

Intestinal helminthes and/or *Toxocara* infection are unrelated to anti-HBs titers in seven-year-old children vaccinated at birth with recombinant hepatitis B vaccine

Helmintos intestinais e/ou infecção por *Toxocara* não tem relação com títulos de anti-HBs em crianças de sete anos de idade vacinadas ao nascer com vacina recombinante para hepatite B

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ABSTRACT

The aim of this investigation was to evaluate the possible effect of nematode infection on anti-HBs antibody levels in the serum of seven-year-old schoolchildren vaccinated at birth with the recombinant hepatitis B vaccine. Anti-HBs and anti HBe antibodies were evaluated in the sera of 100 schoolchildren with at least one intestinal nematode and/or a positive serological reaction for anti-Toxocara antibodies and in 95 schoolchildren without intestinal helminthiasis or serum anti-Toxocara antibodies. Both groups were from public elementary schools located on the urban periphery of Vitória, ES, Brazil. Among these 195 children, the median anti-HBs antibody titer was 31.3IU/ml and the frequency of titers less than 10IU/ml was 33.8% (95% CI: 27.1-40.4%). There were no significant differences between the medians of anti-HBs titers or the frequency of titers less than 10IU/ml between the groups with or without helminthes (29.5 and 32.9IU/ml and 33 and 34.7%, respectively; $p > 0.05$). Even when the children with intestinal nematodes and/or anti-Toxocara antibodies and with blood eosinophil counts over 600/mm³ were compared with children without infection from intestinal nematodes and without anti-Toxocara antibodies, with blood eosinophil counts less than 400 eosinophils/mm³, these differences were not significant. None of the children presented anti-HBe antibodies. In conclusion, infections with intestinal nematodes and/or the presence of anti-Toxocara antibodies did not interfere with the anti-HBs antibody titers in seven-year-old children vaccinated at birth with the recombinant hepatitis B vaccine.

Key-words: Intestinal nematodes. Toxocariasis. Hepatitis B vaccine. Helminthiasis.

RESUMO

O objetivo dessa investigação foi avaliar um possível efeito de infecções por nematóides sobre os níveis de anticorpos anti-HBs no soro de escolares de sete anos de idade, vacinados ao nascer com a vacina recombinante para hepatite B. Anticorpos anti-HBs e anti-HBe foram avaliados no soro de 100 escolares portadores de pelo menos um nematóide intestinal e/ou uma reação sorológica positiva para anticorpos anti-Toxocara e em 95 escolares sem helmintíases intestinais e sem anticorpos séricos anti-Toxocara, todos matriculados em escolas primárias públicas situadas na periferia urbana de Vitória, ES, Brasil. Nas 195 crianças, a mediana dos títulos dos anticorpos anti-HBs foi 31,3UI/ml, e a frequência de títulos inferiores a 10UI/ml foi de 33,8% (IC a 95%:27,1- 40,4%). Não houve diferença significativa entre as medianas dos títulos de anti-HBs ou da frequência de títulos inferiores a 10 UI/ml entre as crianças com ou sem helmintos (29,5 e 32,9 UI/ml e 33 e 34,7%, respectivamente; $p > 0.05$). Mesmo quando comparadas crianças com nematóides intestinais e/ou anticorpos anti-Toxocara com eosinófilos circulantes acima de 600/mm³, com crianças sem infecção com nematóides intestinais e sem anticorpos anti-Toxocara, com menos de 400 eosinófilos/mm³, aquelas diferenças não foram significativas. Nenhuma das crianças apresentou anticorpos anti-HBe. Em conclusão, infecções com nematóides intestinais e/ou presença de anticorpos anti-Toxocara não interferem nos títulos de anticorpos anti-HBs em crianças de sete anos de idade, vacinadas ao nascer com a vacina recombinante para hepatite B.

Palavras-chaves: Nematóides intestinais. Toxocaríase. Vacina para hepatite B. Helmintíases.

