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Análisis de factores de riesgo y tratamiento de la malnutrición en edad escolar. Protocolo de actuación "aquí" para extrapolar "allí"

TESIS DOCTORAL

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"Mucha gente pequeña, en lugares pequeños, haciendo cosas pequeñas, puede cambiar el mundo"

Eduardo Galeano

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RESUMEN

La malnutrición en población escolar es un problema de Salud Pública que aqueja a nivel mundial a millones de niños y niñas y, lamentablemente, sigue existiendo. En el caso de los países de ingresos bajos y medios como Ecuador, la desnutrición infantil era considerada su gran problema a resolver, pero esta realidad lleva cambiando en los últimos años, debido a la transición epidemiológica nutricional que atraviesan; en los países de ingresos altos como España, el exceso de peso alcanza proporciones epidémicas, y que no dejan de incrementar. Para valorar la malnutrición infantil, se utilizan como referencia los indicadores antropométricos (puntuaciones Z o *Z-score*) desarrollados por la Organización Mundial de la Salud, pero la información que proporcionan es limitada; para mejorar el diagnóstico se puede unificar dos de sus indicadores: el BIMZ (del inglés, *Body Mass Index Z-score*) y HAZ (del inglés, *Height-For-Age Z-score*) en la Nutrimetría, la cual combina estas dos variables antropométricas, con la intención de facilitar su interpretación y generar una visión más completa del estado nutricional. Ha sido ampliamente estudiada la relación de la malnutrición con los estilos de vida (alimentarios, actividad física y sedentarismo), pero esto no sucede con un factor importante como es el parasitismo intestinal, el cual históricamente ha afectado a países de ingresos económicos bajos y medios, pero también a países de ingresos altos como España, debido a cambios demográficos, climáticos y de comportamiento. El sector educativo es una piedra angular en la batalla contra la malnutrición infantil. Sin embargo, aún no existen protocolos consolidados que delineen estrategias sobre cómo los programas de nutrición pueden ser ejecutados a través de este sector, adaptados a la realidad local y que sean sostenibles.

Por todo ello, nos propusimos analizar el estado nutricional de poblaciones escolares en diferentes contextos y su asociación con aquellas variables clásicas (dieta, ejercicio físico y otros estilos de vida), así como con otras variables más novedosas como el parasitismo intestinal. Este análisis nos permitió realizar un diagnóstico previo a la propuesta de una intervención nutricional de vital importancia para el éxito de la misma.

En el estudio participaron escolares de 3 a 11 años de edad de la Comunidad Valenciana (España) y de las provincias de Chimborazo (municipios Pallatanga y Penipe) y Guayas (municipio General Antonio Elizalde, GAE) (Ecuador), de los cuales 206 eran de España y 574 de Ecuador. Se recogieron características sociodemográficas, alimentarias, de estilos de vida, hábitos de comportamiento, datos antropométricos y coproparasitológicos. El análisis del estado nutricional se hizo a través de la Nutrimetría. Para el análisis parasitológico, se empleó el examen microscópico convencional como método de cribado, y ensayos moleculares (PCR y secuenciación de Sanger) para investigar más a fondo la epidemiología de protistas intestinales concretos. Se realizaron análisis estadísticos para establecer las asociaciones entre el estilo de vida, las especies parasitarias seleccionadas y el estado nutricional. Se hizo un análisis de regresión logística multivariante para evaluar la fuerza de la asociación de los factores de riesgo sospechosos con la presencia de parasitismo intestinal.

La prevalencia de exceso de peso en los y las escolares fue del 32,6% y 29,2% para España y Ecuador, respectivamente; en España la desnutrición es prácticamente inexistente, mientras que en Ecuador afecta a más del 20,6% de los niños y niñas. La prevalencia de malnutrición infantil estuvo asociada con el nivel socioeconómico en Ecuador. El municipio con un mayor nivel socioeconómico (GAE) presentó cifras mayores de sobrepeso/obesidad. El 43,9% de los participantes españoles y españolas tenía una alta adherencia a la Dieta Mediterránea, cuya ingesta media diaria era de 2.428,7 kcal, un porcentaje mayoritario (72,3%) no cumple con las recomendaciones de actividad física, y el 30,6% dedica más horas de las recomendadas al uso de pantallas. Se identificó parasitismo intestinal en el 49,5% de los niños y niñas de España, siendo las especies más prevalentes *Giardia duodenalis* (28,6%), *Blastocystis* sp. (19,4%) *y Enterobius vermicularis* (19,4%); el agua de bebida consumida en el hogar resultó ser un factor de riesgo de parasitismo intestinal. En Ecuador, se identificó al menos una especie de parásito intestinal en el 63,2% de los escolares, destacando: *Blastocystis* sp. (39,2%), *G. duodenalis* (30,8%), y *Entamoeba coli* (26,3%). Se detectaron los genotipos A (50,0%), B (37,5%) y A+B (12,5%) de *G. duodenalis*, y ST3 (28,6%), ST1 y ST2 (26,2% cada uno) y ST4 (14,3%) de *Blastocystis* sp. y se identificaron tres genotipos, dos conocidos (A: 66,7%;

KB-1: 16,7%) y uno nuevo (HhEcEb1, 16,7%) de *Enterocytozoon bieneusi*; el municipio de origen, el hacinamiento en el hogar y los malos hábitos sanitarios fueron factores de riesgo de parasitismo intestinales en la infancia. Con toda la información analizada, se elaboró un marco de trabajo para la implementación de la educación para la salud, nutricional e higiénica, centrado principalmente en el ámbito de la escuela primaria.

