

# Epidemiological, serological and molecular aspects of hepatitis B and C in children and teenagers of municipal daycare facilities schools and schools in the city of Santos

*Aspectos epidemiológicos, sorológicos e moleculares das hepatites B e C em crianças e adolescentes de creches e escolas municipais na cidade de Santos*

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**ABSTRACT:** *Objective:* To estimate the prevalence of the serological markers anti-HBc, HBsAg and anti-HBs of hepatitis B and anti-HCV of hepatitis C among children and teenagers enrolled at daycare facilities, kindergartens and municipal elementary education network in the city of Santos, São Paulo, Brazil. *Methods:* A cross-sectional study was carried out from June 28 to December 14, 2007, in which 4,680 finger-prick blood samples were collected from children and teenagers. A survey questionnaire was applied to their family members. The sample was dimensioned using the software Epi Info version 6 with expected frequency of 1%, acceptable error of 0.5% and confidence interval of 95%. The serological tests were performed using the ELISA technique. The molecular analysis was performed using the technique of polymerase chain reaction *in House*. *Results:* Age of the studied population ranged from 7 months to 18 years and 1 month. The general prevalence of anti-HBc reagent was 0.1%, HBsAg was 0.02% and anti-HCV was 0.02%. *Conclusions:* In children, the general prevalence of serological markers for hepatitis B and C in the city of Santos was low when compared with literature data.

**Keywords:** Prevalence. Hepatitis B. Hepatitis C. Child. Epidemiology. Serology.

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**Conflict of interests:** nothing to declare – **Financing source:** São Paulo Research Foundation (FAPESP), Process n. 06/599728.

**RESUMO:** *Objetivo:* Estimar a prevalência de marcadores sorológicos anti-HBc, AgHBs e anti-HBs da hepatites B e anti-HCV da hepatite C em crianças e adolescentes matriculados em creches e escolas de ensino infantil e fundamental da rede municipal na cidade de Santos, São Paulo. *Métodos:* Estudo transversal realizado no período de 28 de junho a 14 de dezembro de 2007, no qual foram coletadas 4.680 amostras de sangue colhidas através de punção capilar. Foi aplicado um questionário nos familiares das crianças e adolescentes. Para o cálculo da amostra, foi utilizado o programa Epi Info versão 6 com frequência esperada de 1%, erro aceitável de 0,5% e nível de confiança de 95%. Os exames sorológicos foram realizados utilizando a técnica de ELISA. O estudo molecular foi realizado pela técnica de reação em cadeia de polimerase *in House*. *Resultados:* A idade da população estudada variou de 7 meses a 18 anos e 1 mês. A prevalência geral do anti-HBc reagente foi de 0,1%, do AgHBs foi de 0,02% e do anti-HCV foi de 0,02%. *Conclusão:* A prevalência geral em crianças dos marcadores sorológicos para hepatites B e C na cidade de Santos foi baixa, quando comparada com os dados de literatura.

*Palavras-chave:* Prevalência. Hepatite B. Hepatite C. Criança. Epidemiologia. Sorologia.

## INTRODUCTION

According to the World Health Organization (WHO), 170 million people have chronic hepatitis C, and 350 million people have chronic hepatitis B<sup>1</sup>. In Brazil, the estimation of people with chronic hepatitis B is of approximately 600 thousand, and those with chronic hepatitis C account for 1.5 million<sup>1</sup>.

The hepatitis B virus (HBV) is classified in the HepaDNA family, and its natural reservoir are men and women<sup>2</sup>. Sexual transmission is prevalent in hepatitis B<sup>2</sup>, even though it can also be parenteral, horizontal (close relationship in the family), vertical and, less often, it can be caused by the contamination with body liquids containing the agent<sup>1,2</sup>.

The hepatitis C virus (HCV) belongs to the Hepacivirus gender, and the Flaviridae family. Its genome is constituted of a single strand RNA. The most common genotypes in Brazil are 1, 2 and 3<sup>2</sup>. Among children, up to 1992, parenteral transmission was the most prevalent one for hepatitis C and, after this period, the vertical transmission prevailed. In a significant percentage of cases, it is not possible to identify the form of viral acquisition<sup>3</sup>. Sexual transmission is not very frequent, reaching less than 3% among stable partners; it mostly affects people with multiple partners who do not use protection<sup>3-7</sup>. The vertical transmission of hepatitis C is rare when compared to hepatitis B<sup>4,8</sup>.

