

# Materno-fetal transmission of human coronaviruses: a prospective pilot study

A. Gagneur · E. Dirson · S. Audebert · S. Vallet ·  
M. C. Legrand-Quillien · Y. Laurent · M. Collet ·  
J. Sizun · E. Oger · C. Payan

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**Abstract** This prospective pilot study investigates the possibility of materno-fetal transmission of human coronaviruses (HCoV) responsible for cases of neonatal infection. This vertical transmission was studied with 159 samples from mother-child couples: maternal vaginal (MV) and respiratory (MR) samples during labor; and newborn gastric sample (NG) with detection of HCoV (229E, OC-43, NL-63, HKU1) via real time RT PCR. HCoV was detected in 12 samples (229E: 11; HKU1: 1) from seven mother-child couples. For three couples, only MR tested positive (cases 1–3). For two other couples all three samples (MV, MR and NG) tested positive (cases 4 and 5). For case 6, only MV and NG tested positive. In case 7, only MV was positive. Possible vertical transmission of HCoV was hypothesized in this pilot study and requires further investigation on a larger scale.

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The experiments in this study comply with the current laws of France regarding clinical research.

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A. Gagneur (✉) · E. Dirson · S. Audebert · J. Sizun  
Department of Pediatrics, CHU Morvan,  
2 avenue Foch,  
29609 Brest cedex, France  
e-mail: arnaud.gagneur@chu-brest.fr

S. Vallet · M. C. Legrand-Quillien · C. Payan  
Department of Microbiology,  
EA 3882 Laboratory of Biodiversity and Microbial Ecology,  
Brest cedex, France

Y. Laurent · M. Collet  
Department of Gynecology-Obstetrics,  
Brest cedex, France

E. Oger  
Center for Clinical Investigation,  
INSERM CIC 0502, CHU Brest,  
2 avenue Foch, 29609 Brest cedex, France

## Introduction

Human coronaviruses (HCoV) are positive-stranded enveloped RNA viruses and were discovered by Tyrrel Bynoe in the 1960s [1]. Two serotypes, 229-E and OC-43, are responsible for up to one-third of common colds in adults [2]. Since the identification of a new coronavirus that is responsible for severe acute respiratory syndrome (SARS) [3], new technological tools have enabled the recent discovery of two new HCoV: NL-63 and HKU1 [4, 5].

Transmission of HCoV, like many other viruses with respiratory tropism (rhinovirus, respiratory syncytial virus, human metapneumovirus), is inter-human via rhinopharyngeal secretions [6]. We recently described the role of HCoV in cases of newborn nosocomial infection [7–10]. These viruses may be introduced into the hospital by personnel, visitors, or hospitalized children. A materno-fetal origin must nevertheless also be explored by reason of the frequency of this mode of transmission in animal models [11] and the existence of neonatal infections.

The primary objective of this study was to examine the materno-fetal transmission of human coronaviruses, which may explain the cases of neonatal infection observed in previous studies (unpublished data).

## Patients and methods

This prospective monocentric pilot study was conducted at Brest University Hospital during two epidemiological periods: from July 2003 to June 2004, and from March 2005 to August 2005 with inclusion of mother/child pairs admitted in labor to the Gynecology-Obstetrics Unit. This study was supported by funds from the French Ministry of

Health (Hospital Protocol of Clinical Research) and was approved by the ethics committee. Maternal and/or fetal emergency were considered as exclusion criteria.

Maternal vaginal swabs were taken for *Streptococcus B* detection, conforming to clinical routine. An additional sample was collected for research after written parental consent. Vaginal swabs and maternal nasal aspirates were performed at the beginning of labor; newborn gastric and rhino-pharyngeal secretions were collected during systematic aspiration at birth. All samples were placed in a viral culture medium and taken directly to the laboratory to be stored at  $-70^{\circ}\text{C}$ .

Two real-time RT-PCR assays were carried out for the simultaneous detection of HCoV 229E, OC43, and NL63, and of HCoV OC43, and HKU1, as previously reported by Gunson et al. and Kuypers et al. respectively [12, 13]. Each assay was initially assessed using positive controls. An ABI prism 7000 TaqMan (PE, Foster City, CA, USA) was used to amplify and quantify the amplification product after each cycle. Triplex RT-PCR was initially carried out to test for HCoV-229E, NL63, and OC43; a second real-time RT-PCR was subsequently performed with a set of primers/probe, detecting both HCoV-OC43 and HKU1 as described by Kuypers et al. [13]. In each technique, 5–7  $\mu\text{l}$  of RNA extract were added to a 25- $\mu\text{l}$  one-step reverse-transcription PCR using Superscript<sup>TM</sup> III Platinum<sup>®</sup> One-Step qRT-PCR SuperMix plus ROX (Invitrogen, Cergy Pontoise, France). Respective primers and probes were added to each of the two RT-PCR reactions at 10  $\mu\text{M}$  and 300 nM respectively. The thermocycling conditions were 15 min at  $50^{\circ}\text{C}$ , 2 min at  $95^{\circ}\text{C}$  followed by 40 cycles of 8 s at  $95^{\circ}\text{C}$  and 34 s at  $60^{\circ}\text{C}$ . The data were collected at the annealing step ( $60^{\circ}\text{C}$ ) of each cycle.