Como conclusiones, podemos decir que la Nutrimetría es un buen indicador para un estudio completo del estado nutricional, fácil de utilizar e interpretar y adecuado para su uso a nivel clínico y epidemiológico. El nivel socioeconómico en Ecuador es una variable y está relacionado con la coexistencia de los dos tipos de malnutrición. Los estilos de vida son un aspecto fundamental a tener en cuenta por su relación con el estado nutricional, pero es necesario, sobre todo a nivel de hábitos dietéticos, combinar diversos métodos que mejoren su análisis y faciliten el hallazgo de asociaciones con el estado nutricional. En cuanto al parasitismo intestinal, las cifras encontradas son altas, tanto en España como Ecuador, por lo tanto, este factor necesita más atención para comprender su impacto en la salud de los niños y niñas; además en el caso de Ecuador a pesar de las campañas de desparasitación masivas siguen siendo un problema en las poblaciones pediátricas que viven en entornos de escasos recursos. Los resultados obtenidos de todas las variables demuestran la importancia de un adecuado diagnóstico previo a cualquier tipo de intervención; es importante que dicha intervención identifique los recursos promotores de salud ajustados y adaptados a la realidad de la población, junto con el establecimiento de alianzas para garantizar la sostenibilidad.

Palabras clave: malnutrición; nutrimetría; escolares; estilos de vida; parásitos intestinales; factores de riesgo; intervención nutricional.

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1.1. Definición, Clasificación y Características de la Malnutrición. Epidemiología de la malnutrición. Herramientas de Diagnóstico

La malnutrición representa un problema de salud multifactorial a escala mundial, siendo uno de los factores más relacionado a la morbilidad y mortalidad infantil, más de la tercera parte de las enfermedades de los niños y niñas se le atribuyen (Das *et al.*, 2020; Organización Mundial de la Salud [OMS], 2021a). Las consecuencias en el desarrollo y repercusiones a nivel económico, social y médico son graves y perecederas tanto para la población infantil como para sus familias, la comunidad y el país.

En este primer apartado se desarrollará el concepto de malnutrición con sus peculiares matices y las características que hacen de ésta un gran problema de salud pública (apartado 1.1.1). Se abordará también la epidemiología descriptiva a nivel mundial y en continentes y países como España y Ecuador (apartado 1.1.2). Por último se comentarán las herramientas que se utilizan en la actualidad para su diagnóstico (apartado 1.1.3).

1.1.1. Concepto de malnutrición, clasificación y características de la malnutrición

La malnutrición es las carencias, excesos o desequilibrios de ingesta calórica y de nutrientes de una persona (OMS, 2021a). La malnutrición es un concepto que abarca tanto el sobrepeso, la obesidad y las Enfermedades No Transmisibles (ENT) relacionadas con la dieta, como la desnutrición (la insuficiencia ponderal, las carencias de micronutrientes, la emaciación y el retraso del crecimiento) (Popkin *et al.*, 2020).

A continuación, se comentarán y explicarán con detalle los conceptos de malnutrición.

1.1.1.1. Desnutrición

La Sociedad Americana de Nutrición Parenteral y Enteral (ASPEN) la define como "un desequilibrio entre las necesidades de nutrientes y la ingesta, que provoca déficits acumulativos de energía, proteínas o micronutrientes que pueden afectar negativamente al

crecimiento, el desarrollo y otros resultados relevantes" (Nilesh *et al.*, 2013). En función de su etiología, la desnutrición puede estar relacionada con una enfermedad o causada por factores ambientales o de comportamiento asociados a una disminución de la ingesta o la administración de nutrientes (Dipasquale *et al.*, 2020). Según la OMS existen 3 tipos de desnutrición (Figura 1):

a) Emaciación, es la insuficiencia de peso respecto de la talla. Indica una pérdida de peso reciente y grave, dado por a una ingesta insuficiente de energía y nutrientes, su mala absorción, o una patología frecuente o prolongada y/o a que tiene una enfermedad infecciosa (por ejemplo: diarrea) que ha provocado la pérdida de peso. Un niño o niña que presente una emaciación moderada o grave tiene un riesgo mayor de morir sino recibe un tratamiento adecuado (OMS, 2021a; Organización de las Naciones Unidas para la Alimentación y la Agricultura [FAO], Fondo Internacional de Desarrollo Agrícola [FIDA], Organización Panamericana de la Salud [OPS], Programa Mundial de Alimentos [PMA] y Fondo de las Naciones Unidas para la Infancia [UNICEF], 2023).

b) Talla insuficiente para la edad, se denomina retraso del crecimiento o desnutrición crónica. Es consecuencia de una desnutrición prolongada en el tiempo o recurrente, se asociada a deficientes condiciones socioeconómicas, factores alimenticios/nutricionales, a una salud deficiente de la madre y a la recurrencia de enfermedades. Afecta el desarrollo físico y cognitivo de los niños y niñas aumentando su riesgo de morir por enfermedades infecciosas. También pueden predisponer a niños y niñas a tener exceso de peso y desarrollar a futuro ENT (Kerr *et al.*, 2000; OMS, 2021a; FAO, FIDA, OPS, PMA y UNICEF, 2023).

c) Insuficiencia ponderal, la presentan aquellos niños y niñas que pesan menos de lo que corresponde a su edad y es el estado resultante de una insuficiente alimentación (FAO, 2023). Los y las que lo padecen puede presentar a la vez retraso del crecimiento y/o emaciación (OMS, 2021a).

La desnutrición infantil tiene una relación directa con la disminución de la capacidad cognitiva, desarrollo y crecimiento que afectan la productividad a lo largo de la vida, debilita la inmunidad y con ello se da una mayor incidencia de enfermedades infecciones y parasitarias (Black *et al.*, 2013; Hajri *et al.*, 2020; Nugent *et al.*, 2020; Unicef, 2023). La desnutrición conlleva a una reducción del número de neuronas, sinapsis, arborizaciones dendríticas y mielinizaciones, provocando una disminución del tamaño del cerebro (Georgieff *et al.*, 2020). Los efectos sobre el cerebro en desarrollo pueden ser irreversibles después de los 3-4 años de edad (Koletzko *et al.*, 2015; Dipasquale *et al.*, 2020).

