Infantile hypertrophic pyloric stenosis (IHPS) is characterized by abnormal thickening of the internal circular muscle layer. IHPS is known to be due to a combination of genetic and environmental factors, but its precise causes and pathophysiology are poorly understood. The objective of the study is to determine the prevalence of the principal viruses targeting the respiratory and digestive tracts in children with IHPS. Nasopharyngeal fluids, stools, vomit, and surgical pyloric muscle fragments and swabs were tested by cell culture, viral antigen assay and PCR. IHPS was diagnosed in 23 boys and 8 girls with a mean (±SD) age of 42 ± 15 days (range 20–88 days). There was no seasonal pattern of diagnosis. Twenty-two children (71%) lost weight (mean 246 ± 164 g, range 30–600 g) after the onset of vomiting, and five (16.1%) were dehydrated. Seven (22.6%) infants had been exposed to an infectious contact within 15 days before admission, and one on the day of admission (3.2%). Ear, nose and throat samples and pyloric muscle specimens were negative for all the viruses tested. An adenovirus type 3 was recovered from one stool sample, and RT-PCR was positive for an enterovirus on one vomit sample. This study suggests that the principal viruses targeting the respiratory and digestive tracts are not responsible for IHPS.
(date of onset of vomiting, weight changes, presence of peristaltic waves, palpability of the pyloric olive, and signs of dehydration), plain abdominal radiography and pyloric sonography, and blood electrolyte levels. Peristaltic waves of gastric contraction indicate hypertrophy of the gastric muscle secondary to slowly progressive obstruction: their observation makes pyloric stenosis likely. Information on the pregnancy and delivery, gestational age, breast-feeding and possible neonatal disorders was also collected. Informed consent was granted by the parents of the infants.

Nasopharyngeal aspirates were tested for the principal viruses targeting the respiratory tract, including influenza type A and B viruses circulating at the time of the study, respiratory syncytial virus (RSV) type A and B, metapneumovirus (MPV), Paramyxovirus parainfluenza (PIV) type 1, 2, and 3, rhinovirus (RHV), coronaviruses OC43 and 229E, and adenovirus (ADV).

Stools or rectal swabs, vomit samples, intra-operative pyloric swabs, and biopsy specimens of the pyloric muscle fragment were tested for viruses targeting the digestive tract, including ADV, enterovirus (EV), and rotavirus (RV).

This study was approved by the Institutional Ethics Committee of Poitiers University Hospital, and informed consent of the parents of the infants was obtained in all cases.

Direct Immunofluorescence and Cell Culture

Direct immunofluorescence was performed with fluorescein-labeled monoclonal antibodies against RSV A + B and influenza A and B (Monofluo Screen RSV, Monofluo Kit Influenza; Biorad, Marnes-la-Coquette, France).

Nasopharyngeal aspirates and rectal, vomit and stool samples were inoculated on MRC-5 human diploid cells, LLCMK-2 heteroploid simian cells, HeLa 229 cells, and MDCK heteroploid canine cells. Cell cultures were maintained for at least 3 weeks, and were passaged blindly twice.

Immunochromatography

Stool and vomit samples were tested for RV and ADV antigens with an immunochromatographic method (SD Rota/Adeno BioLine; Standard Diagnostics, Inc., Kyonggindo, Korea), as recommended by the manufacturer. Colored lines appearing after sample migration indicate positivity.

Multiplex PCR for Influenza A and B, and RSV A and B; PCR for ADV, MPV, PIV Types 1, 2, and 3, RHV, and Coronavirus OC43 and 229E

Multiplex PCR was carried out as described elsewhere [Stockton et al., 1998]. This nested PCR approach detects RSV A and B and circulating influenza viruses A(H1N1), A(H3N2) and B. The two PCR rounds were done with primer pairs targeting the hemagglutinin (H) gene of influenza viruses A(H1), A(H3), and B, and a sequence overlapping the RSV N (capsid) and P (phosphoprotein) genes.

