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CASE REPORT

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Fatal Parvovirus B19 Myocarditis in an 8-Year-Old Boy

ABSTRACT: A report is given on an 8-year-old boy who suddenly and unexpected died. Autopsy findings point to acute heart failure. Microscopic examination of the heart showed increased interstitial and perivasal fibrosis and myocarditis with macrophage infiltration. Polymerase chain reaction (PCR) analysis for parvovirus B19 was positive in heart samples and in the spleen. Immunostaining for parvoviral surface antigens was negative. Although the virus does not appear to have infected the cardiomyocytes, we speculate that myocarditis arose from immunological cross-reaction to epitopes shared between the virus and the myocardium.

KEYWORDS: forensic science, myocarditis, parvovirus B19, immunohistochemistry, nested PCR

The family parvoviridae is a group of DNA viruses that are known to infect both animals and humans. Parvoviruses are small (20 to 25 nm in diameter), nonenveloped, single-stranded DNA viruses. Parvovirus B19 is the only known human pathogen in this family of viruses and the causative agent for fifth disease (erythema infectiosum) (1). The infection is relatively common in humans. Period patrons are viremic from Days 6 to 12 after infection, and more than 50% of the population have detectable antibodies by the age of 15 years, increasing to 90% in the elderly (2). A growing number of case reports now show an association between parvovirus B19 infection and myocarditis in previously healthy children (3) or adults (4). In fetal pathology, parvovirus B19 has been shown to cause lethal myocarditis and hydrops (5). Human parvoviral infection usually causes asymptomatic infections or erythema infectiosum ("fifth disease") accompanied by a characteristic facial rash. Cases of lethal myocarditis during the course of a parvoviral infection seem to be rare.

Case History

We report the case of an 8-year-old boy who collapsed shortly after a visit of the family doctor and died before he could be brought to the hospital. The boy had been suffering from watery diarrhea and vomiting for approximately four days. At first, the family doctor had given his recommendations by phone. Erythema infectiosum was not reported. There was no evidence of arrhythmia. The familiar situation was inconspicuous; there were no indications of malnutrition and neglect. An autopsy was performed on behalf of the public prosecutor to clarify the cause of death and possible maltreatment by the family doctor.

Autopsy Findings

The autopsy of the boy with reduced nutritional condition, a length of 139 cm and 20 kg, revealed moderate pulmonary edema and hepatic congestion. An empty small intestine was found and little content in the colon. Dryness of muscles and inner organs point to antemortem dehydration. No preexisting abnormalities and no other pathological findings were discovered during autopsy, nor any evidence for fatal dehydration.

Additional Findings

Conventional Histology

Eight myocardial samples were taken from the heart (right ventricle anterior and posterior, septum interventriculare cranial and caudal, left ventricle anterior wall cranial and caudal, left ventricle posterior cranial and caudal). Conventional histological methods (Hematoxylin&Eosin, Mallory stain) revealed focal myocytolysis and interstitial edema but no findings of an active myocarditis according to the Dallas-criteria (6). Mallory stain presents an interstitial fibrosis and often circular fibers around small intramyocardial vessels (Figs. 1 and 2), both findings in an intensity, which in our experience, is unusual in children of this age. The diffuse and interstitial fibrosis was accompanied by infiltrating mononuclear cells.

Immunohistochemistry

The immunohistochemical qualification and quantification of interstitial myocardial leucocytes can improve the diagnosis of myocarditis and is already established in the diagnosis of myocarditis

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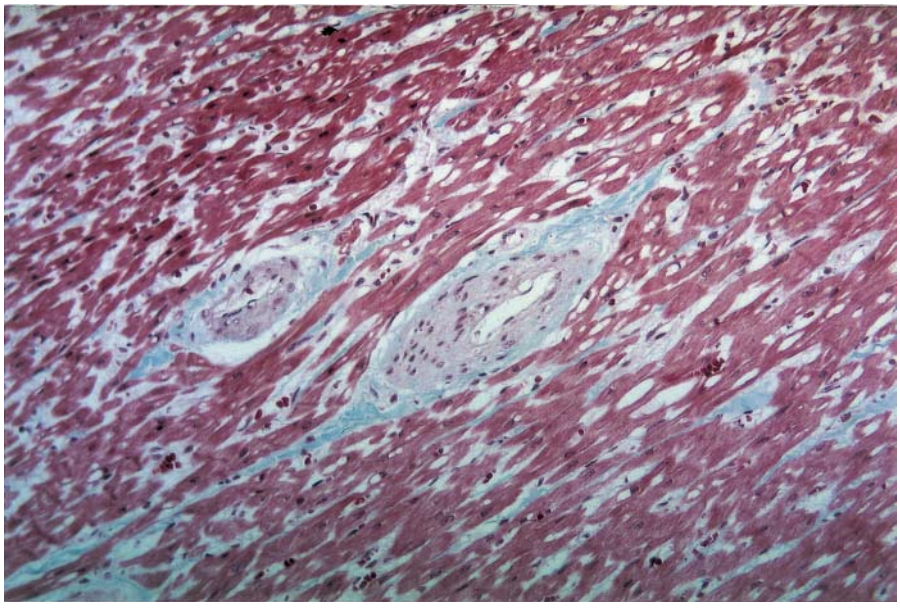

