Prevalence and Risk Factors of Soil Transmitted Helminths Infection and Malnutrition Among School-Aged Children in Ibadan

ABSTRACT

Background: In developing countries, infections caused by soil-transmitted helminths (STH), such as Ascaris lumbricoides, Trichuris trichiura, and hookworms pose major public health problems among school children, resulting in impaired physical growth such as stunting and thinness, and cognitive development.

Objectives: This study aimed therefore to determine the prevalence of STH, malnutrition and factors associated with transmission among school-age children (SAC) in four local government areas in Ibadan.

Methods: A cross-sectional study was carried out in eight primary schools in Ibadan, Oyo State, Nigeria, from May to November 2018. SAC ages 5 to 18 years old were randomly selected from primary one to six, across four Local Government Areas. Stool samples were collected and helminth eggs were quantitatively estimated using the Kato-Katz thick smear technique. Anthropometric data were obtained using a height measuring tape to the nearest 0.1 cm while weights were recorded using a weighing scale to the nearest 0.1 kilograms. Malnutrition indices such as stunting and thinness were defined as Height-for-Age Z-score [HAZ] < -2Standard Deviation (SD) and Body-Mass-Index-for-Age Z-score [BAZ] < -2SD respectively. Multivariable logistic regression was used to assess factors associated with STH, thinness, and stunting.

Results: A total of 458 SAC took part in the study, and an overall prevalence of 9.0%(95% CI 6.6 to 11.6) was observed for STH; 7.6%(95% CI 5.2 to 10.0) for A. lumbricoides, 2.8% (95% CI 1.5 to 4.6) for Trichuris trichiura, and 1.5% (95% CI 5.4755.18) for double infection. Stunting and thinness were 24.7% (95% CI 94.5 to 98) and 27.3% (95% CI 29.5 to 38.6) respectively, based on the WHO reference Growth Standards. Notably, the ages of the children (Adjusted OR= 1.688; 95% CI: 1.412 to 2.018; P<0.001), unhygienic classes (Adjusted OR= 0.729; 95% CI: 0.559 to 0.950; P= 0.019), and improper washing of hands (Adjusted OR= 0.815; 95% CI: 0.381 to 1.741; P = 0.031) were important factors associated with determining, stunting, thinness and STH infestation.

Conclusions: The study has highlighted factor that predisposes SAC to a high risk of STH infection, stunting, and thinness. Improper washing of hands, such as washing without soap, among the sampled children were more likely to be positive for STH infestation and thinness than those who washed their hands properly after defecation. Promotion and supervision of students’ hygiene and improvement in parents’ socio-economic status may further help to reduce the prevalence of STH, stunting, and thinness among school children in these LGAs in Ibadan.

Keywords: Soil-transmitted helminths, School-age-children, Stunting, Thinness, Ibadan

INTRODUCTION

Among the most prevalent neglected tropical diseases worldwide, which affect the poorest and most deprived communities, are those caused by soil-transmitted helminths (STH) [1]. The roundworm (Ascaris lumbricoides), hookworms (Necator americanus and Ancylostoma duodenale), and whipworms (Trichuris trichiura) are the main that infect...
people. In areas where there is poor hygiene, STH transmission is by ingesting eggs present in human faeces, which invariably contaminate the soil, or through skin penetration. World Health Organization [2] estimation indicated that more than five billion people are at risk and over two billion people are infected with these parasites globally [3]. School-Aged Children (SAC) of about one billion are at risk of being infected with at least one STH species [4]. There is a concentration of STH infections in impoverished regions such as tropical and subtropical, where clean water and provision of sanitation are deficient [5,6,7]. Prominent symptoms of infected individuals with STH are anemia, stunted growth, abdominal pain, diarrhea, mental retardation, and death when infections are chronic and untreated [8,9,10].

A common health problem in the world is malnourishment, i.e. a lack of sufficient nutrients in the body, one out of three people are malnourished [11]. The STH infections are found to predispose one to malnutrition; for instance, susceptibility of SAC to parasitic infection can lead to poor growth and development as a result of their poor intake of nutrients [11,12]. When STHs feed on host tissue, it will lead to a deficiency of protein and iron, loss of appetite, diarrhea, dysentery, and an increment in malabsorption, thereby leading to malnutrition [13]. Stunting or low Height-for-Age Z score (HAZ) and thinness or low Body Mass Index (BMI)-for-age Z score (BAZ) are good indicators of malnutrition. Earlier studies from different countries have reported that STHs such as *Ascaris lumbricoides*, *Trichuris*, and *multi-parasitism* were linked with malnutrition in school-age children [14, 15]. Although studies on STH have been conducted in Ibadan [16], there is limited information on this study area. Conducting a study in this area will provide the needed information which will definitely support the ongoing control interventions targeted at STH. This study, therefore, aimed to determine the prevalence of STH infection, stunting, and thinness and their associated risk factors among SAC in four local government areas in Ibadan, Oyo State, from May and November 2018. The city has a population of about 3.8 million people. It has 11 Local Government Areas where the principal inhabitants are the Yorubas.

