load of 320 copies/μg, whereas the two PCR-MS-negative/RT-qPCR-positive samples exhibited viral loads of 672 and 724 copies/μg, respectively. Concerning PVB19 results that were discrepant between the traditional RT-qPCR and PCR-MS detection (Fig. 1A), the four PCR-MS-positive/RT-qPCR-negative samples exhibited viral load values of 34, 112, 120, and 2,000 copies/μg, respectively. Concerning PVB19 results that were discrepant between the traditional RT-qPCR and PCR-MS detection (Fig. 1A), the four PCR-MS-positive/RT-qPCR-negative samples exhibited viral load values of 34, 112, 120, and 2,000 copies/μg, respectively.

Genotyping identification of viruses by PCR-MS analysis of heart tissue samples from study patients. Using the PCR-MS system for broad viral detection, a mass spectrum was obtained from amplified viral DNA/RNA fragments in 27 (40.2%) of the 67 heart samples from IDC patients, allowing the identification of 31 single or multiple viral heart infections by comparative analyses of the base composition signatures. Figure 3 shows examples of mass spectra of single infections in two distinct heart samples. Positive-genotyping CVB strain controls provided by ATCC were included in each serial assay; all were successfully identified as EV.
genus and coxsackievirus B viruses. Only the reference CVB5 strain was misidentified as a CVB3 strain (data not shown).

Among the 14 EV-positive heart samples (Fig. 1A), the PCR-MS system identified 14 strains, including 1 strain belonging to the group B coxsackieviruses (CVB) or echovirus, one CVB5 strain, 9 CVB3 strains, and 3 untyped EV strains. In cases of PVB19 cardiac infection, the primers used did not allow a specific virus genogroup identification. The confidence of genotyping identification was indicated by a Q-score that ranged from 0.93 to 1.00 (mean, 0.98 [SD = 0.02]) (data not shown).

DISCUSSION
Viral infections of the heart have become a central issue in studying the pathogenesis of unexplained dilated cardiomyopathy. There are still controversies on the importance of several common human viruses in idiopathic dilated cardiomyopathy (IDCM), partly due to the lack of standardized and reliable molecular assays for virus detection in cardiac tissues (1–3). In the present study, we evaluated and used a new molecular system capable of detecting a broad range of viral infections and assessing the loads of cardiotropic viruses in heart tissues of IDCM adult patients.

We report here a high prevalence (40.2%) of cardiac virus genomes in IDCM patients, with a major proportion of single virus detections (EV or PVB19 strains) in cardiac tissue samples. The screening of these adult patients identified high proportions of EVs and PVB19, whereas adenoviruses were not detected in the ventricular heart samples tested (Fig. 1). Interestingly, no other common or unexpected human cardiotropic viruses could be detected using the new molecular assay (PCR coupled to electrospray ionization-time of flight mass spectrometry [PCR-MS]), which is capable of specifically detecting 84 known human viruses in only one assay (11). The samples in the current study were formalin fixed and paraffin embedded and then subjected to de-waxing; the effects of such tissue handling on virus genomes remains unknown.

Some previous molecular studies had reported that human EVs could be the potential viral cause of IDCM in 35% of end-stage patients who required a heart graft and that PVB19 may be another major viral cause in 51% of IDCM patients (5, 6, 25–27). Interestingly, we performed our molecular analyses on large ventricular tissues and we confirmed a high prevalence of EV (32.2%) and PVB19 (35.4%) infections in IDCM patients. Thus, restricted
Moreover, this broad viral detection assay allowed a reliable semi-sensitive molecular assays capable of detecting numerous viruses in cardiac samples (3, 28). Finally, these findings reflect the need for immunohistological detection of inflammatory cells in clinical practice according to the Dallas classification and the presence of viral pathogens at the end stage of DCM that leads to the need for a heart graft. Only one previous study reported PVB19 viral loads in cardiac tissues of DCM patients (1, 3). Only half of DCM cases were negative for viral detection in their ventricular tissues. Possible contamination of cardiac tissues by systemic circulating common viruses potentially reactivated at the time of heart transplantation could not be ruled out, but this was not observed in previous studies (5). Our results confirmed the high prevalence of viral detection in cardiac tissue of patients with unexplained and nonfamilial/nongenetic ventricular cardiac dysfunction classified as IDCM according to the ESC report (5, 6, 12). In contrast to clinical practice, we tested large tissue samples, increasing our chances to detect viral genomes. However, we compared the classical and novel molecular techniques using the same amount of total nucleic acids extracted from 5 mg of cardiac tissues, corresponding to the weight of common endomyocardial biopsy specimens (EMBs) sampled in DCM patients (9). This indicates that this new technological approach might be used for the routine identification of single or mixed cardiac viral infections in clinical practice.