1.1.1.2. Sobrepeso, obesidad y enfermedades no transmisibles (ENT) relacionadas con la alimentación

Una persona padece sobrepeso u obesidad cuando tiene un peso superior al correspondiente a su estatura. Una acumulación anormal o excesiva de grasa puede afectar a la salud. Pueden ser consecuencia de un desequilibrio en donde las calorías gastadas son menores a las consumidas (Mayneris-Perxachs & Swann, 2019; OMS, 2021a; FAO, FIDA, OPS, PMA y UNICEF, 2023). Las ENT relacionadas con la alimentación abarcan las enfermedades cardiovasculares, algunos tipos de cánceres y la diabetes tipo 2 (NCD Risk Factor Collaboration, 2017; OMS, 2021a) (Figura 1).

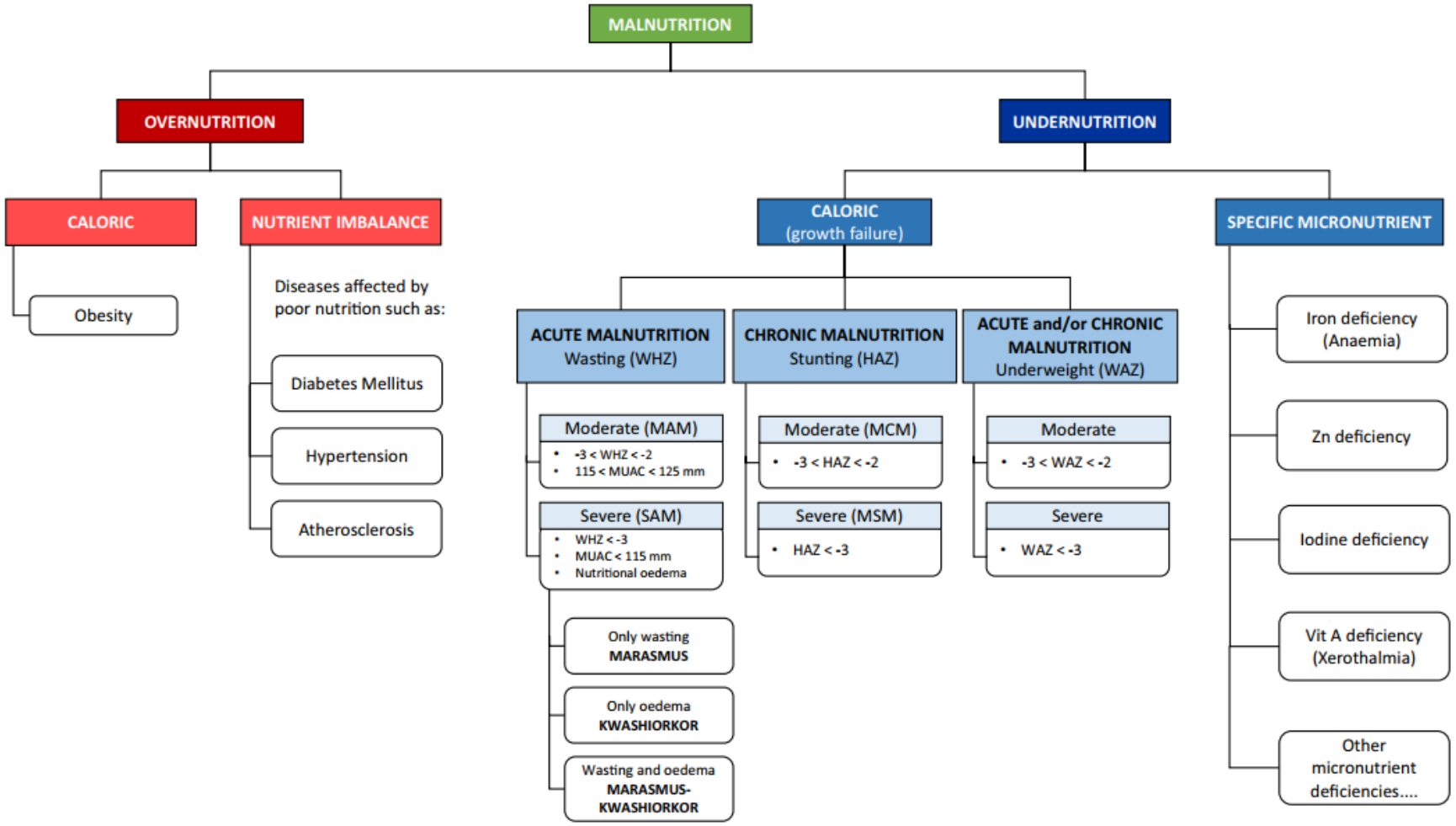

Figura 1. Tipos de malnutrición (tomado de Mayneris-Perxachs y Swann, 2019).

Puntuación Z: altura/edad HAZ (height-for-age Z-score); peso/edad WAZ (weight-for-age Z-score); peso/altura WHZ (weight-for-height Z-score); desnutrición aguda moderada MAM (Moderate Acute Malnutrition); desnutrición crónica moderada MCM (Moderate Chronic Malnutrition); desnutrición aguda grave SAM (Severe Acute Malnutrition); desnutrición crónica grave SCM (Severe Chronic Malnutrition).