The city of Santos, in the state of São Paulo, has most of the studies about the seroprevalence of hepatitis conducted with non-representative population samples, or with adults who had higher chances of acquiring the disease. A study conducted in this city, during three different periods (1992 to 1993; 1994 to 1996; and 1999), involving adults with risk behaviors for the transmission of

the human immunodeficiency virus (HIV) and users of injectable drugs, showed seroprevalence of hepatitis B of 75, 71 and 51%, and of hepatitis C, of 75, 77 and 44%, respectively<sup>9</sup>. In 2004, in the same city, adults infected with HIV showed seroprevalence of hepatitis C of 36.2%<sup>10</sup>. In 2008, 98 blood samples of students aged 4 to 14 years old in the municipal network of Santos showed prevalence of anti-HBc of 2.8%; AgHBs, 1.3%; and anti-HVC, 2.8%<sup>11</sup>.

Santos is the largest city in the coast of São Paulo, and also one of the oldest ones in the country. It is an important tourist spot, and holds the most important port in Latin America. The young population of Santos includes about 97,820 children and adolescents aged between 0 and 19 years old<sup>12</sup>. The city has almost half a million inhabitants, and medical care resources are focused on the Unified Health Systems, besides private health insurance plans. It does not have a representative evaluation about the prevalence of antibodies against hepatitis B and C among children and adolescents.

The objective of this study is to estimate the prevalence of antibodies anti-HBc IgC, anti-HBs and antigen HBs, as well as the antibody anti-HCV among children and adolescents enrolled in day care institutions, kindergartens and the municipal elementary education network in the city of Santos, by using blood withdrawal and filter paper. It also aims at knowing the molecular aspects and the form of acquisition of the hepatitis B and C viruses in cases in which serology is positive.

## METHODOLOGY

This is a cross-sectional study conducted from June 28 to December 14, 2007, when 4,680 finger prick blood samples were collected. Besides, a survey was applied to the families of children and adolescents enrolled in day care institutions, kindergartens and municipal elementary schools from the city of Santos.

The sample was calculated for 29,589 students, being 2,050 from the day care; 6,079, from the kindergarten; 16,026, from elementary school, more specifically from the 1<sup>st</sup> to the 4<sup>th</sup> grades; and 5,434 adolescents from the 5<sup>th</sup> to the 8<sup>th</sup> grades. The Epi Info software, version 6, was used (November, 1996), and for each educational stratum, a sample was calculated with expected frequency of 1%, acceptable error of 0.5% and 95% confidence interval. The total sample was comprised of 4,680 students, being 880 from day care facilities; 1,220 from kindergarten; 1,390 from elementary school, 1<sup>st</sup> to 4<sup>th</sup> grades; and 1,190 from elementary school, 5<sup>th</sup> to 8<sup>th</sup> grades.

Ten day care institutions, ten kindergartens, ten elementary schools from the 1<sup>st</sup> to the 4<sup>th</sup> grades and ten elementary schools from the 5<sup>th</sup> to the 8<sup>th</sup> grades were selected. In each school unit, classrooms and the number of students were raffled, always similarly, until the total number of the sample was reached. After not attending to 3 requests to participate in the study, 482 students (10.3%) were excluded, so new raffles were necessary.

Data were collected regarding identification, age, sex and date of birth of the student, as well as level of education, parents' profession, family income, personal and family history, physical environment, besides the observation of the vaccination card.

Two nursing assistants were trained for blood withdrawal. The technique consisted of a finger puncture made with an appropriate disposable lancet. After collection, the material was placed on a filter paper (Whatman – 1004917) containing 2 circles of 2.5 centimeters in diameter. In the laboratory, the paper was separated in a proper buffer for the posterior conduction of serological tests, with the ELISA technique. For hepatitis B, the following were conducted: AgHBs, anti-HBs and antiHBc and for hepatitis C, anti-HCV.

When the results were undetermined and/or reagents, new venous blood samples were collected, in a gel tube, without anticoagulants, in the patients' houses, being transported on the same day to the laboratory, under refrigeration. The members of the families were also summoned, and, after approval, their samples were collected.

In positive cases of AgHBs, the qualitative detection of DNA from the hepatitis B virus was conducted, by amplifying the S fragment of the B virus by the in-house polymerase chain reaction (PCR), with lower detection limit of  $3 \times 10^2$  copies/mL. The adopted model was the nested PCR, described by Kaneko et al.<sup>13</sup>, in 1989. The primers for the C region generate products of 250 base pairs (bp), according to Kaneko et al.<sup>13</sup>, and those in the S region result in products of 450 bp, according to Sitnik et al.<sup>14</sup>. The positive control was composed of known serum, and negative, of water.