In the present study, conventional histological analyses indicated the presence of borderline inflammation in 10 (35.7%) of the 28 virus-positive heart samples, corresponding to 4 (25%) of the 16 virus-positive patients. This confirms that routine histological analyses were too insensitive and inadequate to detect a myocardial inflammation related to viral infection at the end stage of IDCM in adult patients and, thus, indicates the limitations of histological diagnosis according to the Dallas classification and the need for immunohistological detection of inflammatory cells in cardiac samples (3, 28). Finally, these findings reflect the need for sensitive molecular assays capable of detecting numerous viruses in EMBs taken from adults or infants with unexplained IDCM. In the analyses reported here, the new PCR-MS system appeared to be as reliable as classical PCR assays for the detection of EV and PVB19 genomes in cardiac tissues of IDCM patients (Fig. 1 and 2). Moreover, this broad viral detection assay allowed a reliable semi-quantification of EVs and PVB19. Quantification of viral genomes in cardiac tissues can be of major interest to distinguish ongoing active viral replication from a chronic persistent viral infection (1, 3, 7). This viral genomic quantitative approach is of major interest for the investigation of viral pathogenesis in human cardiac diseases and for routine monitoring of viral load during the natural course of the disease or for clinical management during clinical trials using classical or new antiviral strategies (6, 29). Moreover, the PCR-MS system was able to genotype species, specifically for the EVs. Only group B coxsackieviruses (serotypes 3 and 5) belonging to EV species B were detected in end-stage DCM patients. However, this genotyping approach has to be confirmed by referenced EV genotyping strategies based on partial amplification and sequencing of VP1 or VP4/VP2 capsid genes (30). Nevertheless, EV genotyping identification by PCR-MS technology might be of interest to rapidly confirm the presence of EV in heart samples and to include patients in further clinical trials using specific antiviral strategies. Our group is now developing next-generation sequencing strategies (short PCR assays in VP1 and VP2/VP4 genes followed by next-generation sequencing technologies) for reliable phylogenetic analyses of persistent cardiac viral strains. Finally, our genotyping results indicated the suitability of the PCR-MS for rapid EV genotyping to species but not for EV serotyping identification.

Our quantitative approach using the classical RT-qPCR and PCR coupled with mass spectrometry analysis (PCR-MS) indicated the presence of moderate viral loads per microgram of nucleic acids extracted from ventricular tissues (Fig. 2). The cardiac levels of EVs and PVB19 viral genomes obtained in our IDCM patients are compatible with the hypothesis of persistent cardiac infections that could explain the absence of detectable inflammatory cells by classical histological techniques at the end stage of IDCM that leads to the need for a heart graft. Only one previous study reported PVB19 viral loads in cardiac tissues of DCM patients, with a mean viral load of 709 genomes per microgram of extracted nucleic acids (7). These PVB19 DNA loads levels are quite similar to those detected in our IDCM patients (Fig. 2), but they are significantly lower than those detected in acute PVB19...
diseases, such as hydrops fetalis (31). Our group was the first, to our knowledge, to perform a standardized quantification of EV genomes in endomyocardial tissues of end-stage IDCM patients and to identify low levels of EV cardiac infection in IDCM patients (8).

In further prospective clinical trials, it could be of major interest to perform a reliable detection of cardiotropic viruses in samples from patients with IDCM in order to obtain an etiological diagnosis and to have the possibility to select for specific or broad-spectrum antiviral therapies against one or several cardiotropic viruses detected in cardiac tissues (3, 32, 33). In such therapeutic investigations, the use of quantitative multiple viral genome detection in follow-up biopsy specimens would be of major interest for the reliable monitoring of antiviral efficacy and to analyze a potential relationship between viral clearance and cardiac hemodynamic improvement in IDCM patients (34).

In conclusion, we identified single or mixed EV and PVB19 cardiac infections as leading potential causes of IDCM. The low viral loads were compatible with chronic persistent cardiac infections. The PCR-MS analysis appeared to be a valuable tool to rapidly detect and semiquantify common viruses in cardiac tissues and may be of major interest to better understand the role of viruses in unexplained cardiomyopathies.

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The molecular analyses on the PCR-ESI TOF SI system were performed in the AthoGen laboratory (Irvine, CA, USA) using coded tubes containing the DNA/RNA extracts. M.P.-M. (Abbott GmbH & Co. KG, Europe, Wiesbaden, Germany) supervised the molecular analyses and interpreted the mass spectra obtained from the PCR system. The data obtained for the heart samples were independently analyzed and interpreted in the EA-4684 unit Cardiovir SFR-CAP Sante, Faculty of Medicine, Reims, France, which possesses the entire final data bank. The corresponding author had full access to all the data of the study and had the responsibility for the decision to submit this work for publication with the agreement of all the coauthors.

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