Factors Associated with Dengue Virus Infection and Reinfection in Asymptomatic Children in Two Colombian Municipalities

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Abstract. Dengue is the most important arbovirolosis in the world. In this study, we assessed the knowledge, attitudes, and practices (KAP) regarding dengue in parents from two small Colombian municipalities in the Cundinamarca Province. Parents and their healthy children from 4 to 14 years of age were included in some public elementary schools. After a medical examination, blood samples were taken for diagnosis of dengue using enzyme-linked immunosorbent assays (capture immunoglobulin M and capture immunoglobulin G [IgG], indirect IgG and detection non-structural viral protein 1) and detection of viral RNA by reverse transcription polymerase chain reaction. In addition, a KAP survey was applied to the children's parents or tutors. The indirect IgG test determined that of the 347 examined children, 87.9% had a previous infection with the dengue virus (DENV), 12.7% of them were positive for viral RNA (asymptomatic infection), and 32.0% presented reinfections. Risk factors evaluation showed that children aged 8 years and older living in the municipalities for more than 7 years were more likely to be infected or reinfected by DENV. In the same way, poor nutrition, lack of water supply, sewer service, or waste disposal services could raise the likelihood of dengue infections. The surveys indicated that parents have unhealthy practices and a low knowledge about the transmission of the disease, which could result in an increase of mosquito breeding sites, allowing sustained dengue transmission.

INTRODUCTION

Dengue is considered one of the most important vector-borne diseases worldwide.^{1,2} This viral disease is transmitted by the bite of the *Aedes (Stegomyia) aegypti* mosquito^{3,4} and is the same vector involved in the transmission of other viruses such as yellow fever,⁵ chikungunya, and Zika viruses.⁶ It is estimated that 390 million infections occur every year throughout the world and of these, only 96 million show clinical manifestations. In addition, around 500,000 people are diagnosed with severe dengue and require hospitalization. In 2016, the Americas region reported more than 2,380,000 cases, and in Brazil alone, there were more than 1,500,000 cases, three times more than that reported in 2014. However, it is generally accepted that the total number of cases is underestimated and most of the infections are not correctly classified or are misdiagnosed.^{7,8} In Colombia, dengue represents an important public health problem, not only because of the high prevalence but by the burden of the disease, which represents an annual average of 3,900 disability-adjusted life years (DALYs).^{9,10} In Colombia, rapid and disorganized urbanization, climate changes, migration of rural population to the cities, and weak vector control programs have been associated with greater proliferation of the mosquito and the consequent infection increase.¹¹

For instance, in 2014, the Colombian National Epidemiological Surveillance System (SIVIGILA) reported 107,975 dengue cases, of which 105,356 (95.7%) were classified as dengue cases and 2,619 as severe dengue. The 5- to 14-year-old group was the most affected (28.5% of the cases).¹² These findings evidence weaknesses in vector control programs^{13,14} and explains, for example, the endemic transmission of dengue virus (DENV) since 2010. This same situation is repeated in the Department of Cundinamarca, where 16% of the urban

population is in DENV transmission risk areas. In this province, the vector circulates widely, and the four DENV serotypes are present, which causes it to be classified as a hyperendemic province. With respect to the specific data for Cundinamarca Province, during a 12-year period (1999–2010), approximately 2,000 cases per year (totaling 21,857 cases) were reported. However, in the 2013 epidemic, 4,357 cases of dengue and 92 cases of severe dengue (including two deaths) were reported.¹⁵

According to the strict definition of dengue risk, the two selected municipalities with touristic and economic relevance to the department are at high risk. Anapoima was the first municipality classified as a high-risk area, with incidence rates between 823.5 and 1,904 per 100,000 inhabitants. Although the municipality of Apulo has a lower incidence than Anapoima, it is also considered as a high-risk area, given that incidence rates range between 482.4 and 788.9 cases per 100,000 inhabitants.¹⁶

Recently, the infestation and circulation of all four DENV serotypes in the urban and rural areas of these municipalities was reported, as well as an increase in the number of cases of DENV in the same areas, which could be associated with the lack of prevention campaigns and the difficulty in controlling the vector.^{17–20} These reasons support the need to identify the factors associated with DENV infection and reinfection in a group of school children from 4 to 14 years old in these two Colombian municipalities.