FIG. 1—*Mallory stain: increased circular-perivascular (X400) and diffuse interstitial fibrosis.*

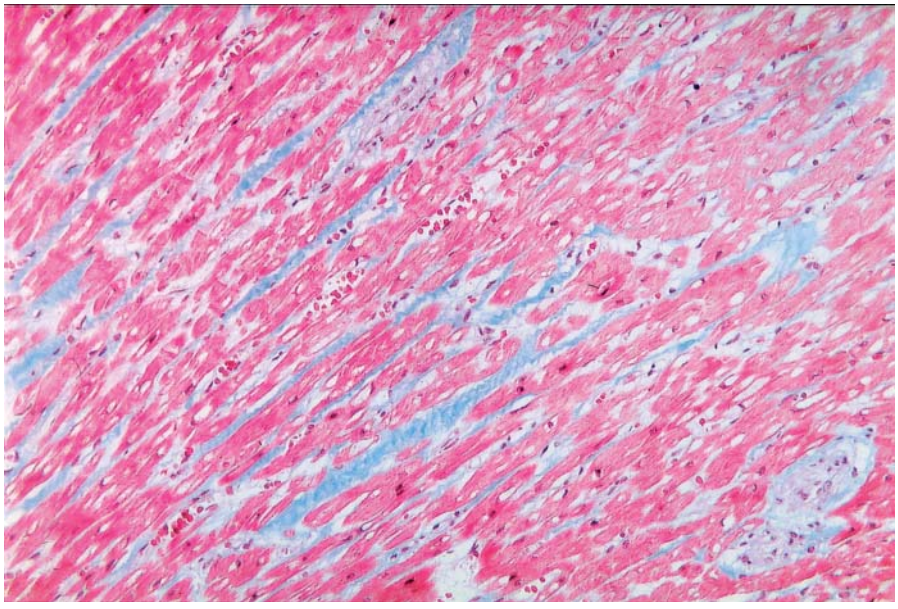

FIG. 2—*Mallory stain: increased circular-perivascular (X200) and diffuse interstitial fibrosis.*

in adults (7). Although control values concerning children are not published, in our experience immunohistologically an increased number of LCA-positive leucocytes are found. While CD3-positive T-lymphocytes were only detectable in a number which can be accepted as normal, numerous CD68-positive macrophages were found within the interstitial and perivascular fibrotic regions (Fig. 3). Immunostaining for parvoviral surface antigens was negative in heart and spleen, but perhaps the levels of surface antigens have dropped below the sensitivity of the stain.

PCR for Parvoviral Genome

To validate the diagnosis of myocarditis, a virus proof was initiated using nested PCR and in-situ hybridization. Paraffin-embedded myocardial samples were dewaxed with xylene and washed with ethanol. The paraffin sections were homogenized using a Miccra D-8 homogenizer (Art-moderne Labortechnik, Müllheim-Hügelheim, Germany). Genomic DNA was extracted with the Gen-ial First DNA Kit (Gen-ial, Troisdorf, Germany) according to the suppliers protocol. The assumption for the specific virus PCR was the successful amplification of the housekeeping gene human alpha fibrinogen locus (FGA) (8). Five microlitres of extracted total DNA was combined with 10 pmol/μL primer, 200 μ*M* of each dNTP, 2.5 μL 10XPCR buffer, 0.5 m*M* $MgCl_2$, 1 U Taq polymerase. Four-min initial denaturation at 94°. Thirty-five rounds of amplification were performed at the following conditions: 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min. A nested PCR was performed and the primers were used according to