The other PCR assays were carried out as reported previously, using the following targets: a 161-bp region of the ADV-2 hexon gene sharing a high degree of nucleotide homology with 47 human ADV serotypes [Hierholzer et al., 1993]; the MPV matrix gene, with an amplicon size of 416 bp [Bellau-Pujol et al., 2005]; conserved regions of the hemagglutinin-neuraminidase gene of PIV types 1, 2, and 3, with respective amplicon sizes of 316, 203, and 477 bp [Echevarria et al., 1998]; a region encompassing the RHV VP4/VP2 and hyper-variable region of the 5’ non-coding region, yielding an amplicon of about 549 bp [Savolainen et al., 2002]; and the M and N genes of coronaviruses OC43 and 229E, with respective amplicon sizes of 335 and 573 bp [Vabret et al., 2001]. The PCR products were separated by electrophoresis on 2.5% agarose gels (NuSieve; FMC BioProduct, Rockland, ME) in Tris-EDTA buffer. GAPDH transcripts were co-amplified with each sample to control for RNA quality and PCR inhibitors [Guedin et al., 2001]. A second internal control corresponding to the β-globin gene was also co-amplified.

Statistical Analysis

Statview 4.5 software (SAS Institute, Berkeley, CA) was used for all analyses. Quantitative variables were expressed as the mean ± standard deviation (SD) and, when appropriate, the range. Qualitative variables were expressed as the absolute number and corresponding frequency.

Quantitative variables were compared between groups by using the Mann–Whitney U test. Correlations between sonographic findings were tested with Spearman’s correlation coefficient (r).

RESULTS

IHPS was confirmed in 23 boys and 8 girls (mean age 42 ± 15 days; range 20–88 days), whose mean weights at birth and on admission were respectively 3.199 ± 0.469 kg and 3.171 ± 0.579 kg. About three cases of IHPS were diagnosed per month, with no significant difference across the seasons.

Vomiting occurred in 87.1% of patients (n = 27), and symptoms lasted from 2 to 41 days (median 9 days). Six infants (19.3%) received anti-reflux medication. Physical examination showed a palpable pyloric olive in 13 cases (41.9%), presence of peristaltic waves in 5 cases (16.1%), and signs of dehydration in 5 cases (16.1%). Symptoms lasted an average of 19.0 ± 9.4 days in dehydrated children and 11.0 ± 9.4 days in the other children (P = 0.04).