METHODS

Study sites. Municipalities of Anapoima and Apulo belong to the state of Cundinamarca. They are located 87 and 101 km southwest of Bogotá, the capital of Colombia, having a population of approximately 13,106 and 7,812 inhabitants, respectively.²¹

Anapoima has a mean altitude of 710 m above sea level, a rainfall of 1,300 mm per year, and a mean temperature of 26°C.²⁰ Aqueduct coverage extends to 81.7% of population and only 43.7% are serviced by sewage disposal systems. Apulo has a mean altitude of 420 m above sea level and a mean

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temperature of 28°C. According to official data from 2011, 76.1% of the population has aqueduct coverage and 50.5% are serviced by sewage disposal systems.²²

These areas have been characterized by rapid urbanization and constant human settlements owing to its proximity to the Colombian capital. In Anapoima, the population is concentrated mainly in the urban area, whereas Apulo is mainly a rural municipality, where construction of houses is less developed and is dispersed in rural areas or concentrated in "inspections" (similar to neighborhoods, although rural).²²

Study population. All procedures were approved by the Institutional Ethics Committee of Universidad El Bosque. During February 2014, the study was presented orally and by a written form to parents, teachers, and administrators of three schools in the Anapoima municipality (two are urban: B and C and one is rural: A) and in the urban school (D school) of the Apulo municipality. The parents interested in the study signed the authorization for the children to be enrolled and the children gave their assent to participate. The children included in the study were selected without any randomization mechanism. An appointment was made with the study staff for a meeting during March 2014 when the study started and the parents signed the consent form and the children signed the assent form. Personal information was collected from the parents and a knowledge, attitudes, and practices (KAP) survey was conducted with each parent. The children were examined by a medical doctor to establish the health status and to obtain the medical history. If signs or symptoms of dengue or other diseases were detected, the parents were informed and medical recommendations were handed to parents to treat/control it. A blood sample was taken from the children; the serum was separated and frozen immediately at -20°C and transported to the Laboratory of Virology located at Universidad El Bosque for processing and diagnostic testing.

Survey on KAP. The questionnaire has been previously used²³ and has a total of 37 questions divided as follows: 11 questions about dengue disease knowledge, its transmission, characteristics, symptoms, treatment, and prevention; two questions about dengue prevention attitudes; three questions corresponding to the practices; and 21 questions on socio-economic aspects such as access to public services and housing characteristics, and demographic data such as ethnicity and educational level.

Laboratory tests and diagnosis. Plasma processing initially involved the application of serological tests for the detection of IgM or IgG antibodies in the participants and later for virological tests (NS1 enzyme-linked immunosorbent assay [ELISA] and reverse transcription polymerase chain reaction [RT-PCR]). The detection of IgM antibodies was performed using an MAC-ELISA test (UMELISA Dengue IgM Plus; Tecnosuma, La Habana, Cuba). This is a capture immunoassay in which the wells are sensitized with anti-IgM antibodies that recognize the IgM of the sample, which can be detected since the third or fifth day after the onset of symptoms and up to 3 months later, and is an indicator of active or recent infection.²⁴ The second test to define active or recent infection was the capture IgG ELISA test (catalog no. 01PE10; Panbio, Alere) in which the surface of the wells is coated with anti-IgG antibodies that interact with the IgG from the sample. This test detects IgG antibodies specific for DENV, which is a high level, indicating an ongoing or very recent secondary infection.²² Finally, the indirect IgG ELISA test (catalog no. 01PE30; Panbio,

Alere) was used in which the wells have adsorbed recombinant antigen for the four DENV serotypes to which the dengue-specific IgG antibodies present in the plasma becomes bound. A positive result indicates that the participant had contact with DENV previously.²⁴ Samples that were positive for capture IgM or capture IgG were processed for detection of the DENV NS1 antigen (NS1 Early ELISA, Panbio, Alere) and for the detection of viral RNA by the RT-PCR protocol previously described.^{25,26}

Calculation of sample size and group definition. OpenEpi V.3.01 software²⁷ was used to calculate the sample size with a 95% confidence interval (CI), a 1.4-design effect and 80% statistical power, giving a final number of 345 participants. We finally enrolled 347 children.