The swabs considered positive had a threshold cycle of 38 for OC43 and 37 for 229-E, respectively corresponding to a ID<sub>50</sub> of 1 and 0.2.

Analysis of results was performed using Epi-info software (CDC, USA; French version ENSP). Chi-squared test and Fisher's exact test were used to compare qualitative variables; Student's *t*-test, Wilcoxon test and analysis of variance (ANOVA) were employed for quantitative variables. Statistical difference was considered significant if  $p < 0.05$ .

## Results and discussion

One hundred and fifty-nine mother/infant pairs were studied, including 159 mothers and 161 infants (two sets of twins). Five mothers had a positive respiratory sample (incidence 3%). Among the 159 pairs, 7 presented at least one swab (respiratory, vaginal or gastric) that was positive for HCoV 229-E or HCoV-HKU1 (Table 1).

For 3 pairs, only the maternal respiratory swab tested positive, with no detection in the newborn (cases 1–3). In 2 pairs, all samples were positive: maternal respiratory (MR) and vaginal (MV) swabs and newborn gastric swabs (NG) (cases 4 and 5). In case 6, only the MV and the NG were positive, with no detection of HCoV in maternal respiratory specimens. In case 7, only the MV was positive.

In the 5 mothers with a positive respiratory swab, the vaginal sample was positive in 2 cases, as well as the newborn gastric swab. Only one case revealed a negative NG sample, despite a positive vaginal swab in the mother. Genital tropism of HCoV was thus found, with four vaginal swabs that were effectively positive, in association with a positive respiratory swab 50% of the time. Vaginal carriage of HCoV appears to be a significant element of transmission, as the 3 infants with a gastric swab positive for HCoV all had mothers with positive vaginal samples. One newborn among the 3 was delivered by cesarean: case 6 (MR-, MV+, NG+). Genital passage thus does not appear indispensable to transmission, leading to the hypotheses of ascending intrauterine transmission or transplacental viremia. However, no viremia has been reported in human coronavirus respiratory infection aside from SARS-CoV. Moreover, transmission to the infant is not consistent, as among the 7 mothers carrying the virus, more than half had infants whose gastric samples were negative.

The limited size of this pilot study does not allow for the formulation of conclusions regarding the symptomatology of HCoV infection in newborns. None of the aforementioned 3 neonates was clinically symptomatic. One had positive C-reactive protein (CRP) at 24 h of life, which was measured due to maternal vaginal carriage of *Streptococcus B*. He was asymptomatic and no other risk factor for materno-fetal infection existed. CRP was not measured for the other newborns. Another presented an icterus with negative etiological testing. No respiratory distress at birth was present in this study and no significant difference was noted in APGAR scores.

Human coronaviruses represent one of the principal viruses responsible for common colds in adults after rhinoviruses; thus, 4 out of 5 mothers with positive respiratory samples had colds on the day of delivery. However, the notion of having a cold during pregnancy does not seem to be relevant, as a predictive element of HCoV infection at delivery; 62% of mothers who reported a cold during pregnancy subsequently produced a negative respiratory sample. Hyperthermia was absent in infected mothers. No differences were observed between the groups with positive samples and those with negative samples, in terms of labor and delivery criteria (Table 2).

In sero-epidemiologic studies, Monto et al. [14] suggested that HCoV community infections occurred every 3 or 4 years, with serogroups alternating between OC43 and 229E. This

**Table 1** Cases of mother/newborn pairs with positive human coronavirus (HCoV) specimens detected in maternal respiratory and vaginal swabs and in newborn gastric swabs

	Mothers		Newborns
	Maternal respiratory (MR) (Ct)	Maternal vaginal (MV) (Ct)	Newborn gastric (NG) (Ct)
Case 1	HCoV-HKU1 (30.5)	–	–
Case 2	HCoV-229E (36)	–	–
Case 3	HCoV-229E (25)	–	–
Case 4	HCoV-229E (27)	HCoV-229E (29)	HCoV-229E (31)
Case 5	HCoV-229E (38)	HCoV-229E (31)	HCoV-229E (31)
Case 6	–	HCoV-229E (32)	HCoV-229E (33)
Case 7	–	HCoV-229E (37)	–

Seven pairs presented at least one swab that was positive for HCoV 229E (11 swabs) or HCoV-HKU1 (1 swab). Cycle threshold (Ct) values were noted in parentheses

study period perhaps corresponds to a higher incidence of serotype 229E in relation to other HCoV serotypes.