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Serum anti-HBs antibodies acquired after a complete course of primary vaccination with the hepatitis B recombinant vaccine fall progressively over the course of the years following vaccination, to a greater or lesser extent^{27,28}. Factors such as birth weight, HBs antigen positivity in the mother and the magnitude of the response to vaccination are related to the rate of anti-HBs decay, but the role of environmental factors like nutrition and parasite infection have not been studied²⁸.

Helminth infection induces immunomodulation, by skewing the immune response to the Th2 pole and inducing the activation of different types of regulatory T cells^{13,14,15}. Corroborating this immune deregulation, increased prevalence of some infectious diseases like staphylococcal infection, tuberculosis and leprosy has been reported in individuals with intestinal worms and/or *Toxocara* infection^{5,17,18,24}. In addition, this immunomodulation may interfere with the response of several vaccines, as demonstrated in experimental models and in humans. In these studies, impairment of the production of protective antibodies, the induction of cell-mediated immunity and the anamnestic response have been reported^{3,4,7,10,16,19,21}. Children with nematode infection presented significantly lower responses to PPD and, when vaccinated with BCG, became less reactive to that antigen but presented an improved response following worm eradication⁷. Thus, there is evidence that helminth infection may interfere with the anamnestic response to vaccines.

With regard to HBV vaccine, Ghaffar et al¹¹ reported lower anti-HBs response in children with schistosomiasis, three and nine months after vaccination. However, Bassily et al² did not find any effects from maternal infection with *Schistosoma mansoni* on the anti-HBs titers of babies vaccinated at birth.

Hepatitis vaccination induces protective anti-HBs antibodies. Although it is accepted that anti-HBs titers higher than 10 IU/l indicate protection, this protection does exist with lower titers because of the anamnestic response involving T cells²⁸. It is not known how the immunological memory and high production of protective anti-HBs titers are maintained, but the mechanisms involved may include: (a) generation of long-lived plasma cells, and (b) frequent stimulation of B and T memory cells by contact with cross-reactive epitopes or through bystander stimulation after nonspecific polyclonal activators have been in contact with the immune system¹². The latter mechanisms, which are dependent on stimulation of memory cell clones, may be influenced by the status of the immune system. This may include immunomodulation induced by worm infection, which would induce bystander suppression of memory cells. Thus, it is plausible that introduction of helminth infection in children who received hepatitis B vaccine at birth could induce impairment of the titers of protective anti-HBs antibodies in the first years of life. To investigate this possibility, we evaluated and compared the anti-HBs titers in seven-year-old children, with or without intestinal helminthes and/or positive serology for *Toxocara*.

MATERIAL AND METHODS

The anti-HBs titers were evaluated in two groups of seven-year-old schoolchildren who had been vaccinated at birth with three doses of recombinant hepatitis B vaccine. These children

were from eight public elementary schools located in low-income neighborhoods. They were separated in two groups: one group with negative serology for anti-*Toxocara* antibodies and without intestinal helminthes and another group including children with at least one intestinal helminth (*Ascaris lumbricoides* in 33 cases, *Trichurus trichiura* in three cases and both worms in one case) and/or a positive serology for anti-*Toxocara* antibodies.

All children who are admitted to the public elementary schools in Vitória undergo clinical examination, complete hemogram and stool examination to investigate intestinal parasites. The excess of blood collected for the hemogram was used to obtain the sera for the present study. All sera were stored at -20°C .

Anti-HBs and anti HbC were investigated using commercial kits (Axsyn AUSAB and Axsyn System Core, Abbot Laboratórios do Brasil Ltda), in accordance with the manufacturer's instructions. The tests were performed at Central Sorologica de Vitória.

Anti-*Toxocara* antibodies were investigated by means of ELISA IgG, using secretion-excretion antigens of second and third-stage larvae of *Toxocara canis*, in accordance with the manufacturer's instructions (CELISA-Toxocara, Cellabs, Australia). This was performed at Núcleo de Doenças Infecciosas, Federal University of Espírito Santo (UFES). The sera were not adsorbed with other helminth antigens before ELISA testing. In the group considered to be negative for *Toxocara*, the optical densities were lower than 0.250, which was considered to be nonreactive by the manufacturer. In the group considered to be *Toxocara*-positive, the optical densities were higher than 0.500, and these values were considered indicative of *Toxocara* infection, according to the manufacturer. The stool examination was performed on a single sample, by the sedimentation method, at the routine laboratory of the municipality of Vitória. The hemogram was performed at the same laboratory, using automated methods.