Of the 347 participating volunteer children, 218 (62.8%) belonged to the municipality of Anapoima and 129 (37.2%) belonged to the municipality of Apulo. The distribution of the population by schools was as follows: institution A with 67 children (19.3%), institution B with 107 children (30.8%), institution C with 44 children (12.7%), and institution D with 129 children (37.2%).

According to the results of the laboratory tests, four diagnostic groups were defined. Group with no history of infection. Individuals who were negative on all tests.

Group with asymptomatic infection. Participants with a positive result for capture IgM or capture IgG (with positive or negative RT-PCR result) who reported not having fever in the last 15 days.

Reinfection group. Children with capture IgM and indirect IgG positive or children with positive capture IgG, in both cases without a report of fever or disease in the last 15 days.

Infection history group. Individuals with a positive indirect IgG test result.

Analysis. The information collected in the field was recorded in a database (Excel 2010, Microsoft Corp., Redmond, WA). According to the responses collected, the study variables that included both sociodemographic and clinical history were defined. The software used for the statistical analysis was STATA 13.0 (StataCorp, College Station, TX); first, the absolute and relative frequencies of the nominal and ordinal variables by univariate analysis were presented. Shapiro-Wilk test was used to verify the normality of the ratio variables and the median and interquartile range were estimated. The χ^2 test was used to compare the dependent categorical variables (negative history, infection, reinfection, and infection history) and independent variables, that is, socio-demographic characteristics, KAP data and data from medical records. A bivariate analysis was performed to identify associations, estimating crude odd ratios and their 95% CIs. To perform the multivariate logistic models, the variables identified in the bivariate analysis were included, even the non-significant ones that had a clinical or virologic interest. Both raw OR and adjusted OR were compared to identify the confounding factors. Again, the individuals were grouped following the diagnosis and compared with the group of subjects negative for the tests. Two collinearity analysis were performed: first one for the aqueduct and sewer variables and second, collinearity analysis for age (8 years age and older) and municipality residency for more than 7 years. The models in which the variables were collinear were rejected.

RESULTS

Study population. A total of 347 children were included, of which 50.7% (176) were female. The median age was 9 years;

53.1% of children lived in the rural area, 73.5% (255) resided longer than 7 years in the municipalities, and 58.7% of the individuals were affiliated to the subsidized health system. At the time of the medical examination, a medical history was taken and 11.2% (39) reported history of lung disease, 9.2% (32) a previous infectious disease, 6.3% (22) reported decreased visual acuity, and 9.0% (31) allergies.

Only 12.0% (40) of the parents reported that their child ever had dengue, of which 24 were hospitalized for this disease, and 3.7% of the parents reported that their children had a fever in the last 15 days. The clinical examination revealed that 26.8% (93) presented some type of visual acuity anomaly and 9.8% (34) tooth cavities. Most of the children (58.8%) had a normal nutritional status and 20.2% were found to be overweight. Only 10 parents reported yellow fever vaccination in their children (Table 1).

Knowledge, attitudes, and practices. The vast majority of parents surveyed were women (317/347), with a median age of 34 years (range 19–74), of which 47.8% (165) were engaged in household activities and 40.2% (139) were self-used. On the other hand, half of the people surveyed (51.4%; 178) attended

high school. Some of the respondents or some of the people with whom they shared housing with (28.1%; 95) visited another municipality in the last 15 days. The municipality that most frequently traveled was Bogotá city which has cold weather (8.6%; 29) and the second one most frequently traveled was Girardot municipality which has hot weather (5.0%; 17). Data on aqueduct, sewage disposal, and garbage collection are shown in Table 2.