**Study population**

School-aged children ages 5 to 18 years old, from primary one to six across four Local Government Areas (LGA), namely: Ibadan South-East (IBSE) LGA, Ibadan North-West (IBNW) LGA, Akinyele (AKIN) LGA and Ido (IDO) LGA, took part in the study. The inclusion criteria include individuals who were willing to participate in the study, and whose parents or guardians gave consent. Children whose parents or guardians failed to give their consent to the study and children who were on drugs for any infection were excluded.

**Sample size determination and sampling**

Two schools, out of about ten schools present in each LGA, and a minimum of 50 pupils in each school (SCH) were randomly selected by the Local Inspector of Education (LIE) per LGA: IBSE-SCH1 and IBSE-SCH2; IBNW-SCH1 and IBNW-SCH2; AKIN-SCH1 and AKIN-SCH2; IDO-SCH1 and IDO-SCH2. With an estimate, the minimum acceptable sample size of 354 participants, using the single population proportion formula, was obtained.

Sample size \( n = \left( \frac{Z^{2} \times p \times q}{d^{2}} \right) \) where

- \( Z \) = standard normal deviate at 95% Confidence Interval = 1.96
- \( p \) = prevalence of 35.9% (0.359 from Ikeoluwa et al. [16])
- \( q = 1 - p = (1 - 0.359) = 0.641 \)
- \( d \) = the margin of error, which is taken as 5% = 0.05

The sample size is 354.

To avoid errors that might arise from the probable occurrence of non-compliance, 41% of the sample size was added to the calculated sample size, thereby making it 500.

**Collection of demographic history and examination of stool samples**

All individuals in the study were interviewed with a structured questionnaire to obtain demographic information such as age, sex, education, history of worm infestation and frequency of treatment, socio-economic status of the parent, mother’s level of education, availability of toilet facilities at schools.
and homes, hand washing practices, etc. Data were mainly obtained from the children with a little help from their teachers. Individuals who met the inclusion criteria were given sterile bottles (100 mL) and were instructed on how to do a proper faecal collection. Helminth eggs in stool were quantitatively estimated using the Kato-Katz thick smear technique. Parasite intensities were determined using direct egg per gram count (epg) according to standard protocols [17]. The intensity of STH infection was expressed as the number of eggs per gram (epg) of faeces: *A. lumbricoides*, 1–4,999 epg, 5,000–49,999 epg, and ≥50,000 epg for light, moderate, and heavy, respectively; while in *T. trichiura* infection the presence of 1–999 epg, 1,000–10,000 epg, and >10,000 epg for light, moderate and heavy, respectively [18]. All children positive for STH infection were treated with benzimidazole (mebendazole) by a health officer.

**Collection of Anthropometric Data**

The children’s height and weight were measured alongside the age and sex data obtained via the questionnaire to determine their anthropometric indices. The heights of the children were measured to 0.1 centimeters (cm) using a height measuring tape while the pupils’ weights were recorded using a scale to the nearest 0.1 kilograms (kg) using a weighing scale. Height and weight were used to determine HAZ (Height-for-Age Z-score) and BAZ (Body-Mass-Index-for-Age Z-score) using ANTHROPLUS software [19]. Stunting and thinness or underweight were defined as HAZ and BAZ <−2 Standard Deviation (SD), respectively [20, 21].

**Ethical Approval**

Approvals for the protocol were obtained from the University of Ibadan/University College Hospital (UI/UCH) Ethical Review Committee, College of Medicine, University of Ibadan (NHREC/05/01/2008a); Oyo State Ministry of Health (AD 13/479/614), Oyo State Ministry of Education (EDU. 215T2/3), and Oyo State Universal Primary Education Board (SUPEB) (SUBEB/G.11571/32). Parent Teacher Association meetings were held before the commencement of the study. Written informed consent and assent were obtained from the parents and their children/ward, respectively.