El exceso de peso es un factor predeterminante para un futuro desarrollo de ENT. Los niños y niñas con sobrepeso y obesidad están expuestos a padecer enfermedades como la resistencia a la insulina, diabetes mellitus tipo 2, enfermedades cardiovasculares, hipertensión arterial, dislipidemia, ciertos tipos de cáncer, esteatohepatitis no alcohólica, apnea del sueño, osteoartritis, y problemas ginecológicos, a edades tempranas y a lo largo de la adultez (Martínez-Alvárez *et al.*, 2013; Williams *et al.*, 2015; NCD Risk Factor Collaboration, 2017; Poti *et al.*, 2017). Padecer sobrepeso y obesidad podría implicar una aceleración en el proceso de envejecimiento vascular (Barton, 2012), además acarrear problemas a nivel psicológico, social (Latzer & Stein, 2013) y disminuir el rendimiento escolar (Caird *et al.*, 2014). En la etapa infantil, también se han descrito asociaciones entre la obesidad y una mala calidad de vida, enfermedad periodontal, alteración de las hormonas prepuberales y trastorno por déficit de atención con hiperactividad (Pulgarón, 2013). Grandes estudios prospectivos de cohorte han demostrado que el tiempo de duración de la obesidad se asociada con un mayor riesgo de diabetes tipos 2, y que por cada año que la persona mantiene este exceso de peso el riesgo de diabetes incrementa hasta en un 14% (Hu *et al.*, 2014). En el estudio de Bjerregaard *et al.* en el 2018 hallaron que un aumento del IMC entre los niños y niñas de siete años de edad y el inicio de la adultez se asoció con un riesgo significativamente mayor de desarrollar diabetes tipo 2 en edades posteriores (Bjerregaard et a., 2018). Asimismo, se ha descrito que aquella población pediátrica obesa a la edad de 15 meses y que permanecieron así durante su niñez, tiene un riesgo mayor de desarrollar enfermedades cardiovasculares en la adolescencia (Boyer *et al.*, 2015). Con respecto a la cardiopatía coronaria, se ha descrito que si la obesidad persiste hasta la adolescencia, el riesgo de desarrollarla aumenta de formar significativa (Tirosh *et al.*, 2011). Todo esfuerzo que implique normalizar el IMC en edades tempranas es de vital importancia, porque sólo el hecho de que un niño o niña tenga un sobrepeso leve puede ya ser un factor asociado a un mayor riesgo cardiovascular a la mediana edad (Weihrauch-Blüher y Wiegand, 2018)

Por otra parte, en los últimos años se ha acuñado un nuevo término: "la doble carga de la malnutrición". Este concepto, se refiere a la coexistencia en la misma persona de uno

o más tipos de desnutrición y/o carencias de micronutrientes junto con el de sobrepeso/obesidad y las ENT relacionadas con la dieta (Freire *et al.*, 2018; Popkin *et al.*, 2020). Este tipo de malnutrición se considera un problema emergente sobre todo en países de ingresos medios y bajos donde cada vez parece ser más frecuente y estar asociada a factores económicos, sociales y demográficos como son la inequidad, pobreza, inseguridad alimentaria, las alteraciones en los patrones alimentarios y de estilo de vida caracterizado por una mayor producción, distribución y consumo de alimentos procesados de alto contenido energético pero deficiente en proteínas, micronutrientes y fibra, junto con una disminución de la actividad física (Freire *et al.*, 2014; Freire *et al.*, 2018; Perez-Escamilla *et al.*, 2018; Hajri *et al.*, 2021; Santos *et al.*, 2021). La doble carga de la malnutrición es un problema mucho más grave cuanto más temprana es la edad a la que se padece. Los y las lactantes que padecen desnutrición crónica y que luego presentan un incremento rápido de peso corren el riesgo de padecer obesidad y sus comorbilidades en la edad adulta (Victora & Rivera, 2014; Freire *et al.*, 2018). La relación entre el retraso del crecimiento y la composición corporal posterior es compleja. A corto plazo el aumento de peso a manera compensatoria inmediatamente después de la desnutrición podría dar prioridad a una acumulación de adiposidad sobre la masa magra a través de mecanismos de ahorro de energía (Dulloo *et al.*, 1997; Wells *et al.*, 2020). Un hallazgo consistente es que la desnutrición temprana reduce de forma permanente la masa corporal magra (Dodds *et al.*, 2012; Wells *et al.*, 2012; Lelijveld *et al.*, 2016; Wells *et al.*, 2020). Estudios refieren que una exposición a la desnutrición en los primeros años de vida podría agravar el desarrollo de exceso de peso y ENT más adelante (Wells, 2011; Wells *et al.*, 2020).

1.1.2. Epidemiología de la malnutrición infantil

En este apartado se describirá y comentará cual es la situación actual de la malnutrición a nivel mundial y en contextos más específicos como Europa o América Latina ya que España y Ecuador van a ser países objeto de estudio en este trabajo. Se tratará específicamente la malnutrición infantil ya que como se ha comentado en el apartado anterior ésta es especialmente importante a nivel de salud pública.

En el informe de 2022 de El estado de la seguridad alimentaria y la nutrición en el mundo, afirman que a nivel mundial en el año 2020, 149 millones (22%) de niños y niñas menores de cinco años padecían retraso en el crecimiento, 45 millones (6,7%) sufrían emaciación y 39 millones (5,7%) presentaban sobrepeso/obesidad. Alrededor del 45% de las muertes de menores de cinco años tienen que ver con la desnutrición, situación que ocurre en países de ingreso bajos y medios (FAO, FIDA, OMS, PMA y UNICEF, 2022). La doble carga de la malnutrición se concentra especialmente en el África subsahariana, Asia meridional y Oriental y en el Pacífico (Popkin *et al.*, 2020).

