Article/Artigo

# Gastroenteric virus detection in fecal samples from women in Goiânia, State of Goiás, Brazil

## Detecção de vírus gastroentéricos em amostras fecais de mulheres em Goiânia, Estado de Goiás, Brasil

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**ABSTRACT**

**Introduction:** This was a prospective study that included women seen in the obstetrics and gynecology sector of Hospital das Clínicas, Federal University of Goiás, in Goiânia, State of Goiás, with the aim of detecting rotaviruses, adenoviruses, caliciviruses and astroviruses. Eighty-four women participated in the study and from these, 314 fecal samples were collected. Out of all of the women, 29 were seropositive for HIV and 55 were seronegative, and 45 and 39 were pregnant and non-pregnant, respectively. **Methods:** Fecal samples were collected from each woman once every two months over the period from July 2006 to June 2007, and they were screened for rotaviruses by means of polyacrylamide gel electrophoresis and immunoenzymatic assays, for caliciviruses and astroviruses by means of RT-PCR and for adenovirus by means of immunoenzymatic assays. The astroviruses were genotyped using nested PCR. **Results:** Among the 84 patients, 19 (22.6%) were positive for either calicivirus (14/19) or astrovirus (6/19), while one women was positive for both viruses in fecal samples collected on different occasions. Most of the positive samples were collected during the months of July and August (astrovirus) and September and October (calicivirus). None of the samples analyzed was positive for rotavirus or adenovirus. Gastroenteric viruses were detected in 13/19 (68.4%) of the pregnant women, whether HIV-seropositive or not. **Conclusions:** The results from the present study showed that neither pregnancy nor HIV-seropositive status among the women increased the risk of infection by any of the gastroenteric viruses studied. This study presents data on gastroenteric virus detection among pregnant and/or HIV-positive women.

**Key-words**: Gastroenteric viruses. Calicivirus. Astrovirus. HIV-positive. Pregnant women.

**RESUMO**

**Introdução:** Este foi um estudo prospectivo que incluiu mulheres atendidas no setor de obstetrícia e ginecologia do Hospital das Clínicas da Universidade Federal de Goiás, em Goiânia, Estado de Goiás com o objetivo de detectar rotavírus, adenovírus, calicivírus e astrovírus. Oitenta e quatro mulheres participaram no estudo e destas, 314 amostras fecais foram coletadas. Do total de mulheres, 29 eram soropositivas para HIV, 55 soronegativas, 45 e 39 estavam grávidas e não-grávidas, respectivamente. **Métodos:** Amostras fecais foram coletadas de cada mulher uma vez a cada dois meses pelo período de Julho-2006 a Junho-2007, foram triadas para rotavírus pela metodologia de eletroforese em gel de poliacrilamida (EGPA) e através de ensaio imunoenzimático (EIE), para calicivírus e astrovírus por RT-PCR e por EIE para adenovírus. Os astrovírus foram genotipados por Nested-PCR. **Resultados:** De 84 pacientes, 19 (22,6%) foram positivas para calicivírus (14/19) ou astrovírus (6/19), sendo que uma mulher foi positiva para ambos os vírus em amostras fecais coletadas em diferentes ocasiões. A maioria das amostras positivas foi coletada no período de Julho a Agosto (astrovírus) e de Setembro a Outubro (calicivírus). Nenhuma das amostras analisadas foi positiva para rotavírus ou adenovírus. Os vírus gastroentéricos foram detectados em 13/19 (68,4%) mulheres grávidas, as quais eram HIV-soropositivas ou não. **Conclusões:** Os resultados do presente estudo mostram que nem o estado gravídico das mulheres nem a soropositividade para HIV aumentaram o risco para a infecção por nenhum dos vírus gastroentéricos estudados. Este estudo apresenta dados sobre a detecção de vírus gastroentéricos entre mulheres grávidas e/ou HIV-positivas.

**Palavras-chaves:** Virus gastroentéricos. Calicivírus. Astrovírus. HIV-positivo. Mulheres grávidas.

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## INTRODUCTION

Acute gastroenteritis is a major public health issue around the world, and is associated with high morbidity-mortality rates, especially among children. Gastroenteric viruses constitute the single most important cause of acute gastroenteritis in children up to five years of age, worldwide[1,2]. Among the more than twenty known gastroenteric viruses, the most important etiological agents of acute gastroenteritis are rotaviruses, enteric adenoviruses, astroviruses and human caliciviruses[3-5].

Elderly and immunosuppressed individuals are among those with the highest susceptibility to infection and reinfection by gastroenteric viruses[6-9]. It is also believed that pregnant women and HIV-seropositive individuals are more vulnerable to viral infections than is the general population[7,10].