Knowledge. Of the parents interviewed, 96.5% (335) recognized that fever was one of the first and main symptoms associated with the disease, although vomiting and diarrhea were also recognized by 61.6% (214) and 40.6% (141) of the parents, respectively, as symptoms of dengue. Eighty-four percent (283) of the parents reported that in the last 12 months, none of the inhabitants of their homes had dengue. A high number of adults knew that dengue was transmitted by a mosquito bite (90.5%) and 90.2% knew it could happen more than once. However, 22.6% (78) did not know how many days the disease could last. Just more than half of the parents (56.8%) used self-medicated pills and syrups to treat dengue; however, 83.8% (291) recognized that if a person did not receive care and treatment of the symptoms, that person could die. Up to 43.5% (146) of the parents reported that the information about dengue was received from communication media and 23.5% (79) from a relative.

Attitudes and practices. Of the total number of participants, 98.5% (342) stated that they were disturbed by the presence of mosquitoes and 98.3% (340) reported that having standing water on objects such as tires and tanks facilitated the transmission of the disease. Finally, regarding the practices, 80.0% (276) of the participants thought that the best way

TABLE 1
Sociodemographic characteristics and clinical history

Characteristics	N	%	CI (95%)
Sex (N = 347)			
Male	171	49.3	44.0–54.5
Female	176	50.7	45.4–55.9
Age years (N = 347)			
4–7	92	26.5	22.1–31.3
8–9	104	30.0	25.3–34.9
10–11	77	22.2	18.0–26.7
12–14	74	21.3	17.2–25.8
Median 9 (4–14)			
Affiliated health system (N = 329)			
Subsidized	193	58.7	53.2–63.9
Contributory	136	41.3	36.1–46.7
Home location (N = 326)			
Rural	173	53.1	47.6–58.4
Urban	153	46.9	41.5–52.3
Medical history (N = 347)			
Infectious disease	32	9.2	6.5–12.6
Lung disease	39	11.2	8.2–14.9
Decreased visual acuity	22	6.3	4.1–9.3
Hospitalization	88	31.1	20.1–30.1
No history	166	42.2	42.6–53.1
Dengue history (N = 341)	42	12.0	9.1–16.3
Hospitalization by dengue (N = 341)	24	7.0	4.6–10.1
Signs and symptoms of last 15 days (N = 347)			
Headache	11	3.1	1.7–5.4
Fever	13	3.7	2.1–6.1
Vomit	4	1.2	0.4–2.7
Diarrhea	4	1.2	0.4–2.7
Abdominal pain	9	2.6	1.3–4.7
No disease	306	88.2	84.4–91.2
Anomalies found (N = 347)			
Decreased visual acuity	93	26.8	22.3–31.6
Tooth cavities	34	9.8	6.7–13.2
Other	32	9.2	6.5–12.6
No anomalies	188	54.1	48.9–59.4
Nutritional status (N = 347)			
Normal	204	58.8	53.5–63.8
Obesity	33	9.5	6.7–12.9
Overweight	70	20.2	16.2–24.6
Low weight risk	34	10.0	7.0–13.2
Moderate malnutrition	5	1.4	0.5–3.1
Severe malnutrition	1	0.3	0.01–1.4

CI = confidence interval.

TABLE 2
Sociodemographic characteristics of respondents

	N	%	CI (95%)
Gender (N = 347)			
Male	30	8.7	6.0–11.9
Female	317	91.7	88.0–93.9
Age (N = 326)			
19–30	95	29.1	24.4–34.2
31–34	75	23.0	18.6–27.8
35–41	78	23.9	19.5–28.7
42–74	78	23.9	19.5–28.7
Median 34 (19–74)			
Work dedication (N = 345)			
Household chores	165	47.8	42.5–53.1
Self-employee	139	40.2	35.2–45.5
Other	41	11.9	8.8–15.6
School grade (N = 346)			
None	16	4.6	2.7–7.2
Elementary	103	29.8	25.1–34.7
High school	178	51.4	46.1–56.6
Technical/university	49	14.2	10.8–18.1
Public services (N = 345)			
Garbage collection	246	71.3	66.3–75.8
Aqueduct	298	83.6	82.4–89.7
Sewerage system	207	60.0	54.7–65.0
Type of toilet service (N = 345)			
Connected to the sewer	217	62.9	57.7–67.8
Connected to septic tank	123	35.7	30.7–40.8
Source of water for preparing food (N = 345)			
Aqueduct	239	69.3	64.2–73.9
Water well	8	2.3	1.1–4.3
Pile	3	0.8	0.2–2.3
Rain water	58	16.8	13.1–21.0

CI = confidence interval.

to prevent the disease was to avoid water stagnation, although 29.4% (99) did not take any action if a relative or neighbor was sick and only 24.1% (81) decided to isolate the patient. On the other hand, 57.9% (200) said that they would self-medicate a family member who had fever. In regard to this, 70.8% (158) of the respondents did not consider it necessary to consult the doctor and only 48.7% (168) of the respondents suggested that they should attend the hospital.