RESULTS

The results are shown in Tables 1, 2, 3 and 4. The two groups (with and without helminth infection) were homogeneous, without significant differences in age, gender distribution, lymphocyte counts or hemoglobin concentration (Table 1).

Table 1 - Age and gender distribution of schoolchildren, separated according to the presence or absence of at least one intestinal nematode and/or positive serology for anti-*Toxocara* antibodies, in which the serum levels of anti-HBs antibodies were tested.

Variables	Intestinal nematode and/or <i>Toxocara</i>		p
	negative (N = 95)	positive (N = 1 00)	
Gender			
male (N = 103)	46	57	
female (N = 92)	49	43	0.788 ^a
Age (years; mean \pm SD)			
male	7.25 \pm 0.54	7.16 \pm 0.48	
female	7.27 \pm 0.66	7.42 \pm 0.39	
Hemoglobin (mg/dl, mean \pm SD)	11.95 \pm 0.78	12.17 \pm 0.76	0.188 ^b
Lymphocyte/mm ³ (mean \pm SD)	2522.8 \pm 608.4	2625.5 \pm 667.1	0.934 ^b

^aChi-square test; ^bStudent's t test

There was no undernutrition in either group. This was demonstrated by direct inspection as well as by lymphocyte counts and hemoglobin concentrations (Table 1). The latter are considered to be indirect markers for nutritional status.

The median for the anti-HBs titers for the 195 children studied was 31.3IU/ml and the prevalence of titers less than 10IU/ml was 33.8%, without gender differences (Table 2).

When the means of anti-HBs titers and the frequency of titers less than 10IU/ml were compared between the groups with and without helminth infection, the differences were not significant (Table 3).

Since blood eosinophil counts may provide indirect evidence of stronger Th2 reaction induced by worms, anti-HBs titers were compared between the children without intestinal helminthes and negative for anti-*Toxocara* antibodies whose blood eosinophil counts were less than 400/mm³, and the children with helminth infection and with blood eosinophil count higher than 600/mm³ (Table 4). Even in this situation, the difference in anti-HBs titers was not significant.

No child in either of the two groups was reactive to anti-HBc.

Table 2 - Serum anti-HBs titers in seven-year-old schoolchildren vaccinated at birth with recombinant anti-HBV vaccine in Vitória, ES.

Groups	anti-HBs (IU/ml) ^a			
	mean ± SD	median	GM ^b	<10 / >10 (%; 95% CI)
All cases (N = 195)	150.9 ± 345.2	31.3	22.7	66/129 (33.8; 27.1 – 40.4)
male (N = 103)	174.5 ± 392.1	32.0	25.3	32/71 (31.1; 22.1– 40.3)
female (N = 92)	124.5 ± 283.1	29.8	20.1	34/58 (36.9; 27.0 – 46.7) ^c

^aInternational Units; ^b Geometric Mean; ^cChi-square test: p = 0.470

Table 3 - Anti-HBs titers in sera of seven-year-old children vaccinated at birth with recombinant hepatitis B vaccine, according to the presence of at least one intestinal helminth and/or positive serology for *Toxocara*, in Vitória, ES.

Intestinal helminth and/or serology for <i>Toxocara</i>	Anti-HBs (IU/ml) ^a			
	mean ± SD	median	GM ^b	<10 / >10 (%; 95% CI)
Negative (N=95)	154.3 ± 59.2	32.9	23.7	33/62 (34.7; 25.1 – 44.2)
Positive (N=100)	147.4 ± 331.9	29.5	21.6	33/67 (33.0; 23.1 – 42.2) ^c

^aInternational Units ^b GM = Geometric mean; ^c Chi-square test; p = 0.916

Table 4 - Anti-HBs titers in serum of seven-year-old children vaccinated at birth with recombinant anti-HBV vaccine, according to the presence of at least one intestinal helminth and/or positive serology for *Toxocara*, and with or without eosinophilia (blood eosinophils higher than 600/mm³) in Vitória, ES.