**Data analysis**

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 20.0 and Microsoft Excel version 2009. Overall descriptive statistics was carried out to select variables for consideration in multivariate regression models, *A. lumbricoides* and *T. trichiura* status as dependent variables. Anthropometric data were analyzed using World Health Organization Child Growth Standards (Anthroplus) [18]. Multivariate analyses of the prevalence and intensity of STH infection were done with socio-demographic indicators of age, sex, risk behaviors such as handwashing, previous history of deworming treatment, and use of toilets. Multivariate logistic regression analysis was used to evaluate factors independently associated with STH, stunting, and thinness. The probability value was set at p<0.05

**Results**

**Socio-demographic characteristics of study participants**

Out of the four Local Government Areas that were studied, 458 out of the 500 recruited children were eligible to partake in this study; this is a result of some parents not giving their consent and the inability of some pupils to submit faecal samples. The characteristic of the studied population is represented in Table 1. 10.4 years (range 5 – 18) was the mean age; most of the participants were from ages 9-12 (P= 0.018), boys were slightly more than girls (51.2% vs 48.8%, P=0.452) and most of them claimed to wash their hands regularly after using the toilet (P= 0.002). The participants had access to sewer systems at home (96.3%) and at school (85.8%). The majority of the children (81.4%) had been treated with an anthelminthic drug in the past 12 months (P= 0.013).

**Prevalence and intensity of soil-transmitted helminthiasis**

The prevalence of soil-transmitted helminthiasis with at least one parasite was 9.0% (95% CI 6.6 to 11.6). Most frequent infections were with *A. lumbricoides* 7.6% (95% CI 5.2 to 10.0) followed by *T. trichiura* 2.8% (95% CI 1.5 to 4.6) and 1.5% (95% CI 5.47 to 55.18) for double infection. Hookworm was not detected in this study. In this study,
18 (3.9%) and 17 (3.7%) participants had light and moderate *A. lumbricoides* infection, respectively, while for *T. trichiura* infection, 11 (2.4%) and 2 (0.4%) participants had light and moderate infections, respectively. We observed that there was no record of heavy infection found for the two STHs. The median intensity of infection was 4824 eggs/g (epg) with a range of 24–36000 and 72 eggs/gram (epg) with a range of 24–3696 for *A. lumbricoides* and *T. trichiura*, respectively.

Of the eight selected schools in the four Local Governments in Ibadan, the prevalence of STH infection was significant (p<0.001) (Fig. 1). In this study, IBSE-SCH1 was observed to have the highest rates of ascariasis and trichuriasis (15% and 10%, respectively) while 0–8% prevalence was observed for each of the two parasites in the other schools. Among infected children, light and moderate infection intensities with *A. lumbricoides*, 3.9%, and 3.7%, respectively, and *T. trichiura*, 2.4%, and 0.4% respectively, were detected. The intensity of both infections was generally higher among males but not statistically significant; for instance, in *A. lumbricoides*, light infection: male=28.6%, female=22.9%; moderate infection: male=25.7%, female=22.9%; for *T. trichiura* males=46.2%, females=38.5% (light infection) and males=15.4%, females=0% (moderate infection). The prevalence and intensity patterns for *A. lumbricoides* and *T. trichiura* vary by age of the SAC in this study, children between the ages 9–12 years had a high prevalence of *Ascaris lumbricoides* and *T. trichiura* but was lower in other children.

### Factors associated with STH infestation

After multivariate logistic regression of factors associated with STH, there was strong evidence that children from ages 9 to 12 were more likely to be infested with

**Table 1: Socio-demographic characteristics of the studied population**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Soil Transmitted Helminths (STH)</th>
<th>Df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (%)/Absent (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>5-8</td>
<td>41</td>
<td>0.018*</td>
</tr>
<tr>
<td></td>
<td>7.3%/15.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9-12</td>
<td>319</td>
<td></td>
</tr>
<tr>
<td></td>
<td>73.1%/76.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13-18</td>
<td>37</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>19.5%/8.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class</td>
<td>1-3</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39.0%/39.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>25</td>
<td>0.452</td>
</tr>
<tr>
<td></td>
<td>71.0%/60.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51.2%/45.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>20</td>
<td>0.854</td>
</tr>
<tr>
<td></td>
<td>48.8%/54.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilet at School</td>
<td>Yes</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>87.8%/84.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5</td>
<td>0.402</td>
</tr>
<tr>
<td></td>
<td>12.2%/15.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilet at Home</td>
<td>Yes</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>92.7%/95.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td>7.3%/4.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment of STH within one year</td>
<td>Yes</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.4%/80.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>7</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td>1.5%/19.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand-washing after toileting</td>
<td>Regularly</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>82.9%/82.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sometimes/Never</td>
<td>7</td>
<td>0.402</td>
</tr>
<tr>
<td></td>
<td>17.1%/17.9%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DF: Degree of freedom, P value in *- statistically significant (P <0.05)
STH infestation compared to other ages (Adjusted OR = 0.814; 95%CI: 0.650 to 1.019; P= 0.05) while children who do not wash their hands or washed only with water after using the toilet were also prone to STH infestation (Adjusted OR = 0.815; 95%CI: 0.381 to 1.741; P= 0.031).