Laboratory tests. Among the children evaluated by serology, independently of the results for other tests, 87.9% of children had had previous contact with DENV (indirect IgG positive). Interestingly, 17.0% (59) of the analyzed samples were positive for capture IgM, whereas 29.1% (101) were positive for capture IgG, indicating in both cases a recent infection. Positive samples for capture IgM or IgG (147) were processed to detect the NS1 protein and viral RNA. We found in this way that 44 (29.9%) of them were positive by RT-PCR; however, the NS1 antigen could not be detected in any of them. The DENV-2 serotype had the highest frequency (33 samples, 75.0%), followed by DENV-3 with 11.4% (5), DENV-4 (4.5%), and DENV-1 (2.3%) (1). Three serum samples were also double serotype positive by RT-PCR; two of them were positive for DENV-1/DENV-2 and the other was positive for DENV-2/DENV-3.

Classification of the groups by laboratory results. According to laboratory tests and the classification of specific

groups, it was found that only 38 children (10.9%) were negative for all serological tests. Of the sampled children, 28 (8.1%) had an asymptomatic primary infection at the time of sampling and 32.0% (111) had an asymptomatic secondary infection (reinfection group) (Figure 1). With these serological tools, a total of 139 children (40.1%) were identified in the infection group (IgM and/or IgG capture and/or positive RT-PCR) and a further 208 children (59.9%) were included in the no-infection group.

Regarding the group of asymptomatic infections, the most affected age group was 8–9 years (13.5%) and there were no differences by gender. The percentage of children with asymptomatic infections was slightly higher in the urban area than in the rural area (20.3% versus 17.9%).

Multivariate analysis between diagnostic groups and knowledge and practices on dengue. To identify the confounding variables and mitigate their impact, a logistic regression analysis was performed. We considered the following groups: infection and reinfection, asymptomatic infections, infection history, and only reinfection to analyze the relationship with significant and nonsignificant variables. By adjusting the models, recurrently we found those children aged 8 years and older living for more than 7 years in the municipalities could have a higher risk of DENV infection or reinfection. Other variables such as low weight or malnutrition, and living

FIGURE 1. Diagnosis algorithm and its classification applied to the participating children. The scheme shows the algorithm that was applied to the participating children. Enzyme-linked immunosorbent assays (ELISAs) for indirect IgG, and capture IgM and IgG were performed on all samples; if the capture IgM or IgG tests, independent of the polymerase chain reaction (PCR) result were positive or indeterminate, complementary tests such as viral antigen detection (NS1) and reverse transcription PCR (RT-PCR) were applied. In samples that detected viral RNA (RT-PCR: positive), the serotype or serotypes involved were identified.

in homes with no solid waste collection service could also be related with increased likelihood of infection (Table 3).

In addition, other KAP survey data variables were evaluated as risk factors for DENV infection or reinfection with different mathematical models. For example, asymptomatic infection in children and belonging to subsidized health service providers had a weak positive relationship (adjusted OR: 1.60; 95% CI: 0.96–2.68). Have improper practices for the disposal of solid waste (adjusted OR: 1.12; 95% CI: 0.56–2.24), lacking waste collection service (adjusted OR: 1.14; 95% CI: 0.63–2.09), or lacking sewage collection service (adjusted OR: 1.12; 95% CI: 0.44–2.84) could raise the likelihood of acquiring DENV infection. On the other hand, living in a rural area showed a nonsignificant reduction in the likelihood to be infected or reinfected (adjusted OR: 0.61; 95% CI: 0.34–1.08) (Table 4).