For positive anti-HCV cases, the qualitative reaction of RNA from the hepatitis C virus was performed by amplifying the fragment 5'NCR with the PCR technique, with detection limit of  $10^3$  copies/mL.

Serological and molecular examinations were conducted in the Laboratory of Hepatitis at Instituto Adolfo Lutz, in São Paulo.

Data analysis was conducted with the software Epi Info, version 6 (November, 1996). Proportions were compared through the Mantel-Haenszel  $\chi^2$  test.

In order to be operational, the project was presented to the principals of the selected day care institutions and schools, after the approval by the Ethics Committee for the Analysis of Research Projects in the Clinical Board of Hospital das Clínicas and the Medical School of *Universidade de São Paulo*, by the Ethics Committee from the Secretariat of Health in Santos and by the Secretariat of Education in Santos. The selected students were summoned, as well as their parents, with a vaccination card. The Informed Consent Form of the research was presented to the parties in charge of the children and adolescents and, after approval, data collection began.

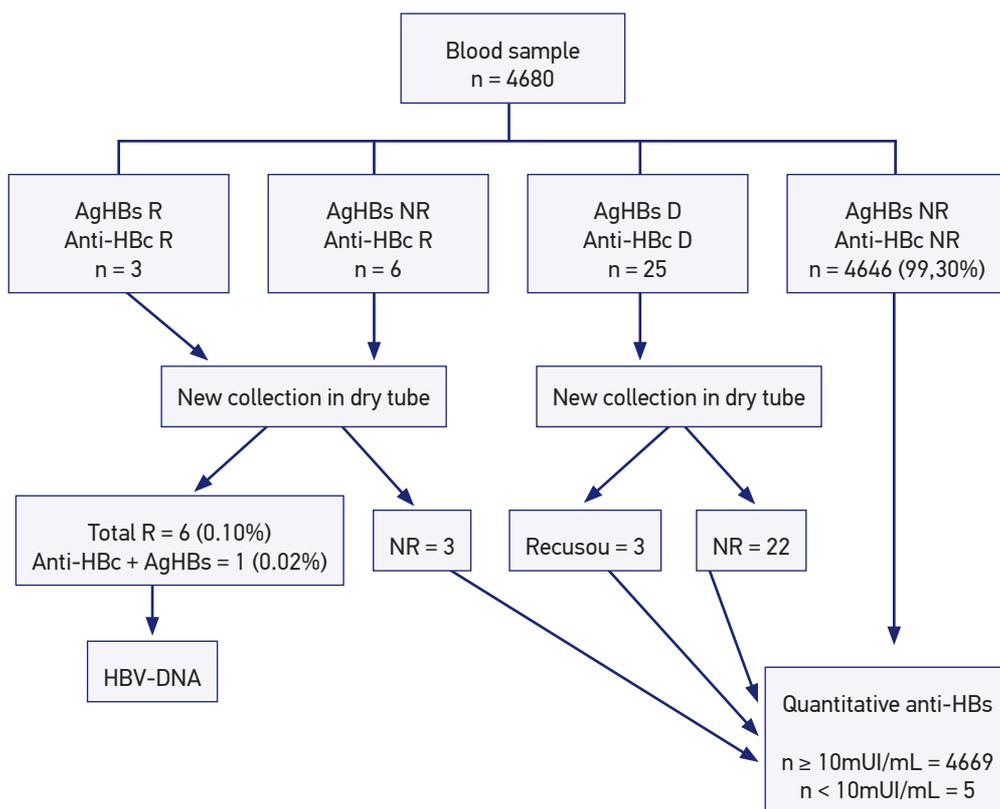
## RESULTS

The age of the study population ranged from 7 months old to 18 years and 1 month old. The median age in years and interquartile intervals in the different education groups, for day care participants, was of 2.5 (2 – 3.2); for kindergarten, 5.3 (4.5 – 6.1); for elementary school, from 1<sup>st</sup> to 4<sup>th</sup> grades, 9.2 (8.2 – 10.2); and for elementary school, from 5<sup>th</sup> to 8<sup>th</sup> grades, 12.5 (11.5 – 13.8).