En Europa la malnutrición infantil destaca por sus altas prevalencias de sobrepeso y obesidad, que actualmente alcanza proporciones epidémicas y constituyen un importante problema en la salud pública (Martínez-Álvarez *et al.*, 2013). La OMS en su Informe Regional Europeo de la Obesidad 2022 declara que la prevalencia del sobrepeso y la obesidad aumenta en el grupo de edad de 5 a 9 años, con uno de cada ocho niños/as (11,6%) con obesidad y casi uno de cada tres (29,5%) con sobrepeso (incluida la obesidad); la prevalencia disminuye temporalmente en el grupo de edad de 10 a 19 años, el 7,1% vive con obesidad y el 24,9% con sobrepeso, los mismos datos demuestran que la prevalencia de sobrepeso y obesidad entre los niños de 5 a 19 años se multiplicó casi por tres entre 1975 y 2016, y por más de dos en las niñas de la misma edad; los niveles de obesidad aumentaron a un ritmo más rápido y fueron alrededor de cinco veces más altos en promedio entre los niños, niñas y adolescentes de 5 a 19 años en 2016 en comparación con 1975, y estos grandes aumentos se deben en parte a los niveles muy bajos de exceso de peso presentes en 1975 (OMS, 2022a). Las prevalencias más altas de exceso de peso se registran en aquellos países de Europa del Este y los Mediterráneos, donde las inequidades a nivel educativo están muy extendidas y se relaciona un menor nivel educativo con mayores cifras de sobrepeso y obesidad (OMS, 2019a, 2022a). En la población adulta de más de 18 años de edad el problema de sobrepeso y obesidad también alarmante, y está incrementando a un ritmo acelerado en la mayoría de países miembros de la Unión Europea, se estimó en el 2019 que el 52,7% presentó un exceso de peso (Eurostast, 2019).

Como ejemplo de país mediterráneo con altas prevalencias de sobrepeso y obesidad, se describirá a continuación la situación epidemiológica de España. España es un país Mediterráneo y actualmente la cuarta economía de la Unión Europea (ICEX España Exportación e Inversiones, 2020), por tanto, se considera un país con ingresos altos. Su Coeficiente de Gini (mide las desigualdades, valores entre 0 y 1, mientras más el valor se aleja de 0 implica mayor desigualdad) en el 2020 fue de 0,349 (Banco Mundial, 2023). Estas características pueden estar relacionadas con sus altas cifras de exceso de peso en niños y niñas, donde ocupada unos de los primeros puestos de sobrepeso y obesidad infantil en el continente europeo (OMS, 2021b). Estudios previos en población infantil española estiman que las cifras de prevalencia de sobrepeso y obesidad oscilan entre el 21,2% al 45,2% (Serra-Majem *et al.*, 2003; Gómez *et al.*, 2015; Ministerio de Sanidad, Consumo y Bienestar Social, 2017; Agencia Española de Seguridad Alimentaria y Nutrición [AESAN] y Ministerio de Consumo, 2020; Bibiloni *et al.*, 2022; de Bont *et al.*, 2022). Los resultados del estudio "EnKid" (1998-2000) muestran que la prevalencia de exceso de peso infantil en España es del 26,3% (Serra-Majern *et al.*, 2003). El programa "Thao" (2007-2015) estimó en su último estudio realizado entre 2014-2015 sobre una muestra de 20308 niños/as de entre 3 y 12 años, una prevalencia del 33,2% (Gómez *et al.*, 2015). El Estudio ALADINO del año 2019, realizado en escolares de 6 a 9 años encontró un 40,6% de sobrepeso y obesidad (AESAN y Ministerio de Consumo, 2020).

En América Latina y el Caribe hace más de dos décadas llevan enfrentando el grave problema de desnutrición en niños y niñas que ha disminuido, pero sigue sin erradicarse, a esta situación en los últimos años hay que sumarle las prevalencias cada vez más elevadas de sobrepeso y obesidad, y sin dejar aún lado el emergente problema de la doble de carga de la malnutrición (Comisión Económica para América Latina y el Caribe [CEPAL], 2018). La pobreza, desigualdades, inseguridad alimentaria son prevalentes en esta región siendo algunas de ellas las principales causas de su malnutrición infantil (Heltberg 2009; Perez-Escamilla *et al.*, 2018).

Los datos del documento Panorama regional de la seguridad alimentaria y nutricional - de América Latina y el Caribe 2022, muestran que la prevalencia del retraso del crecimiento en población infantil menor de cinco años en esta región entre el 2000 y 2020 disminuyó en un 37,0%, siendo equivalente a 6,7 puntos porcentuales, aunque la reducción se ha ralentizado en los últimos años. Con respecto a la emaciación en los niños y niñas menores de cinco años su prevalencia en el 2020 fue del 1,3%, y las cifras de sobrepeso en esta población aumentaron, siendo su prevalencia del 7,5%, casi dos puntos porcentuales por encima del promedio mundial. (FAO, FIDA, OPS, PMA y UNICEF, 2023). El exceso de peso (sobrepeso y obesidad) en niños y niñas mayores de 5 años y adolescentes también presenta un alarmante incremento, más del 30,0% tenían sobrepeso y un 12,0% obesidad (Figura 2) (FAO, FIDA, OPS, PMA y UNICEF, 2020).

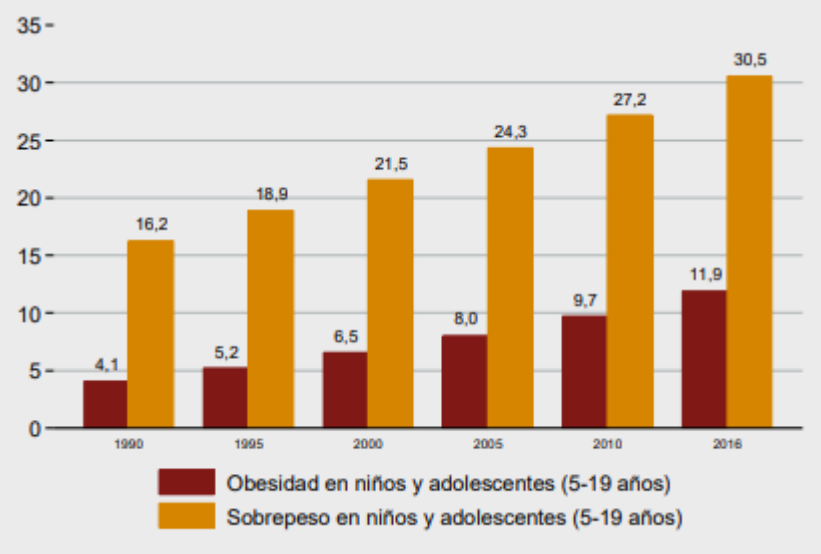

Figura 2. Prevalencia de personas afectadas por sobrepeso y obesidad (5 a 19 años) en América Latina y el Caribe, 1990-2016 (FAO, FIDA, OPS, PMA y UNICEF, 2020).