Prevalence of malnutrition

In this study, the prevalence of stunting and thinness were 24.7% (95% CI 94.5 to 98) and 27.3% (95% CI 29.5 to 38.6), respectively. The prevalence of stunting and thinness between sampled schools varied significantly (P= 0.003 and P= 0.013 respectively), and IBSE-SCH2 was observed to have the highest prevalence of malnutrition, (23% stunting) and (20% thinness) respectively (Fig. 2).

Factors associated with stunting and thinness

The result of the multivariate analysis is represented in Table 2. Associated factors for stunting were: Ages (Adjusted OR = 1.688; 95%CI: 1.412 to 2.018; P< 0.001) and unhygienic classrooms (Adjusted OR = 0.729;
Table 2: Multivariate analysis of factors associated with stunting, thinness, and STH among school-aged children in Ibadan

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stunting (n=458) Adjusted OR (95% CI)</th>
<th>B</th>
<th>P-value</th>
<th>Thinness (n=458) Adjusted OR (95% CI)</th>
<th>B</th>
<th>P-value</th>
<th>STH (n=458) Adjusted OR (95% CI)</th>
<th>B</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages</td>
<td>1.688 (1.412, 2.018)</td>
<td>0.523</td>
<td>&lt;0.001**</td>
<td>1.189 (1.020, 1.386)</td>
<td>0.173</td>
<td>0.027**</td>
<td>0.814 (0.650, 1.019)</td>
<td>-0.206</td>
<td>0.05</td>
</tr>
<tr>
<td>Classes</td>
<td>0.729 (0.559, 0.950)</td>
<td>-0.317</td>
<td>0.019**</td>
<td>0.875 (0.678, 1.116)</td>
<td>-0.133</td>
<td>0.282</td>
<td>1.216 (0.837, 1.765)</td>
<td>0.195</td>
<td>0.305</td>
</tr>
<tr>
<td>Sex</td>
<td>0.885 (0.559, 1.402)</td>
<td>-0.122</td>
<td>0.603</td>
<td>0.998 (0.651, 1.529)</td>
<td>-0.002</td>
<td>0.992</td>
<td>1.121 (0.576, 2.179)</td>
<td>0.114</td>
<td>0.737</td>
</tr>
<tr>
<td>Toilet at school</td>
<td>0.840 (0.471, 1.500)</td>
<td>-0.174</td>
<td>0.556</td>
<td>0.430 (0.209, 0.885)</td>
<td>-0.845</td>
<td>0.022**</td>
<td>1.505 (0.517, 4.377)</td>
<td>0.409</td>
<td>0.453</td>
</tr>
<tr>
<td>Hand-washing after toiletting</td>
<td>1.022 (0.582, 1.793)</td>
<td>0.022</td>
<td>0.940</td>
<td>2.107 (1.281, 3.467)</td>
<td>0.745</td>
<td>0.003**</td>
<td>0.815 (0.381, 1.741)</td>
<td>-0.205</td>
<td>0.031**</td>
</tr>
<tr>
<td>Father’s</td>
<td>0.888 (0.767, 1.028)</td>
<td>-0.119</td>
<td>0.111</td>
<td>1.012 (0.887, 1.154)</td>
<td>0.011</td>
<td>0.864</td>
<td>1.167 (0.434, 1.459)</td>
<td>0.155</td>
<td>0.174</td>
</tr>
</tbody>
</table>

P value in ** are statistically significant (p<0.05)
95% CI: 0.559 to 0.950; P = 0.019); while thinness were: ages (Adjusted OR = 1.189; 95% CI: 1.020 to 1.386; P = 0.027), unhygienic school toilet/poor toilet practice (Adjusted OR = 0.430; 95% CI: 0.209 to 0.885; P = 0.022) and children who do not wash their hands or washed only with water after using the toilet were prone to lose weight twice compared to those who washed their hands properly with soap and water (Adjusted OR = 2.107; 95% CI: 1.281 to 3.467; P = 0.003).