No published articles have put forth the existence of materno-fetal transmission of human coronaviruses. Verti-

cal transmission was not detected in pregnant women infected with SARS during the Asian epidemic of 2002–2003 [15]. Nevertheless, women infected during pregnancy had a higher incidence of miscarriage, premature delivery,

**Table 2** Maternal, delivery, and neonatal characteristics associated with an HCoV-positive gastric swab

Maternal and delivery characteristics	Positive newborn gastric swab, <i>n</i> =3	Negative newborn gastric swab, <i>n</i> =156	<i>p</i>
Age at delivery, years			
Mean (SD)	29 (2.6)	29.5 (5.4)	ns <sup>b</sup>
Range	27–32	16–45	
Gravida (median)	1 to 2 (2)	1 to 7 (2)	ns <sup>b</sup>
Para (median)	1 to 2 (2)	1 to 6 (1)	ns <sup>b</sup>
Rupture of membranes, hours, mean (SD)	8.6 (8.1)	12.8 (46.1)	ns <sup>b</sup>
Common colds during pregnancy, <i>n</i> (%)	3 (100)	97 (63)	ns <sup>a</sup>
Common cold week before delivery, <i>n</i> (%)	0 (0)	27 (17)	ns <sup>a</sup>
Common cold during delivery, <i>n</i> (%)	0 (0)	16 (10)	ns <sup>a</sup>
Fever week before delivery, <i>n</i> (%)	0 (0)	5 (3)	ns <sup>a</sup>
Fever during delivery, <i>n</i> (%)	1 (33)	10 (6)	ns <sup>a</sup>
Toxemia, <i>n</i> (%)	0 (0)	6 (4)	ns <sup>a</sup>
Threatening premature delivery, <i>n</i> (%)	0 (0)	13 (8)	ns <sup>a</sup>
Eclampsia, <i>n</i> (%)	0 (0)	2 (1)	ns <sup>a</sup>
Meconial liquid, <i>n</i> (%)	0 (0)	25 (16)	ns <sup>a</sup>
Mode of delivery, <i>n</i> (%)			
Vaginal	2 (66)	129 (83)	ns <sup>a</sup>
Cesarean section	1 (33)	27 (17)	
Neonatal characteristics			
Gestational age, weeks (SD)	40.7 (1.5)	39.6 (2)	ns <sup>b</sup>
Apgar score, median, (SD)			
1 min	9 (1.5)	10 (2)	ns <sup>b</sup>
5 min	10 (0)	10 (0.6)	
Birth weight, g, mean (SD)	3,903 (92)	3320 (545)	0.02 <sup>b</sup>
Male (%)	3 (100)	76 (49)	ns <sup>a</sup>
Intubation at birth, <i>n</i> (%)	0 (0)	3 (2)	ns <sup>a</sup>
Ventilation at birth, <i>n</i> (%)	0 (0)	9 (6)	ns <sup>a</sup>
Respiratory distress syndrome, <i>n</i> (%)	0 (0)	11 (7)	ns <sup>a</sup>
C- reactive protein > 10 mg/l at 24 h, <i>n</i> (%)	1 (33)	3 (2)	ns <sup>a</sup>
Feeding issues in the first 3 days of life, <i>n</i> (%)	0 (0)	15 (10)	ns <sup>a</sup>
Icterus issues in the first 3 days of life, <i>n</i> (%)	1 (33)	36 (23)	ns <sup>a</sup>
Maternal vaginal swab HCoV positive, <i>n</i> (%)	3 (100)	1 (1)	< 0.001 <sup>a</sup>
Maternal respiratory swab HCoV positive, <i>n</i> (%)	2 (67)	3 (2)	0.01 <sup>a</sup>

<sup>a</sup> Fisher exact test, <sup>b</sup> Wilcoxon test

and stunted growth [16]. On the other hand, vertical transmission was shown in enteroviruses responsible for sometimes severe neonatal infections [17].

If no materno-fetal transmission has been observed in humans, this mode of contamination is well-known among veterinary services. Numerous coronaviral strains have been isolated in different animals, each virus being named as a function of its host and possible associated pathology: avian infectious bronchitis virus (IBV), mouse hepatitis virus (MHV), bovine coronavirus (BCV), transmissible gastroenteritis virus of swine (TGEV), and rat coronavirus (RCV), among others [11]. These coronaviruses are responsible for sporadic infections and seasonal outbreaks among breeders. Adult animals present limited or unapparent infection and transmit the virus to newborns, which then show a far more severe pathology. The majority of coronaviral strains are excreted in respiratory secretions and feces, sources of post-natal transmission. However, certain strains can replicate in the macrophages, lymphocytes, hepatocytes, neurons, endothelial cells or in the urogenital tract, which can result in materno-fetal infections [11]. Experimental infections with the mouse hepatitis virus resulted in fetal death or neonatal infection [18]. Transmission of MHV in utero following oronasal or intravenous inoculation of gestating mice was found by Barthold et al. However, this vertical transmission of MHV occurred in different percentages depending upon MHV strain and host genotype. This could explain our detection of only two HCoV-229E infections in newborns. The rat coronavirus (RCV) infects the respiratory epithelium and the lachrymal glands, but also the genital tract of females, causing perturbations of the hormonal cycle, miscarriage, and neonatal mortality. The IBV strain infects the oviduct in chickens and perturbs egg production [2].

The possibility of materno-fetal transmission of HCoV was suggested in this pilot study, requiring further investigation on a larger scale. It is advisable to analyze the genomic profile of the HCoV detected in the three positive mother/infant pairs.

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