Como ejemplo de país de América Latina y cuya población infantil será objeto de estudio en esta Tesis, se comentará la epidemiología de la malnutrición infantil en Ecuador.

Ecuador es un país perteneciente a América del Sur atravesado por la Cordillera de los Andes dividendo la zona continental en tres regiones (Costa, Sierra y Amazonía), cuya

renta es considerada media pero su pobreza y desigualdad siguen siendo sus principales inconvenientes (Agencia Española de Cooperación Internacional para el Desarrollo [AECID], 2022). Su Coeficiente de Gini en el 2021 fue de 0,458 (Banco Mundial, 2023). Las características socioeconómicas de este país se asocian a sus existentes y persistentes problemas de malnutrición. Según la Encuesta de Salud y Nutrición 2018 (ENSANUT, 2018) de Ecuador, la tasa de desnutrición crónica en los y las en menores de cinco años disminuyó de 23,9% a 23,0% entre 2014 y 2018; en el área rural, este indicador se redujo de 31,9% a 28,7% durante el mismo periodo. La tasa de sobrepeso y obesidad en niños y niñas de 5 a 11 años aumentó de 29,9% a 35,4%. También se observaron diferencias según la zona de residencia; los valores fueron del 36,9% en las urbanas y del 32,6% en las rurales (Instituto Nacional de Estadística y Censos, 2019). Estos datos muestran que, aunque la prevalencia de desnutrición crónica está disminuyendo lentamente, las de sobrepeso/obesidad está aumentando de forma alarmante (Freire *et al.*, 2018). Ecuador ocupa el segundo lugar en América Latina y el Caribe en prevalencia de desnutrición crónica en menores de cinco años, el sexto en emaciación y es el de mayor incremento de sobrepeso y obesidad (FAO, FIDA, OPS, PMA y UNICEF, 2023). La transición nutricional que experimenta Ecuador es una realidad, donde la doble carga de la malnutrición no es ajena y es cada vez más preocupante cuando se tiene en cuenta que la desnutrición crónica no disminuye considerablemente, mientras que el exceso de peso crece de forma acelerada (Freire *et al.*, 2014; Freire *et al.*, 2018; Huiracocha *et al.*, 2019; Ramírez-Luzuriaga *et al.*, 2020; Hajri *et al.*, 2021). Las poblaciones rurales indígenas de las regiones Sierra y Amazonía históricamente son las más olvidadas, pobres y que mayores cifras de malnutrición acumulan.

1.1.3. Herramientas de diagnóstico en la malnutrición

Evaluar y diagnosticar el estado nutricional de los niños y niñas tanto a nivel clínico como poblacional es esencial para llevar un control y vigilancia de su crecimiento. Los indicadores antropométricos son valores de composición corporal usados para el diagnóstico nutricional de un individuo

La OMS en su Estudio Multicéntrico de Referencia sobre el Crecimiento (MGRS) que llevó a cabo entre 1997-2003, recopiló datos de crecimiento e información relacionada de aproximadamente unos 8500 niños y niñas de orígenes étnicos y entornos culturales diferentes (Brasil, Ghana, India, Noruega, Omán y EE.UU) (OMS, 2023a). Así desarrolló y publicó en el 2006 unos patrones de crecimiento de referencia mundial (las puntuaciones Z o en inglés *Z-score* y percentiles) que incluye tablas y curvas separadas para niños, niñas y adolescentes desde el nacimiento hasta los 19 años de edad (OMS, 2006). El Z-score es el número de desviaciones estándar de la media que es 0 (tanto por encima como por debajo); el percentil es el rango de posición de una persona sobre una distribución de referencia establecida en porcentaje, donde los valores van de 0 a 100, siendo 50 la media. Tanto la puntuación Z como el percentil, en el caso de los indicadores antropométricos infantiles permiten valorar su estado nutricional al comparar con valores previamente establecidos de una población de referencia (Díaz, 2009).

La OMS también desarrolló dos software denominados: *WHO Anthro* (0-5 años) y *WHO AnthroPlus* (5-19 años) para ordenadores personales que permite el ingreso y procesamiento de datos antropométricos.

A continuación, se comentarán con detalle los índices antropométricos más utilizados en la actualidad.

1.1.3.1. Longitud/talla para la edad

La longitud/talla para la edad es un indicador del crecimiento alcanzado en longitud o talla para la edad del niño o niña. Este indicador permite identificar retraso en el crecimiento (longitud o talla baja) provocado por un prolongado aporte deficiente de nutrientes o enfermedades recurrentes. Puede también identificar a los niños y niñas que son altos/as para su edad, una longitud o talla elevada rara vez es un problema a menos que sea excesivo, en ese caso podría reflejar algún desorden de tipo endócrino (OMS, 2008). Las puntaciones Z o *Z-score* correspondientes se pueden ver en la Tabla 1. Este indicador también se denomina *HAZ*, siglas que provienen del inglés *Height-For-Age Z-score.*