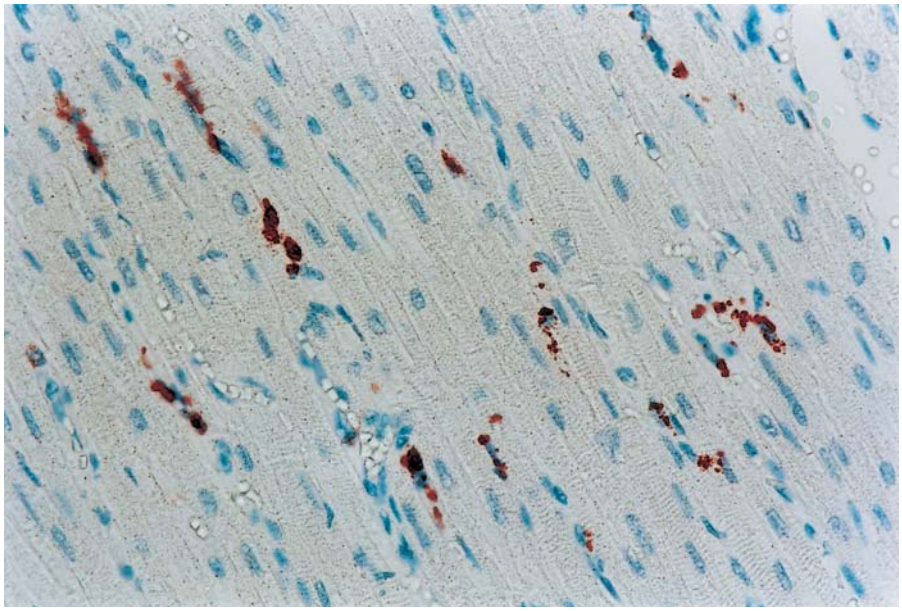

FIG. 3—*Immunochistochemically increased infiltration of CD68-positive macrophages (X400) in the myocardium.*

Cassinotti et al. (9). Primer sequence:

P1: 5′- TTCTTTTCAGCTTTTTAGG-3′
P2: 5′- GTACTTCTGGTACGTTAAGT-3′
P3: 5′- TATAAGTTTCCTCCAGTGCC-3′
P4: 5′- TGTAATCCTCCACTGGGTTT-3′

Four microlitres of each reaction were analyzed on an 8% polyacrylamidgel, cast on Gelbond (Biozym), with 0.07 *M* Tris sulfate in the gel and 0.28 *M* Tris borate buffer in the agarose plugs. The electrophoresis was run for 8 cm. The DNA fragments were detected by silver staining. All samples were run with a simultaneous positive control and negative control (reaction mixture minus template).

While in-situ hybridization was negative, Parvovirus B19-DNA could be detected in three out of eight myocardium samples and the spleen (Fig. 4). The virus could not be detected in the liver or kidneys. The nested PCR results were controlled by two external investigators. Detection was possible using formalin-fixed paraffin-embedded samples. In our case, IgG antibodies were positive and IgM antibodies negative. Additional investigations using nested (RT-)PCR to detect enteroviruses (EV), adenoviruses (ADV), human cytomegalovirus (HCMV), Epstein-Barr-Virus (EBV), and human herpes viruses type 6 (HHV6) were negative as well as nested PCR for chlamydia pneumoniae and borrelia burgdorferi.

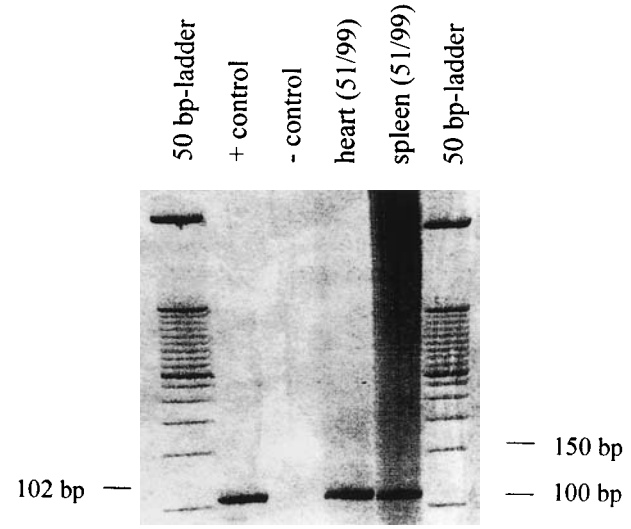

FIG. 4—*Detection of PVB19-DNA by nested PCR in samples from myocardium and spleen; 102 bp PCR fragment: Acc. No.: AB030694; Nucl. Pos.: 3124-3225.*

Discussion

The etiological diagnosis of myocarditis is often difficult. For a long time, enteroviruses, especially group B coxsackieviruses, have been considered the most common cause of viral myocarditis. Meanwhile, we know that several other viruses may also cause this disorder in children, and cases of sudden death in young dogs with myocarditis caused by parvovirus are published (10). The B19 receptor (erythrocyte P antigen) has been identified on fetal myocardial cells, suggesting that intrauterine myocarditis contributes to the development of fetal hydrops after parvovirus B19 infection (11).