## HEPATITIS B SEROLOGY

Out of the 4,680 finger prick blood collections, 4,646 (99.3%) were negative for anti-HBc and agHBs; 9 (0.2%) were reagent, being 6 isolated anti-HBc, 4 reagent antiHBc and AgHBs; and 25 were dubious (0.5%). New samples were collected for 22/25 dubious cases (three refused to), and results were negative for all hepatitis B markers. Out of the 9 cases of positive serology for anti-HBc and/or AgHBs, 6 (0.1%) confirmed the result. After new venous blood collection for anti-HBc, 1 (0.02%), out of these 6, was also positive for AgHBs, as demonstrated in Figure 1.

Out of the six cases with any positive hepatitis B marker, four were male and two were female. Age ranged from 12 years and 11 months old to 14 years and 10 months old. Five out of the six students with anti-HBc reagents presented at least one family member with positive serology in the same household, as shown in Table 1.



NR: non-reagent; R: reagent; D: dubious.

Figure 1. Prevalence of serological markers of anti-HBc, HBsAg and anti-HBs.

After the assessment of the vaccination cards of 6 students with reagent anti-HBc, three of them had not been vaccinated against hepatitis B (one of these had positive AgHBs), and three of them had been vaccinated in 2002 (five years before collection), and had anti-HBs above 10 mUI/mL.

Out of the 4,680 analyzed blood samples, only 5 presented with anti-HBs lower than 10 mUI/mL, and they were aged more than 10 years and 6 months old. Out of these five, one did not bring the vaccination card, therefore, it was not possible to know if he had been vaccinated, three presented an adequate vaccination schedule and one of them had not been vaccinated against hepatitis B. The absence of vaccination against hepatitis B was observed in 116 cases (2.7%), from the 4,306 who brought the card.

## HEPATITIS C SEROLOGY

There are only 2 cases of positive anti-HCV (0.04%), in the 4,680 finger prick samples. After new collection, in a dry tube, a child presented negative anti-HCV, and one family member had reagent anti-HCV with undetected PCR-HCV. For another child, anti-HCV was reagent (0.02%) and PCR-HCV was undetectable. This was a teenage girl aged 11 years and 3 months old, who had been treated for chronic hepatitis C at the time of collection. The mother had positive serology for hepatitis C and PCR-RNA of the undetectable C virus.

Table 1. Family study of six cases with confirmed positive serology.

| N. | Anti-HBc | AgHBs | DNA HBV | Family        | Anti-HBc | AgHBs | DNA HBV |
|----|----------|-------|---------|---------------|----------|-------|---------|
| 1  | R        | NR    | ND      | Father        | Refused  |       |         |
|    |          |       |         | Mother        | R        | NR    | ND      |
|    |          |       |         | Sister        | NR       | NR    | ND      |
|    |          |       |         | Sister        | Refused  |       |         |
| 2  | R        | NR    | ND      | Grandmother   | NR       | NR    | ND      |
|    |          |       |         | Mother        | NR       | NR    | ND      |
|    |          |       |         | Two brothers  | NR       | NR    | ND      |
| 3  | R        | NR    | ND      | Mother        | R        | NR    | ND      |
|    |          |       |         | Four brothers | NR       | NR    | ND      |
| 4  | R        | NR    | ND      | Father        | R        | NR    | ND      |
|    |          |       |         | Mother        | R        | R     | ND      |
| 5  | R        | NR    | ND      | Father        | R        | R     | ND      |
|    |          |       |         | Mother        | R        | NR    | ND      |
|    |          |       |         | Brother       | R        | R     | ND      |
| 6  | R        | R     | ND      | Father        | R        | R     | ND      |
|    |          |       |         | Mother        | R        | NR    | ND      |
|    |          |       |         | Sister        | R        | NR    | ND      |

HBV: hepatitis B virus; R: reagent; NR: not reagent; ND: not detected.

## DISCUSSION

The prevalence of reagent serological markers for hepatitis B (0.128%) was lower than that found in other regions of Brazil and in the world. According to the Ministry of Health<sup>15</sup>, in 2010, the prevalence of anti-HBc in the age group from 10 to 19 years old, referring to the set of capitals in Brazil, was of 1.10%, and the AgHBs was of 0.01%, for the same age group. A multicenter study conducted in the capitals of the Northeast and Center-West of Brazil<sup>16</sup>, in 2009, estimated the prevalence of anti-HBc in individuals aged between 13 to 69 years old: 10.00% for non-vaccinated people and 4.30% for vaccinated participants. The AgHBs ranged from 0.19%, in the Northeast region, to 0.60%, in the Federal District. Aquino et al.<sup>17</sup>, in 2008, found the prevalence of 3.6% of AgHBs and 37.7% of anti-HBc for the age group between 20 and 29 years old, among individuals assisted in the Central Laboratory of Public Health in Pará. Assis et al.<sup>18</sup>, in 2004, reported that 11.1% of the children aged 3 to 9 years old, enrolled in day care facilities and public schools in the city of Peixoto de Azevedo, have already been in touch with the hepatitis B virus, and 1.2% had AgHBs.