1.1.3.2. Peso para la edad.

El peso para la edad indica el peso corporal de un niño o niña respecto a su edad (sólo para menores de diez años). Se utiliza para determinar si un niño o niña tiene bajo peso o bajo peso severo, pero no para el caso de sobrepeso y obesidad. Hay que tener en cuenta que no es confiable cuando no se sabe con exactitud la edad/fecha de nacimiento (OMS, 2008). Los *Z-score* correspondientes se pueden ver en la Tabla 1. Este indicador también se denomina WAZ, siglas que provienen del inglés *Weight-For-Age Z-score.*

1.1.3.3. Peso para la longitud/talla

El peso para la longitud/talla es el peso corporal proporcional al crecimiento conseguido en longitud o talla (sólo para menores de cinco años). Este indicador tiene gran utilidad cuando no se conoce la edad/fecha de nacimiento. Permite identificar a niños y niñas con bajo peso para su longitud o talla que pueden estar emaciados, severamente emaciados o en riesgo de presentar sobrepeso u obesidad (OMS, 2008). Los Z-score correspondientes se pueden ver en la Tabla 1. Este indicador también se denomina WHZ, siglas que provienen del inglés *Weight-For-Height Z-score.*

1.1.3.4. Índice de Masa Corporal (IMC) para la edad

El índice de Masa Corporal (IMC) para la edad determina la presencia de sobrepeso y obesidad en los niños y niñas, pero también permite identificar emaciación y emaciación severa. La curva de IMC para la edad y la de peso para la longitud/talla tienden a mostrar resultados semejantes (OMS, 2008). Los Z-score correspondientes se muestran en la Tabla 1. Este indicador también se denomina BMIZ, por sus siglas en inglés *Body Mass Index Z-score.*

Tabla 1. Indicador de crecimiento en *Z-score* basado en las referencias de la OMS 2007 (Adaptado de: OMS, 2008; Granado *et al.*, 2017).

Z-score	INDICADORES DE CRECIMIENTO			
	Longitud/talla para la edad	**Peso para la edad**	**Peso para la talla**	**IMC para la edad**
> 3	*	**	Obesidad	Obesidad
> 2	Normal	**	Sobrepeso	Sobrepeso
> 1		**		
0 (mediana)		Normal	Normal	Normal
< -1				
< -2	Baja talla	Bajo peso	Emaciación	Emaciación
< -3	Baja talla severa***	Bajo peso severo	Emaciación severa	Emaciación severa

*Un niño/a en este rango rara vez representa un problema (descartar problemas endócrinos).
** Un niño/a en este rango puede presentar un problema de crecimiento, esto es posible evaluar mejor con peso para la longitud/talla o IMC para la edad.
*** Es posible que un niño/a en este rango desarrolle sobrepeso (doble carga de la malnutrición).

Para el diagnóstico del estado nutricional infantil el BMIZ y la HAZ son dos indicadores de fácil uso e interpretación Existen numerosos trabajos (Serra-Majem *et al.*, 2006; Ortiz *et al.*, 2014; Roche *et al.*, 2017; Instituto Nacional de Estadística y Censos, 2018; Freire *et al.*, 2018; Huiracocha-Tutiven *et al.*, 2019; Ramírez-Luzuriaga *et al.*, 2020; Gato-Moreno *et al.*, 2021; de Bont *et al.*, 2022; Comeras-Chueca *et al.*, 2022) en los que se han utilizado uno de éstos dos indicadores, o los dos pero por separado para obtener un diagnóstico a nivel clínico y/o poblacional. Sin embargo utilizándolos de esta manera puede suponer limitaciones; si se usa sólo uno de ellos el diagnóstico quedaría incompleto, si se analizan los dos pero por separado el diagnóstico no se unificaría, además teniendo en cuenta la transición nutricional que atraviesan diversos países, tener un diagnóstico en conjunto facilitaría determinar y analizar los resultados. Selem-Solís *et al.* (2017) desarrollaron un método denominado "Nutrimetría" que combina estas dos variables antropométricas (BMIZ y HAZ), con la intención de facilitar su interpretación conjunta y generar una visión más amplia del estado nutricional de una población, y de esta manera

conseguir llegar a una evaluación mucho más precisa que pueda ser más eficaz a la hora de plantear programas de intervención comunitarios (Selem-Solis *et al.*, 2017, 2018). La Nutrimetría evitaría las limitaciones mencionadas con respecto a un diagnóstico incompleto y separado del estado nutricional.

En la Figura 3, se muestra cómo la Nutrimetría combina ambos indicadores antropométricos para originar 9 subgrupos diferentes.

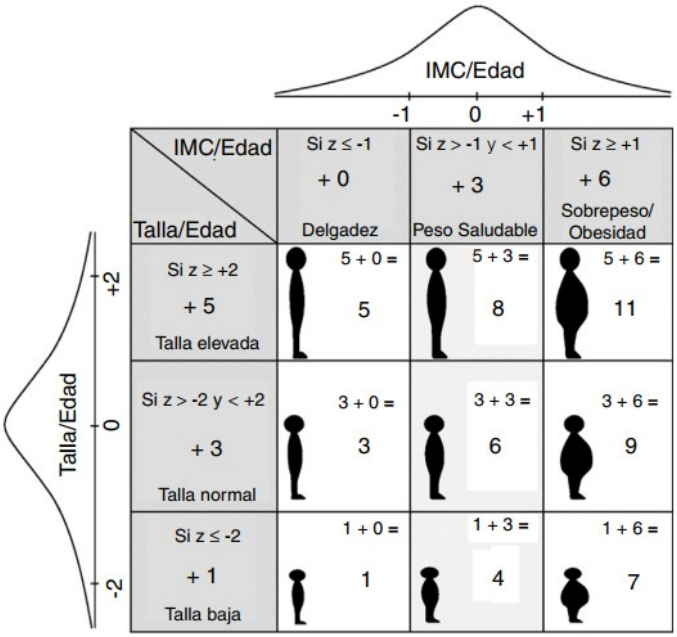

Figura 3. Nutrimetría y sus nutricódigos (Selem-Solis *et al.*, 2017).