We decided to conduct this study because of the epidemiological importance of viral gastroenteritis and the assumption that pregnant women, whether HIV-seropositive or not, could constitute a group potentially at risk of viral infection and the development of gastroenteric disease, and also due to the scarcity of information on the incidence of gastroenteric viruses among the adult female population

Our study presents novel data on gastroenteric viruses (astroviruses and caliciviruses) among adult pregnant women (either HIV-seropositive or not) from the central-western region of Brazil.

## METHODS

This prospective study aimed to detect the prevalence rate of gastroenteric viruses (rotavirus, adenovirus, astrovirus and calicivirus) among women who were either pregnant or non-pregnant and either HIV-seropositive or HIV-seronegative. They were all seen at the obstetrics and gynecology (Ob-Gyn) service of Hospital das Clínicas, Federal University of Goiás (HC-UFG), in Goiânia, between July 2006 and June 2007.

From sample size analysis, the appropriate sample size for this study was estimated to be 84 women (p = 0.24; significance level of 5%, and 9% error). Therefore, 84 women were selected to participate in this study, out of all of the women who were seen at the ObGyn service of HC-UFG over a one-year period. The women had spontaneously sought assistance or had been referred for consultation at the selected services. In accordance with the predefined criteria for inclusion and continuation in the groups, women with chronicle gastrointestinal conditions that could progress to diarrhea episodes (Crohn's disease, Lupus or irritable bowel syndrome) were excluded from the study. All the participants confirmed their participation in the study by signing a written consent form, and the study was approved by the Ethics Committee for Human and Animal Medical Research of Hospital das Clínicas, Federal University of Goiás.

A data collection system provided the database for identifying the patients (age, obstetric history and present pregnancy) and for information about previous diarrhea episodes and HIV viral load. The CD4+ and CD8+ T cell counts of the HIV-positive women who were positive for any of the gastroenteric viruses studied were also recorded.

Fecal samples were collected every two months over a 12-month period, and at the time of every diarrhea episode, in appropriate plastic receptacles, at HC-UFG or in patients' homes when necessary. The samples were transported in ice and delivered to the virology laboratory of the Institute of Tropical Pathology and Public Health of the Federal University of Goiás, where the material was stored at -20°C until further processing.

The fecal samples were initially processed to obtain a 20% solution in phosphate-buffered saline (PBS; pH 7.4). The fecal suspensions were then homogenized and clarified by centrifugation at 9,000rpm for 10 minutes. The supernatant were then stored at -20°C, until further testing.

To detect rotavirus A (RVA), polyacrylamide gel electrophoresis (PAGE) was performed as described by Pereira et al[11], and the gels were stained using silver nitrate[12]. The samples were also screened for RVA using a commercial immunoenzymatic assay (RIDASCREEN® Rotavirus), following the manufacturer's instructions (R-Biopharm, Germany). To detect adenovirus, a commercial immunoenzymatic assay (RIDASCREEN® Adenovirus) was used in accordance with the manufacturer's instructions (R-Biopharm). To detect calicivirus and astrovirus, the viral RNA was first extracted as described by Boom et al[13], with modifications[14], and the samples were screened for calicivirus by means of reverse-transcriptase polymerase chain reaction (RT-PCR) using the primer pairs Ni/E3[15] and 289/290[16], and for astroviruses using the primer pair Mon 269/270[17]. Astrovirus-positive samples were further genotyped by means of nested PCR, as described by Sakamoto et al[18]. To determine possible associations between positive findings of gastroenteric viruses, pregnancy and/or HIV-seropositivity, Fisher's exact test was applied at a significance level of 0.05.

## RESULTS

A total of 314 fecal samples were collected from 84 women who were seen at the Ob-Gyn service of HC-UFG, between July 2006 and June 2007. These women were distributed into four groups characterized as follows: HIV-seropositive women (pregnant and non-pregnant) and HIV-seronegative women (pregnant and non-pregnant). **Table 1** shows the distribution of the women regarding clinical characteristics. It was found that out of the 84 women, 45 were pregnant and HIV-positive.

**TABLE 1- Distribution of the women seen at the Ob-Gyn service of HC-UFG between June 2006 and July 2007, according to the sampling groups.**

| | Pregnant | | Non-pregnant | | Total | |
|---|---|---|---|---|---|---|
| HIV | n | % | n | % | n | % |
| Positive | 19 | 42.2 | 10 | 25.6 | 29 | 34.5 |
| Negative | 26 | 57.8 | 29 | 74.4 | 55 | 65.5 |
| **Total** | **45** | **100.0** | **39** | **100.0** | **84** | **100.0** |

P = 0.053

In terms of age distribution, 43.7% of the women were up to 30 years of age, five (7.8%) were considered teenagers (up to 19 years of age), 32.8% were aged between 20 and 30 years and 23.5% were more than 40 years old. No statistical difference (p = 0.053) was found between the groups of HIV-seropositive women and HIV-seronegative women, in relation to their pregnancy status.