As to the seroepidemiologic variations of hepatitis B in the world, Ali et al.<sup>19</sup>, in 2007, in Pakistan, observed that the mean of AgHBs in the pediatric population is of 2.4%. Alizadeh et al.<sup>20</sup>, in 2006, in the Islamic Republic of Iran, observed 2.3% for AgHBs and 7.8% for anti-HBc among individuals aged more than 5 years old.

It is worth to mention the importance of vaccination, which led to decreasing numbers of people with hepatitis B in the past decade. It is a known fact that the implantation of vaccines in Brazil took place in the 1990s, which leads us to the observation that maybe that is why no children aged from 0 to 10 years old have been found to have positive serology in our study; most of them had anti-HBs above 10 mUI/mL, and only 5 adolescents had levels lower than 10 mUI/mL. De la Hoz et al.<sup>21</sup>, in 2007, in Colombia, in an area close to the Amazon, reported a 60 to 75% reduction in hepatitis B 8 years after the implantation of vaccination. Dong et al.<sup>22</sup>, in China, concluded that vaccination strategies against hepatitis B were successful 15 years after it has been implemented, by comparing the results of the serological marker AgHBs of 1.52% among children aged 0 to 8 years old, in 2007, with the already known prevalence of 5.50% in children who were born in 1992.

With regard to the three students who presented with reagent anti-HBc and had been vaccinated in 2002, we can infer there has been vaccine failure, or that they had been in contact with the virus previously, however, clearing the virus.

It was difficult to compare the reagent serology between socioeconomic groups, due to the low number of children with reagent anti-HBc and AgHBs. The association of hepatitis B with low socioeconomic status was published in many studies, all over the world. As an example, Alam et al.<sup>23</sup>, in 2007, in Pakistan, demonstrated higher

prevalence of AgHBs in the low socioeconomic status group (33%), by comparing it to the group with higher status (19%), among people aged from 8 to 53 years old.

By analyzing positive serological markers for hepatitis B between genders, a higher prevalence was observed among male participants, and the same was found in other studies in literature. As an example, Aquino et al.<sup>17</sup>, in 2008, found total prevalence of anti-HBc of 37.7%; out of these, 62.6% were male participants assisted in the Central Laboratory of Public Health in Pará. Assis et al.<sup>18</sup>, in 2004,

Assis et al.<sup>18</sup>, in 2004, in a city of the Brazilian Amazon, did not find significant differences between the male and female genders aged between 3 and 9 years old, which indicates another important form of transmission for this age group.

A higher prevalence of anti-HBc was found in the highest age group, as well as Aquino et al.<sup>17</sup>, in 2008, found a 0.9% prevalence of anti-HBc among children aged 0 to 9 years old; of 6.0% among people aged from 10 to 19 years old; and of 20.8% among people aged 20 to 29 years old, in Pará. Clemens et al.<sup>24</sup>, in 2000, found significant increase of anti-HBc in adolescence in the four regions of Brazil.

In this cross-sectional study, due to the very small number of students with reagent serology for hepatitis B, it became impossible to determine mechanisms of transmission of the B virus. Five of the six students with reagent serology for hepatitis B had at least one family member with positive marker, which suggests that family environment may have contributed with the dissemination of the virus. Two students, who were siblings, may characterize a horizontal and interpersonal transmission, even though the mechanism of transmission between them is not clear. With these data, the possibility of intrafamily transmission is suggested, without any definition about the involved mechanisms. Tavares-Neto et al.<sup>25</sup>, in 2004, in the state of Amazonas, showed high prevalence of the viral infection between siblings (23.6%). Gupta et al.<sup>26</sup>, in 2008, in the north of India, analyzed horizontal transmission in household contacts, and observed there was a higher number of AgHBs in age groups between 1 and 15 years old (37.0%), especially among siblings (48.3%).