Helminth and/or serology for <i>Toxocara</i>	Anti HBs (IU/ml)			
	Mean ± SD	Median	GM	<10 / >10 (%; 95% CI)
(-) and <400 eosinophils/mm ³	191.1 ± 401.2	40.9	26.7	15/36 (29.4; 16.8 – 41.9)
(+) and >600 eosinophils/mm ³	174.6 ± 400.4	20.2	18.6	18/28 (39.1; 24.9 – 53.2) ^a

^aChi-square test; p = 0.427

DISCUSSION

The utilization of a group with intestinal nematodes and/or *Toxocara* infection as a sample of children with helminth infection can be justified because the general mechanisms of the immune response to nematodes are similar, as demonstrated in experimental and human studies^{14 15}.

None of the children in either group presented significant symptoms or signs of any disease or undernutrition. The only manifestation was the presence of eosinophilia (eosinophil counts over 600/mm³), which was more frequent in the group with helminth infection (76.4 and 48.2% in the groups with and without helminthes, respectively; p=0.000).

The results demonstrate that 33.8% of these healthy seven-year-old children who were vaccinated at birth with recombinant HBV vaccine had anti-HBs titers lower than 10IU/l, without significant gender differences. This is the first report from Brazil on HBs titration seven years after vaccination of newborns, and the results are similar to those observed in other developing countries, in similar samples. The frequencies of anti-HBs titers lower than 10IU/l, five to 12 years after newborn vaccination with recombinant HBV vaccine have been reported to be lower in children from Canada⁶, Spain¹, Italy⁹ and the United States²⁵ than in children from Samoa²⁶, Alaska²⁰, Iran²² and Hawaii²³.

Although still a matter for discussion, booster doses of vaccine for children with low anti-HBs titers do not seem necessary for ensuring long-term protection, even in endemic countries, despite

waning or undetectable anti-HBs levels²⁹. Studies on anti-HBs titers ten years after vaccination have demonstrated that strong immunological memory persists in infants and adolescents with a primary course of vaccination. Consensus groups in Canada, Europe and the United States have not recommended the need for booster doses under these circumstances, in immunocompetent individuals who responded to a primary course of vaccination⁸.

In the sample studied, none of the children had anti-HBc in the serum. It is difficult to interpret this finding because Vitória has a low prevalence of HBV infection, and it is therefore difficult to confirm whether this result indicates protection or absence of contact with the HBV virus.

The results demonstrate that the presence of helminth infection (intestinal worm, *Toxocara* infection or both) is unrelated to the anti-HBs titer decay in the sample studied. Even in children with helminth infection and blood eosinophil counts higher than 600/mm³, the anti-HBs titers were not significantly different from those observed in children without helminth infection and with blood eosinophils lower than 400/mm³. Although the results from stool examination were based on a single sample, the caveats originating from the assumption that false negatives might exist would be minimized by the low probability of helminth infection in children with blood eosinophils lower than 400/mm³.

Although there was no relationship between the presence of worm infection and the anti-HBs titers, we cannot rule out the possibility that worm infection might have an effect on memory cell response after a secondary antigen challenge. In fact, Ghaffar et al¹¹ observed that the titers of anti-HBs were lower in children with schistosomiasis who received the vaccine after they acquired *Schistosoma*. The presence of *Schistosoma* infection reduced the response after primary vaccination.

In conclusion, the possible immunoregulatory effect of helminth infection does not seem to interfere with the maintenance of anti-HBs levels in children vaccinated at birth with the HBV recombinant vaccine. Further investigation is necessary to study the impact of helminth infection on the response of vaccinated children after a secondary challenge with HBV antigen.

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