Nineteen (22.6%) of the 84 women participating in the study had at least one fecal sample that was positive for either astroviruses or caliciviruses. Thus, out of the total of 314 fecal samples collected, 33 (10.5%) were positive for either calicivirus or astrovirus. Caliciviruses were detected in 14 (16.6%) women and astrovirus in six (7.1%) women. One woman was positive for astrovirus and calicivirus in different samples collected on different occasions. RVA and adenovirus were not detected in any of the fecal samples collected.

The results in **Table 2** show that 68.4% (13/19) of the women who were positive for gastroenteric viruses were pregnant (six were HIV-seropositive and seven were seronegative), although no statistical difference was observed when this group was compared with the group of women who were positive for gastroenteric viruses and were HIV-seronegative but were not pregnant (p = 0.199).

**TABLE 2 - Distribution of the women seen at the Ob-Gyn service of HC-UFG between July 2006 and June 2007, according to immunological status (pregnant and/or HIV-seropositive) and positive findings of gastroenteric viruses.**

| Pregnant/HIV-positive | Gastroenteric viruses | | | |
|---|---|---|---|---|
| | yes | | No | |
| | n | % | n | % |
| Yes | 13 | 68.4 | 41 | 63.1 |
| No | 6 | 31.6 | 24 | 36.9 |
| Total | 19 | 100.0 | 65 | 100.0 |

p = 0.199

Among the 19 women that were positive for gastroenteric viruses, 13 had only one positive fecal sample and six had more than one positive fecal sample collected at different times, as observed in **Table 3**. One of these women had three fecal samples, collected with two-month intervals between them, which were positive for gastroenteric viruses, and another woman had two positive fecal samples, with a six-month interval between them.

The circulation patterns of calicivirus and astrovirus were characterized by detection peaks during the months of June and August, for astrovirus, and during the months of September and October for calicivirus.

Genotyping of the six astrovirus-positive samples revealed predominance of genotype 1 (66.6%), followed by genotypes 3 and 8 (16.7% each).

Out of the 19 patients who had at least one fecal sample positive for calicivirus or astrovirus, three (15.8%) had diarrhea episodes frequently, one was HIV-seropositive and was pregnant and two were

**TABLE 3 - Distribution of the gastroenteric viruses in the various samples collected from the women seen at the Ob-Gyn service of HC-UFG between July 2006 and June 2007.**

| | Gastroenteric viruses | | | | | |
|---|---|---|---|---|---|---|
| | calicivirus | | astrovirus | | Total | |
| Sample | n | % | n | % | n | % |
| First | 1 | 7.1 | 4 | 66.7 | 5 | 25.0 |
| Second | 3 | 21.4 | 0 | 0,0 | 3 | 15.0 |
| Third | 1 | 7.1 | 2 | 33.3 | 3 | 15.0 |
| Fourth | 2 | 14.3 | 0 | 0.0 | 2 | 10.0 |
| Fifth | 1 | 7.1 | 0 | 0.0 | 1 | 5.0 |
| First and second | 1 | 7.1 | 0 | 0.0 | 1 | 5.0 |
| First and third | 2 | 14.3 | 0 | 0.0 | 2 | 10.0 |
| Second and fifth | 2 | 14.3 | 0 | 0.0 | 2 | 10.0 |
| First/second/third | 1 | 7.1 | 0 | 0.0 | 1 | 5.0 |
| **Total** | **14** | **100.0** | **6** | **100.0** | **20** | **100.0** |

HIV-seronegative and pregnant. Among the 65 women who did not have any positive fecal samples for gastroenteric viruses, four (6.2%) had sporadic diarrhea episodes (three were HIV-seropositive, of whom two were pregnant and one was non-pregnant, and the fourth patient was non-pregnant and HIV-seronegative).

The HIV viral load was accessed at the time of the first fecal sample collection, and was found to be undetectable (< 50 copies/cell) in five (71.4%) of the women who were HIV-seropositive (either positive or negative for gastroenteric viruses). From analysis on the cell immune status data of the 19 women for whom at least one fecal sample was positive for gastroenteric viruses, it was observed that the CD4+ T cell counts were low in only five (26.3%) of them.

## DISCUSSION

In the present study, calicivirus and astrovirus were detected in fecal samples collected from 84 adult women. Data from the literature show that the detection rates for these viruses among children with gastroenteritis in Brazil vary between 12 and 47%[4,14,19-21]. However, there is no information available on the prevalence of these agents among the adult female population in Brazil.