Discussion
This study recorded a prevalence of 41 (9.0%) for helminth infestations, the low prevalence of STH infestation may not be farfetched from the mass drug administration (MDA) that recently occurred all over the state around four months before the commencement of this study, thereby showing the effectiveness of MDA in reducing the prevalence of STHs infestation. The low prevalence of STH infestation in this study is in contrast with earlier studies conducted by Ikeoluwapo et al., [16], Ibrahim et al., in Igbo-Ora, a semi-urban town in Oyo State, Nigeria [22], and Ojurongbe et al., [23] where 35.9%, 28.8%, 40.2%, 55.0% [24] and 84.4% [25] prevalence of STH infestation were observed, respectively. We found that Ascaris lumbricoides was the most prevalent helminth, followed by T. trichiura. This was expected, as both parasites exhibit a similar mode of entry into human beings, their definitive host, when embryonated eggs are ingested from the environment. These embryonated eggs are known to require similar conducive conditions in the environment for embryonation such as warmth, a shady environment, and moisture in the soil [26]. Therefore, this could be a further reason for their co-existence in the schools that were sampled. Hookworm was not recorded in this study, as about 76% of the pupils were observed to wear their shoes when playing on soil surfaces, thereby preventing the spread of hookworm which is acquired when the infective larvae in the soil penetrate bare feet, unlike in Igbo-Ora, where a relatively low percentage of students (38.7%) wear shoes and hookworm was reported as the highest STH.

In this study, the prevalence of STHs infection varied significantly between schools. Ibadan South-East was observed to have the highest prevalence of STH infestation. In agreement with another study done by Ikeoluwapo et al., [16], Ibadan South-East could be tagged as a hotspot for STH infestation. This may be a result of the schools being located in a densely unhygienic populated area and the unhygienic lifestyle of both the pupils and their parents may enhance the spread of the infection.

We found that the ages of the children, and improper washing of hands after toileting were important associated factors determining STH infestation, stunting, and thinness. The age group of the studied SAC, found in unhygienic environments, is known to be active and may engage in activities, such as not washing hands after toileting or using water without soap to wash their hands, unconscious geophagy, etc, which may promote the spread of the infection. This is corroborated by Ikeoluwapo et al., and Moncayo et al., [16, 27].

Although the study recorded a low prevalence of STH infestation, stunting and thinness had a high prevalence, and IBSE-SCH2 was found to have the greatest prevalence of thinness (20%) and stunting (23%). Unlike low prevalence of STH recorded, each school visited has at least a case of stunting and thinness; this may be as a result of prolonged history of being malnourished, or probably due to other infections that can predispose them to malnutrition[28]. STH infestations, according to Stephenson et al.,[29] are generally known to be a major contributor to malnutrition which could influence the height and weight of infected pupils. STH could affect growth in SAC by several mechanisms which may include a reduction in food intake, malabsorption, and reduced appetite [30]. However, this study did not investigate how STH infestation could impact stunting and thinness among SAC. Therefore, to confirm this observation in this area, further studies will be needed.

In this study, hand-washing habits after toileting, unhygienic toilet at school, and the age of the sampled children were important factors associated with the spread of STH, stunting, and thinness.

Thus, if teachers are better grounded/educated on Water, Sanitation and Hygiene (WASH) and proper health and nutrition behaviours, they will definitely help in transferring the knowledge to both the pupils and
their parents. This is corroborated by Tefera et al., and Moncayo et al., [24, 25] who reported that students who do not wash their hands after toileting are more likely to develop STH infection than those who wash their hands after defecation. Most importantly, regular treatment with benzimidazole anthelmintic drugs in school children which reduces and maintains the worm burden below the threshold associated with the disease [31] should be sustained. This will definitely aid in reducing and/or eradicating STH infections among SAC. Therefore, educational interventions on health and nutrition with teachers and SAC cannot be overemphasized.

Conclusion
In conclusion, the study reported a low prevalence of STH infestation among SAC in four LGAs in Ibadan due to the recent administration of MDA. Schools in Ibadan South-East could be tagged as a hotspot for STH infestation, stunting, and thinness as they recorded the highest prevalence among all schools that were sampled. The ages of the sampled children, the unhygienic toilet, and their habits of not washing hands/improper hands washing after using the toilet were associated factors for stunting, thinness, and STH infestation. Nonetheless, there is a need for more research into the health impact of STH-polyparasitism and other infections that can predispose SAC to malnutrition. In addition, the government should sustain MDA and improve the socio-economic status of the pupils’ parents as about 74.2% of fathers were either unemployed or pettry traders, this will aid in curbing STH infestation, stunting, and thinness among SAC.

Authors’ contributions
AGI conceived the study, drafted the proposal, carried out data collection and laboratory examination, carried out analysis and interpretation of the results, and finalized the write-up of the manuscript. MKT revised the proposal and the manuscript, supervised data collection and laboratory examination. RIN revised the proposal, supervised data collection, and laboratory examination. All authors read and approved the final manuscript.

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