In addition, the analysis of the group of children with a history of infection revealed that it is possible that certain variables such as implementing incorrect practices for solid waste disposal (adjusted OR: 2.07; 95% CI: 0.51–8.32), having parents who attended only elementary school (adjusted OR: 2.15; 95% CI: 0.66–6.96), living in areas without garbage collection service (adjusted OR: 1.13; 95% CI: 0.40–3.16) and accumulate rainwater or use water wells for food preparation (adjusted OR: 4.37; 95% CI: 0.86–22.03). These inappropriate conditions could explain the early exposition to DENV in studied children (Table 5).

Finally, we analyzed other variables related to reinfections by DENV, for instance, those people who did not consider standing water to be a risk factor (adjusted OR: 1.31; 95% CI: 0.26–6.55) and who did not have a waste collection service (adjusted OR: 3.44; 95% CI: 0.79–14.89) could rise the likelihood of being reinfected (Table 6).

DISCUSSION

This research used both serological and virological tests to describe the behavior of DENV circulation and infection in children of two small Colombian municipalities. Strikingly, molecular and serological techniques enabled us to establish that 40.1% of children younger than 14 years living in these areas were suffering continuous DENV infection and reinfection. This study showed that these continuous infections are significantly more frequent in children older than 8 years and those living for more than 7 years in these municipalities. Other variables such as poor water supply and inadequate sanitation and waste disposal services, and low knowledge level about dengue disease or poor practices to control mosquitoes, could also explain the DENV infection and reinfection as reported previously.^{28,29}

Historically, the Department of Cundinamarca annually contributes a high number (average of 3,610 in the last 5 years)

of dengue cases in the country, and 62 municipalities, including those evaluated in the present study, are considered endemic.^{30,31} Despite this situation, it is emphasized that the factors that most affected the constant infection and reinfection by DENV in the children of these areas were mainly the poor education and awareness by the inhabitants about the disease, its transmission, and control strategies, highlighting how inefficient the control policies of the disease and the vector have been.³²

Poor housing development and poor socioeconomic conditions are often associated with a higher prevalence of dengue infections, mainly owing to poor management of waste (which become breeding grounds) and the constant shortage of aqueduct services which forces villagers to store water in containers for several days, allowing the proliferation of vectors.^{31,32} These unfavorable conditions added to the rapid and disorganized urbanization of these small municipalities, and the phenomena associated with global warming make mosquito breeding more efficient, even reaching areas previously mosquito free, such as distant rural areas³³ and allowing permanent circulation of the four viral serotypes as previously reported.²⁴ This could explain why we found similar prevalence of infections in our study among children coming from urban and rural areas. Previously, a high *Aedes aegypti* infestation was reported in rural schools, although with differences according to rainfall patterns, increased entomological rates, and a simultaneous increase in the number of dengue cases in the area.^{20,34}

Although the transmission of dengue is mainly in urban areas, in Colombia, the rapid and disorganized growth of the municipalities has allowed the mixing between rural and urban population to favor the spread of the mosquitoes, thus homogenizing the risk of DENV infection between the zones.³⁵

The increasing circulation of the mosquitoes and the virus and the sustained increase in these cases generates a high burden of the disease in Colombia. For example, DALYs lost for 2010 epidemic were 1,198 per million inhabitants with a total financial cost of U.S. \$167.8 million, U.S. \$129.9 million for 2011, and U.S. \$131.7 million for 2012,¹⁰ considering that these amounts were based on the individuals who were attended to or diagnosed, and the values can change if the contribution of the asymptomatic infections in the transmission dynamics are included. We found that residents of Anapoima and Apulo municipalities have basic knowledge about dengue disease, but they did not show the ability to transfer them toward everyday habits of collection and disposal of solid waste or container water storage habits, revealing a courtesy bias of parents during the surveys answering. Having in mind that the main factor associated with breeding site reduction in endemic areas is the commitment of inhabitants, it is imperative to promote their active participation in the control of both the mosquito proliferation and dengue disease.³⁶

TABLE 3
Multivariate analysis of infection and reinfection groups

Variable	Raw OR	CI 95%	(P value)	Adjusted OR	CI 95%	(P value)
> 8 years old	5.21	2.26–12.0	(0.00)	4.62	1.68–12.74	(0.003)
Low weight risk, moderate or severe malnutrition	2.06	0.88–5.13	(0.07)	2.04	0.72–5.82	(0.78)
No waste disposal service	0.81	0.35–1.96	(0.60)	1.30	0.42–3.96	(0.64)
More than 7 years living in municipalities	8.90	3.70–22.7	(0.000)	6.97	2.52–19.26	(0.000)

CI = confidence interval.