It is known that in developing countries, infection by gastroenteric viruses, especially rotavirus, adenovirus and astrovirus, usually occurs during childhood, and mainly among children under five years of age[3,2,21-24]. Caliciviruses infect people of all ages and constitute important etiological agents for diarrhea, while noroviruses are the main causative agents of non-bacterial epidemic acute gastroenteritis, worldwide[5]. Although uncommon, infection by gastroenteric viruses can generally also occur among adults, especially elderly and immunosuppressed individuals[6-8]. Acute gastroenteritis due to astrovirus has been reported in immunosuppressed (transplanted or HIV-seropositive) adults and children[9,25]. Walter & Mitchell also reported positive findings of these viruses in nurseries[23].

In our study, the only gastroenteric viruses found in the adult female population were calicivirus and astrovirus, while rotavirus or adenoviruses were not detected in any of the samples collected. Additionally, none of the fecal samples collected from the youngest group of women (teenagers) were positive for any of the viruses studied. The reasons for this epidemiological pattern have not yet been elucidated, and require further investigation. From analysis on virus positivity according to the women's ages, we observed that the women with positive findings were predominantly aged 20-30 years, followed by 40-50 years, with prevalence rates a little higher than those detected for calicivirus[4,20] and astrovirus[14,19] in children with acute gastroenteritis under five years of age.

Regarding virus seasonality, caliciviruses and astroviruses were mainly detected during the rainy season in our region. This circulation pattern corroborates the seasonality data obtained from other studies in the region, which have shown higher incidence rates for astroviruses and caliciviruses during periods with elevated relative air humidity[19,20].

The focus on public health and preventive measures (even when treatment is not available) has given greater visibility to pregnant women (whether HIV-seropositive or not), because of the greater vulnerability of their immune system[10]. The inclusion of HIV-seropositive women in this study was justified by the assumption that this condition is associated with impaired immune status that could consequently predispose such individuals to infection by other pathogens, such as gastroenteric agents.

Although our results suggested that there was an association between HIV-seropositivity and/or pregnancy and positive findings of calicivirus and astrovirus (68.4%), especially astroviruses, this was not statistically significant. We hypothesize that the immunological impairment of these HIV-positive women may have been attenuated by the antiretroviral therapy and clinical follow-up, considering that more than one third of these women presented undetectable viral load and CD4+ cell counts higher than 250 cells/mm$^3$. The fecal samples were obtained at two-month intervals (up to five samples per women), and all of them were screened for rotavirus, adenovirus, calicivirus and astrovirus.

For some women, more than one sample was positive for the same agent, while for one woman, more than one sample collected at different times was positive for calicivirus and astrovirus. It is known that gastroenteric viruses such as noroviruses can be excreted by immunocompetent individuals for more than two weeks after infection[24]. Additionally, the immunosuppressive state induced during pregnancy may contribute towards higher susceptibility or even reactivation of viral infection[10].

In addition, positive findings of calicivirus in different samples from the same women, collected up to six months apart, could be due to reinfection by a different calicivirus genotype. Genotyping and sequencing of the samples might have helped elucidate this matter, but this could not be performed in the present study because of to the low RNA concentration in the samples.

The genomic characterization of the astrovirus detected in this study revealed predominance of genotype 1, which corroborates with data from the literature showing that serotypes 1 and 2 are the most prevalent worldwide[18,21,23,25]. In a study conducted in Goiânia in 2002, Cardoso et al. observed that the overall prevalence of genotype 1 astrovirus in children with acute diarrhea, was 2.8%[14]. In a more recent study, Silva et al detected genotype 1 in 42.8% of the positive samples, followed by genotype 2 (23.2%) and genotype 4 (14.3%)[14]. Santos et al reported similar astrovirus detection rates (3%) among children less than five years of age who were hospitalized due to acute gastroenteritis in Goiânia and Brasília (7%)[19]. In another study, Andreasi et al[4] reported a detection rate of 3.1% for astrovirus, among children in Campo Grande (MS), and found that out of the total of 10 samples, six belonged to serotype 1[4]. It is important to note that

although serotype 1 was the most prevalent in our study, samples of serotypes 3 and 8 were also detected.

The clinical follow-up on the women enrolled in our study did not reveal any clinical peculiarities of relevance when we compared the women who were positive for gastroenteric viruses with those who were not. However, a higher frequency of diarrhea episodes was observed among the women who were positive for gastroenteric viruses (15.8%), compared with those who were negative (6.2%).

Considering the close contact between the mothers and their newborns, it is recommended that mothers should maintain good personal hygiene and handle the baby carefully, especially among women who are infected with gastroenteric viruses, so that virus transmission to the baby is prevented.

In analyzing positive findings of gastroenteric viruses among pregnant women, not only the immunological vulnerability of the gestational process but also the possibility of vertical transmission of these agents should be taken into consideration. We believe that our findings and will also help to elucidate some aspects of the epidemiological features of these agents, which are important causes of morbidity-mortality worldwide, especially among children.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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