Seventeen infants had plain abdominal radiography, which revealed abnormalities in eight cases (47%), with two cases of gastric distension, five cases of gastric stasis, and one case of small bowel air-fluid levels. Sonographic examination of the pyloric region always confirmed IHPS, showing a mean pyloric muscle thick-
| No. | Age (days) | Sex (M/F) | Duration of symptoms (days) | Birth weight (kg) | Weight loss (g) | Gestation (weeks) | Delivery | Mother's parity | Breast feeding | Family history | Thickness/length of pyloric muscle |
|-----|------------|-----------|----------------------------|------------------|----------------|------------------|----------|----------------|---------------|---------------|----------------------------------|
| 1   | 27         | F         | 5                          | 3.400            | 120            | 38               | Cesarean | 2              | No            | No            | 4.0 mm/18.0 mm                   |
| 2   | 42         | F         | 31                         | 3.890            | 185            | 41               | Vaginal  | 1              | Yes           | Cardiac souffle | 4.0 mm/20.0 mm                   |
| 3   | 44         | M         | 7                          | 3.450            | 40             | 40               | Vaginal  | 2              | Yes           | Hypercholesterolemia             | 5.0 mm/20.0 mm                   |
| 4   | 31         | F         | 5                          | 2.860            | 100            | 37               | Vaginal  | 5              | No            | No            | 4.0 mm/16.0 mm                   |
| 5   | 32         | M         | 18                         | 3.360            | 130            | 39               | Vaginal  | 1              | Yes           | No            | 5.0 mm/28.0 mm                   |
| 6   | 58         | M         | 2                          | 2.200            | 300            | 33               | Vaginal  | 1              | Yes           | Pylonephritis               | 4.0 mm/15.0 mm                   |
| 7   | 34         | F         | 9                          | 2.140            | 200            | 38               | Vaginal  | 3              | No            | Colonic polyp   | 7.0 mm/30.0 mm                   |
| 8   | 36         | M         | 9                          | 3.120            | 0              | 40               | Vaginal  | 1              | Yes           | No            | 25.0 mm/5.0 mm                   |
| 9   | 33         | M         | 8                          | 2.990            | 230            | 39               | Vaginal  | 3              | Yes           | No            | 5.0 mm/20.0 mm                   |
| 10  | 31         | M         | 5                          | 3.220            | 140            | 38               | Vaginal  | 2              | No            | No            | 3.4 mm/16.0 mm                   |
| 11  | 69         | M         | 25                         | 3.280            | 600            | 40               | Vaginal  | 1              | No            | No            | 6.0 mm/28.0 mm                   |
| 12  | 20         | F         | 7                          | 3.460            | 100            | 41               | Vaginal  | 1              | No            | No            | 3.4 mm/16.0 mm                   |
| 13  | 63         | M         | 15                         | 2.690            | 400            | 39               | Vaginal  | 2              | No            | IHS            | 4.0 mm/19.0 mm                   |
| 14  | 63         | M         | 32                         | 3.590            | 600            | 40               | Vaginal  | 2              | No            | Asthma         | 5.0 mm/20.0 mm                   |
| 15  | 48         | M         | 7                          | 3.640            | 220            | 37               | Cesarean | 1              | No            | No            | 4.0 mm/20.0 mm                   |
| 16  | 39         | M         | 4                          | 3.880            | 0              | 39               | Vaginal  | 1              | Yes           | No            | 6.0 mm/15.0 mm                   |
| 17  | 53         | M         | 25                         | 4.000            | 200            | 40               | Vaginal  | 1              | No            | GER            | 4.5 mm/16.0 mm                   |
| 18  | 43         | F         | 11                         | 3.100            | 300            | 40               | Vaginal  | 1              | No            | IHS            | 5.7 mm/25.0 mm                   |
| 19  | 38         | M         | 4                          | 3.180            | 0              | 39               | Vaginal  | 2              | No            | No            | 3.6 mm/25.0 mm                   |
| 20  | 48         | M         | 13                         | 3.480            | 90             | 41               | Vaginal  | 2              | Yes           | No            | 5.0 mm/23.0 mm                   |
| 21  | 42         | M         | 9                          | 3.160            | 0              | 39               | Vaginal  | 1              | Yes           | No            | 5.0 mm/18.0 mm                   |
| 22  | 22         | M         | 8                          | 2.900            | 200            | 38               | Vaginal  | 1              | Yes           | No            | 4.5 mm/19.0 mm                   |
| 23  | 29         | M         | 3                          | 2.780            | 305            | 39               | Vaginal  | 1              | No            | No            | 5.0 mm/17.0 mm                   |
| 24  | 58         | M         | 20                         | 3.480            | 0              | 39               | Vaginal  | 3              | No            | No            | 5.0 mm/16.0 mm                   |
| 25  | 88         | M         | 17                         | 1.920            | 400            | 33               | Cesarean | 2              | No            | No            | 7.0 mm/28.0 mm                   |
| 26  | 33         | F         | 41                         | 3.720            | 0              | 39               | Vaginal  | 2              | No            | No            | 8.0 mm/25.0 mm                   |
| 27  | 38         | F         | 10                         | 3.210            | 500            | 39               | Vaginal  | 1              | No            | IHS            | 5.0 mm/18.0 mm                   |
| 28  | 22         | M         | 4                          | 3.280            | 30             | 39               | Vaginal  | 1              | No            | No            | 4.0 mm/18.0 mm                   |
| 29  | 31         | M         | 3                          | 3.100            | 400            | 40               | Vaginal  | 2              | No            | No            | 5.4 mm/19.0 mm                   |
| 30  | 28         | M         | 11                         | 2.960            | 170            | 36               | Vaginal  | 2              | No            | No            | 3.0 mm/12.0 mm                   |
| 31  | 51         | M         | 13                         | 3.500            | 100            | 39               | Vaginal  | 2              | No            | Hypertension | 5.0 mm/18.0 mm                   |

IHS, infantile hypertrophic stenosis; GER, gastroesophageal reflux.
ness of $4.9 \pm 1.1$ mm and a mean pyloric length of $20.4 \pm 4.6$ mm on longitudinal sections. Thickness and length of the pyloric muscle correlated weakly ($r = 0.54$; $P = 0.003$). Gestation was significantly shorter among infants with a pyloric thickness of less than 5 mm on sonography than in those with a thickness of 5 mm or more ($37.9 \pm 2.1$ vs. $39.2 \pm 1.7$ weeks; $P = 0.03$).

The main characteristics of the 31 patients and their mothers are shown in Table I.

The mean blood electrolyte levels were as follows: potassium $4.4 \pm 0.8$ mmol/L [$2.5–5.5$]; chloride $96.1 \pm 10.0$ mmol/L [$66–108$]; and bicarbonate $28.8 \pm 7.8$ mmol/L [$16–49$].

The parents were interviewed and asked about recent exposure to infectious contacts (Table II). A history of antibiotic therapy was only mentioned in one case. All virologic tests of ENT samples and pyloric specimens (pyloric muscle fragments and intra-operative swabs) were negative. The only positive samples were a stool specimen (ADV 3 detected by cell culture) and a vomit sample (EV detected by RT-PCR) (Table II).