TABLE 4
Multivariate analysis of asymptomatic infection group

Variable	Raw OR	CI 95%	(P value)	Adjusted OR	CI 95%	(P value)
> 8 years old	1.76	1.03–3.07	(0.02)	1.94	1.06–3.56	(0.03)
Subsidized regimen	1.44	0.89–2.34	(0.11)	1.60	0.96–2.68	(0.07)
Rural area	0.66	0.41–1.06	(0.07)	0.61	0.34–1.08	(0.09)
Risk of low weight, severe or moderate malnutrition	1.18	0.74–1.87	(0.45)	1.32	0.79–2.19	(0.27)
Incorrect disposal and collection of solid waste	1.12	0.59–2.16	(0.69)	1.12	0.56–2.24	(0.73)
No garbage collection service	0.90	0.53–1.49	(0.67)	1.14	0.63–2.09	(0.64)
No aqueduct	0.96	0.47–1.88	(0.89)	1.12	0.44–2.84	(0.79)
More than 7 years living in municipalities	1.90	1.13–3.22	(0.009)	1.30	0.74–2.29	(0.34)

CI = confidence interval.

Our findings showed an association between the parents' low level of schooling and the risk of exposure to the virus from an early age which was reflected in the high seroprevalence (87.9%) of dengue in children, which has been associated with poor practices of vector control. In the same way, studies conducted in Panama,³⁷ Iquitos, Perú,³⁸ and Caribbean region in Colombia³⁹ have shown that people with higher educational levels reported having better knowledge and practices of dengue control, resulting in lower numbers of dengue cases. Many studies reported that high number of dengue disease cases in a region are associated with poor social and economic conditions, generally related with an increase in mosquito breeding sites.^{25,40} However, other factors also favor the continuous transmission of the disease, such as unplanned urbanization with low sanitary service coverage and inefficient management of those financial resources destined to vector control. In addition, frequently large gaps in KAP of people from endemic areas are identified, which point out the inability of health authorities to transmit effective messages, adapted to the sociocultural patterns of those populations to which the actions are intended, undermining the DENV control results.¹⁶

Another aspect we highlight is that there was confusion among the residents about the signs and symptoms of dengue because a high percentage of parents or caregivers indicated diarrhea and vomiting as symptoms which do not normally occur during dengue (although vomiting can be considered part of the dengue signs of alarm).^{41,42} Parents' lack of knowledge about the main signs and symptoms could be associated with the fact that the disease is only recognized at an advanced stage which is associated with severe dengue, as reported in the epidemic in Iquitos, Peru, between 2010–2011.⁴³ In addition, the percentage of parents who considered it necessary to consult the doctor was very low, and self-medication was identified as a bad practice. Avoiding proper treatment of cases and not recognizing severe cases early are some of the most important factors that affect the increase in the number of cases of severe dengue and fatal cases.^{44–46}

As a result of the large panel of diagnostic tests used, we have been able to report a high proportion of children with

asymptomatic infections (40.1%). This is of particular importance because the Colombian surveillance system has reported in recent years that children younger than 14 years is the group with the highest number of cases of dengue, severe dengue, and dengue lethality,^{12,15,47–49} and that the subgroup aged 5–9 years are the most affected, similar to what was found in this study. The phenomenon of infections and reinfections in the children of Anapoima and Apulo municipalities is partly explained by bad practices and inadequate care by parents and caregivers because children are being infected in their homes or schools. Being passive carriers of the virus, they can transmit it to other children or adults in these same environments, increasing transmission of dengue and possibly other arboviruses.^{50–54} Keeping in mind that severe forms of dengue occur mainly during secondary infections, early infection of these children with DENV increases their risk for developing severe signs and symptoms in a future infection.⁴³ Furthermore, these asymptomatic carriers are not detected by surveillance systems, which makes it even more difficult to carry out prevention and control measures in their homes or schools.