Finding parvoviral DNA in myocardial tissue does not necessarily mean that the heart is infected, because the viruses could be present simply due to viremia. The severe, increased interstitial and perivascular fibrosis within the myocardium as well as an increased number of CD68-positive macrophages suggest a chronic inflammatory process leading at least to acute heart failure. Considering the age of the boy, these histological findings must be regarded as impressive. We are aware of cases positive for parvovirus B19 by PCR without signs of myocarditis, without such interstitial and perivascular fibrosis, and also without an increased number of CD68-positive macrophages. Recent parvovirus B19 infection, i.e., acquisition of infection during the last two or three months, is not very likely because of the lack of anti-B19 IgM antibody in our case, while positive IgG-antibodies also indicate a chronic, remote infection. Infection may have occurred some months earlier, indi-

cated by the presence of B19 DNA found in the patient's blood serum.

It is known that B19 DNA can be detected for several months longer after primary infection in serum than IgM antibody, although less probable, reactivation of a persistent infection cannot be excluded. However, a more precise determination of the time of infection from serological data is not possible. According to the histological, immunohistochemical, serological, and molecularpathological results in our case, we interpret the reported findings as parvovirus B19 induced lethal myocarditis as other authors do. Also, it remains open to discussion that myocarditis may have arisen from immunological cross-reaction epitopes shared between the virus and the myocardium.

We want to point out that every case of suspected myocarditis should be investigated for viruses as etiologic agents to confirm the individual diagnosis and to provide further information on the epidemiological importance. Therefore, we need more case reports showing the association between parvovirus B19 infection and myocarditis (12–15).

In our case, all jurisdictional investigations were ended. Because of the diagnosed "chronic myocarditis," it could not be stated with the certainty necessary for a penal court that the boy would have survived if he had been brought to the hospital earlier.

References

1. Cherry JD. Parvovirus infections in children and adults. Advances in pediatrics 1999;46:245–69.
2. Cohen BJ, Buckley MM. The prevalence of antibody to human parvovirus B19 in England in Wales. J Med Microbiol 1988;25:151–3.
3. Saint-Martin J, Choulot JJ, Bonnaud E, et al. Myocarditis caused by parvovirus [letter; comment]. J Pediatr 1990;116:1007–8.
4. Orth T, Herr W, Spahn T, et al. Human parvovirus B19 infection associated with severe acute perimyocarditis in a 34-year-old man [letter]. Eur Heart J 1997;18:524–5.
5. Morey AL, Keeling JW, Porter HJ, et al. Clinical and histopathological features of parvovirus B19 infection in the human fetus. Br J Obstet Gynaecol 1992;99:566–74.
6. Aretz HT. Myocarditis: the Dallas criteria. Hum Pathol 1987;18:619–24.
7. Dettmeyer R, Schlamann M, Madea B. Immunohistochemical techniques improve the diagnosis of myocarditis in cases of suspected sudden infant death syndrome (SIDS). For Sci Int 1999;105:83–94.
8. Barber MD, McKeown BJ, Parkin BH. Structural variation in the alleles of a short tandem repeat system at the human alpha fibrinogen locus. Int J Legal Med 1996;108:180–5.
9. Cassinotti P, Weitz M, Siegl G. Human parvovirus B19 infections: routine diagnosis by a new nested polymerase chain reaction assay. J Med Virol 1993;40:228–34.
10. Hayes MA, Russell RG, Babiuk LA. Sudden death in young dogs with myocarditis caused by parvovirus. J Am Vet Med Assoc 1979;174: 1197–203.
11. Porter HJ, Quantrill AM, Fleming KA. B19 parvovirus infection of myocardial cells. Lancet 1988;1:535–6.
12. Enders G, Dotsch J, Bauer J, et al. Life-threatening parvovirus B19-associated myocarditis and cardiac transplantation as possible therapy: two case reports. Clin Infect Dis 1998;26:355–8.
13. Murry CE, Jerome KR, Reichenbach DD. Fatal parvovirus myocarditis in a 5-year-old girl. Hum Pathol 2001;32:342–5.
14. Chia JKS, Jackson B. Myopericarditis due to parvovirus B19 in an adult. Clin Inf Dis 1996;23:200–1.
15. Schowengerdt KO, Ni J, Denfield SW, et al. Association of parvovirus B19 genome in children with myocarditis and cardiac allograft rejection. Circulation 1997;96:3549–54.

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