**DISCUSSION**

The precise frequency of IHPS is poorly documented [Wang et al., 2008]. In Europe, an overall incidence of $2.0$ per $1,000$ live births has been reported, with a range of $0.86–3.96$ per $1,000$ [Pedersen et al., 2008].

The clinical and epidemiological characteristics of the patients were in keeping with those reported in the literature [Murtagh et al., 1992; Hernanz-Schulman, 2003; Wang et al., 2008], including some cases with a positive family history. A positive maternal history has been linked to a higher risk of IHPS in infants [Carter and Evans, 1969; Panteli, 2009]. The only noteworthy difference with other series is the lack of a seasonal pattern of IHPS in the patients in the present study [Webb et al., 1983]. Sonographic examination showed a larger muscle thickness and pyloric channel length than reported usually, ranging from $3.0$ to $4.5$ mm and $14$ to $20$ mm, respectively [Hernanz-Schulman, 2003].

The risk of IHPS has been linked to a variety of factors, with sometimes contradictory results [Panteli, 2009]. The main potential contributing factors are maternal treatment in early pregnancy with an anti-emetic [Aselton et al., 1984] or macrolide [Srøensen et al., 2003], pyloric muscle defects, such as smooth muscle cells [Guarino et al., 2000], increased growth factor expression [Jabłoński et al., 2006], abnormal distribution and organization of extracellular matrix proteins [Oue and Puri, 1999], defective pyloric innervation [Kobayashi et al., 2001], absence or rarity of interstitial cells of Cajal [Vanderwinden et al., 1996], and low nitric oxide synthase expression in fibers innervating the pyloric circular muscle, leading to decreased plasma nitrile levels [Huang et al., 2006]. However, to our knowledge, the only microbial pathogens to have been sought in this setting are *Helicobacter pylori* [Daeshan et al., 2006; Sherwood et al., 2007] and Epstein–Barr virus [Jenson et al., 2001]. *H. pylori* antigens were not detected by an enzyme immunoassay technique in series of 39 stool specimens from children with IHPS [Sherwood et al., 2007]. A recent study of gastric biopsies from 16 patients showed three cases of chronic active gastritis and six cases of mild focal gastritis [Daeshan et al., 2006]. However, histopathological examination and immunohistochemical staining revealed no bacteria, and the urease test was negative in all samples. Jenson et al. [2001] failed to detect EBV in smooth muscle cells from 10 patients with IHPS diagnosed clinically and histopathologically, by means of in situ hybridization of EBV-encoded RNA1 (EBER1).

The results described above argue against the involvement of a virus in IHPS. The case in which an enterovirus was detected by RT-PCR in a vomit sample which was negative by cell culture could be explained either by a small inoculum or by a non-culturable enterovirus. After oral infection, enteroviruses multiply with the oropharyngeal and intestinal mucosae, leading to abundant orofecal excretion of virions. Our case of PCR positivity was therefore probably fortuitous, especially as asymptomatic enterovirus infection is frequent in newborns [Tebruegge and Curtis, 2009]. Likewise, detection of an ADV 3 strain in the stools of one of the patients does not by itself suggest a role in IHPS, as ADV can be excreted for very long periods in the stools of newborns with no other signs of infection [Madeley, 1979]. This finding also appears to be incidental, although strains of serotype 3 can cause frequently fatal disseminated disease in previously healthy children [Munoz et al., 1998]. Thus, the negative results of all virological investigations of pyloric samples from the patients exclude the involvement of the viruses examined.

This study suggests that the principal viruses targeting the respiratory and digestive tracts are not responsible for IHPS in infants. However, larger studies

**TABLE II. Exposure to Infectious Contacts During Pregnancy (Mother) and After Birth (Infant); Virus Detection in Infant Samples**

| During pregnancy | Up to 15 days before admission | <15 days before admission | At admission | Virus-positive infant samples |
|------------------|-------------------------------|--------------------------|-------------|-------------------------------|
| 5 (16.1%)        | 4 (12.9%)                     | 7 (22.6%)                | 1 (3.2%)    | ENT 0 (0%) Vomit 1a (3.2%) Muscle 0 (0%) Stools 1b (3.2%) |

*Enterovirus.

†Adenovirus type 3.
including other viruses are needed to rule out definitively viral involvement in IHPS.

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