The prevalence of 0.02% of anti-HVC was low and much lower than the estimates for the Brazilian population, which ranges between 1 and 2%<sup>2</sup>. According to the Ministry of Health<sup>15</sup>, in 2001, the positive anti-HCV in the general population of Brazil is of 1.38%, being 0.75% for the age group between 10 and 19 years old. According to the Report of the Study Group of the Brazilian Society of Hepatology, with data from the States of Amazonas, Bahia and Mato Grosso, anti-HVC in the population of students was of 0.20%, and none of the children in day care facilities had anti-HCV<sup>27</sup>. Focaccia et al.<sup>28</sup>, in 1998, found prevalence of anti-HCV in the city of São Paulo, of 0% among children aged 2 to 4 years old, 0.93%, in the age group from 5 to 9 years old, and of 0%, in adolescents aged 10 to 14 years old.

As to the variations in the epidemiology of hepatitis C in the world, in the region of Madrid, Spain<sup>29</sup>, in 1996, the prevalence was of 0.36% among 560 students aged 4 to 17 years old. In Saudi Arabia<sup>30</sup>, in 2009, it was of 0.01% among children aged less

than 15 years old. In Cairo<sup>31</sup>, in 2007, they found 1.43% (15/1042) of the children aged 1 to 9 years old with positive serology for the hepatitis C virus.

Many studies have been trying to clarify the mechanisms of transmission of the hepatitis C virus. In this study, only one case presented reagent serology for hepatitis C, thus not allowing measuring possible associations with risk factors. Paraná et al.<sup>32</sup>, in 2000, in Salvador, assessed the frequency of genotype and epidemiological aspects in 232 adults with the C virus, and observed that, among the risk factors for viral infections, 40% had history of transfusion, 6% were users of venous drugs, 18% inhaled cocaine, 12% had a tattoo, 7% were working in the health field, 2% reused disposable syringes, 2% had multiple risk factors and, for 23%, it was unknown. Alter et al.<sup>5</sup>, in 1999, showed that the use of illegal drugs and high risk sexual behavior were the main forms of acquiring the hepatitis C virus, in the United States. Plancoulaine et al.<sup>7</sup>, in 2008, in a village from the Nile river delta, strongly correlated the seroprevalence between first degree relatives, suggesting intrafamily viral transmission and genetic predisposition for the infection with the hepatitis C virus. Cavaleiro et al.<sup>6</sup>, in two reference centers of hepatitis, in *Universidade de São Paulo* and in Hospital Guilherme Álvaro, in Santos, concluded that the high level of genomic similarity between the viruses of hepatitis C supports the hypothesis of transmission between couples.

The only case with positive serology for hepatitis C in this study was caused by vertical transmission, according to data provided by the mother in the survey. As to vertical transmission, which is the main form of acquisition during childhood, many studies have been conducted since the hepatitis C virus was discovered, in 1989. Literature points out to the high prevalence of anti-HCV among adults in the city of Santos<sup>10</sup>, and the source of infection by vertical transmission was very rare. A systematic review about the vertical transmission rate, published between 1992 and 2000<sup>33</sup>, showed 1.7% of positive results among children with women with positive anti-HCV, regardless of the RNA-VHC, 4.3% when the mother was positive RNA-VHC, and 19.4% in the coinfection with HIV<sup>34</sup>.

The collection of blood samples with filter paper for serological tests offers an excellent opportunity for epidemiological studies, since it facilitates collection, transportation and storage of samples, and also because the obtained results are comparable with the use of serum or plasma, in terms of sensitivity and specificity<sup>35</sup>.

In literature, since the past decade, there were studies about the research of antibodies for hepatitis C using filter paper, and also discussing its efficacy, in the attempt to obtain an alternative method to test the anti-HCV, with similar specificity and sensitivity to the conventional methodology. The Center of Specialty in Virology, in the West of Scotland<sup>36</sup>, from 2009 and 2010, using filter paper, found 100% sensitivity and 94.7% specificity, and observed that when the cutoff point of trials, it is possible to obtain 100% specificity and sensitivity. Therefore, we can infer that the use of filter paper to research for anti-VHC in epidemiological studies is valid, with good

sensitivity and specificity, however, further data are required to prove the efficacy of the study about the blood drawn in filter paper

## CONCLUSIONS

The general prevalence among children of serological markers for hepatitis B and C in the city of Santos was low, when compared to data from literature. It was not possible to know the molecular aspects or to study the form of acquisition of the viruses of hepatitis B and C because the number of positive cases was very low.

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Received on: 10/22/2012

Final version presented on: 11/10/2013

Accepted on: 02/13/2014