The description of the conditions that are associated with reinfections and asymptomatic cases should motivate us to rethink strategies for the control and epidemiological surveillance of dengue in hyperendemic countries. This would include molecular detection in school children, based on our results, which showed great underreporting and the most efficient routes of vector and virus spreading via asymptomatic individuals as reservoirs. It is necessary to include in the surveillance strategies, the combined use of several serological and molecular tests that allow the detection of symptomatic or asymptomatic secondary infections to program control actions in a better way.²⁵ It is very likely that new approaches and surveillance strategies will help reduce DALYs, the financial cost,⁵⁵ and the likelihood that these children could face a new infection that evolves into severe dengue forms.⁴³

The control and eradication of dengue depends mainly on the community. Therefore, an additional effort is required in educational campaigns that sensitize the population of the endemic

TABLE 5
Multivariate analysis of the group with infection history

Variable	Raw OR	CI 95%	(P value)	Adjusted OR	CI 95%	(P value)
> 8 years old	4.32	1.94–9.65	(0.00)	2.60	0.91–7.41	(0.07)
Risk of low weight, severe or moderate malnutrition	2.15	0.94–5.28	(0.05)	1.35	0.47–3.84	(0.56)
Incorrect disposal and collection of solid waste	1.25	0.41–3.30	(0.63)	2.07	0.51–8.32	(0.30)
Elementary education of parents	1.20	0.52–2.91	(0.64)	2.15	0.66–6.96	(0.19)
No garbage collection service	0.89	0.39–2.10	(0.76)	1.13	0.40–3.16	(0.82)
More than 7 years living in municipalities	17.46	3.17–18.0	(0.000)	9.43	3.15–28.2	(0.000)
Food preparation with rainwater or well water	1.20	0.46–3.51	(0.68)	4.37	0.86–22.03	(0.07)

CI = confidence interval.

TABLE 6
Multivariate analysis of reinfection group

Variable	Crude OR	CI 95%	(P value)	Adjusted OR	CI 95%	(P value)
> 8 years old	6.65	2.73–16.2	(0.00)	7.74	1.98–30.16	(0.03)
Rural area	0.53	0.22–1.25	(0.11)	0.45	0.11–1.82	(0.26)
Risk of low weight, severe or moderate malnutrition	2.29	0.96–5.79	(0.04)	2.81	0.78–10.0	(0.11)
Standing water	0.47	0.17–1.32	(0.10)	1.31	0.26–6.55	(0.73)
No waste collection service	0.69	0.29–1.73	(0.37)	3.44	0.79–14.8	(0.09)
More than 7 years living in municipalities	10.15	4.0–26.4	(0.000)	13.27	3.54–49.7	(0.000)

CI = confidence interval.

municipalities with greater force to improve their participation in mosquito and disease elimination. To strengthen the impact of dengue control strategies in Colombia, the community must be directly and actively involved in the generation, elaboration, and development of campaigns and projects that seek to reduce breeding sites. Although these types of interventions require more time and resources, they have been found to generate stronger links between protagonists, supporting program sustainability and an eco-health approach in populations.³⁶

Limitations. As with any cross-sectional study, our study was not alien to the limitations of this type of study design. It was not possible to establish causal associations; however, it was possible to generate hypotheses that need to be proven in later prospective investigations.

The effect of bias by the participants was minimized; although a convenience sampling was performed, the tests that were used for the detection of the cases were highly sensitive and specific and in turn an established and tested diagnostic algorithm was applied. There was probably a courtesy bias at the time of applying the KAP survey to the parents. Furthermore, because of the fact of the KAP survey not being applied in the family environment, it was not possible to verify the housing conditions and their answers.

It has been shown that the test for NS1 antigen detection in asymptomatic patients is not very sensitive in low viremic conditions; thus, all samples were negative. However, this was corrected by molecular viral detection tests.

The probability of information bias was decreased because all the people who were invited to participate in the study agreed; memory bias may have been present because parents more accurately recall the medical history of dengue fever with signs of alarm or severe dengue in those children who presented them compared with those who had mild signs.

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