Así pues, los nueve nutricódigos obtenidos: 1, 3, 4, 5, 6, 7, 8, 9 y 11; representan los diferentes diagnósticos del estado nutricional (Selem-Solis *et al.*, 2017). Se evita la estigmatización de los individuos al utilizar un lenguaje neutro, ya que informa de los resultados en cifras, evitando el uso de palabras como "gordo/a" y "flaco/a", lo que facilita la comunicación de resultados y objetivos a los escolares sin carga semántica emocional (OMS, 2008; Selem-Solis *et al.*, 2017, 2018).

1.2. Factores asociados a la malnutrición infantil

La malnutrición infantil es un problema multidimensional y muy complejo provocado por diversos factores ambientales, culturales, sociales, económicos, alimentarios/nutricionales, educativos, conductuales e higiénico-sanitarios (Hackett *et al.*, 2009; Kwami *et al.*, 2019; Cuevas-Nasu *et al.*, 2022).

En este segundo apartado, se tratará lo referente a factores clásicos asociados (alimentación, actividad física y sedentarismo) y ampliamente estudiados que pueden desencadenar el desarrollo de algún tipo de malnutrición en la etapa infantil. Se abordará el tema de dieta saludable, destacando a la Dieta Mediterránea (DM) y la herramienta que permite su análisis; así como el Cuestionario de Frecuencia de Consumo de Alimentos (CFCA) por ser una herramienta válida que recaba información alimentaria. Y finalmente, se comentará respecto a la actividad física y el sedentarismo, y su relación con la malnutrición y salud de los niños y niñas.

Las mayores prevalencias de malnutrición en niños y niñas son especialmente frecuentes en cuyas madres y/o padres no han recibido educación formal o es muy baja, y que pertenecen al quintil de menor ingreso (OMS, 2022a; FAO, FIDA, OPS, PMA y UNICEF, 2023). El factor alimentario/nutricional como "causa" de malnutrición es muy frecuente pero actúa de diferente manera según el contexto. En países de ingresos bajos suele causar desnutrición, mientras que en aquellos de ingresos altos, el factor alimentario/nutricional suele causar sobrepeso y obesidad (Murphy *et al.*, 2019). El patrón alimentario de estos países caracterizado por un exceso de calorías asociado al consumo de productos ultraprocesados (PU) altamente calóricos que desplazan al consumo de frutas y verduras (FV) favorece el aumento de la prevalencia de sobrepeso/obesidad entre la población infantil (Martínez-Alvárez *et al.*, 2013; Férnández-Gómez & Díaz-Campo, 2014; AESAN y Ministerio de Consumo, 2020; FAO, FIDA, OPS, PMA y UNICEF, 2020). Se ha descrito también la relación existente entre la ingesta de PU caracterizados por contener aditivos, sal, azúcares, grasas saturadas y trans, con algunos tipos de cánceres y ENT, además de con sobrepeso/obesidad (Poti *et al.*, 2017; FAO, FIDA, OPS, PMA y UNICEF, 2020). Estos

productos provocan un incremento automático de la actividad del sistema de recompensa del cerebro, anulando así los mecanismos homeostáticos y que conlleva a su mayor ingesta (Alonso-Alonso *et al.*, 2015). Por el contrario una dieta con alto contenido de FV es imprescindible para un correcto crecimiento y desarrollo infantil, preventiva de ciertas enfermedades y mejora el bienestar mental (Birch *et al.*, 1998; McMartin *et al.*, 2016; Folkvord *et al.*, 2022). Se estima que sólo el 8,8% de los niños y niñas consumen las cinco raciones al día recomendadas de FV en Europa (Kovács *et al.*, 2014). En España, los resultados del Estudio ALADINO (2019) mostraron que 53,9% de los niños y niñas incluyen en su desayunos bollería, zumos o batidos comerciales (AESAN y Ministerio de Consumo, 2020). La infancia es una etapa primordial para establecer los hábitos dietéticos y preferencias de alimentos que se mantendrán en gran medida en la etapa adulta (Mikkila *et al.*, 2004).

En América Latina y Caribe, la malnutrición presenta características diferentes. En 2021, la inseguridad alimentaria afectaba al 40% de su población y tener una dieta adecuada suponía un mayor coste en comparación con otras (FAO, FIDA, OPS, PMA y UNICEF, 2022). En 2019 en esta región 57,7 millones, es decir aproximadamente un 10% de su población, se quedaron sin alimentos, pasaron hambre o estuvieron más de un día sin comer (FAO, FIDA, OPS, PMA y UNICEF, 2020).

Se destaca además, que en América Latina y el Caribe se está produciendo actualmente una transición nutricional, que en otros países ha tenido lugar antes, caracterizada por la disminución del consumo de sus alimentos tradicionales (Lee *et al.*, 2021). El patrón alimentario, basado en un alto consumo de cereales integrales, frutas y verduras, está siendo reemplazando por alimentos de alto contenido energético y bajo contenido nutricional como los UP (Freire *et al.*, 2018; Hajri *et al.*, 2021; Santos *et al.*, 2021).

La escasez de recursos económicos y de oportunidades para comprar alimentos sanos puede contribuir a que los niños y niñas no sigan una dieta nutricionalmente adecuada (Rao *et al.*, 2013). El acceso a PU altamente calóricos es cada vez mayor, muchas veces de menor coste que las FV (Bento *et al.*, 2018). En consecuencia, la población infantil

de familias con bajos ingresos puede tener menos acceso a alimentos saludables en el hogar (Pechey *et al.*, 2021; Pereira *et al.*, 2021). Adicionalmente, una correcta alimentación debe ir acompañada de estilos de vida saludables como realizar actividad física diaria y reducción al mínimo posible actividades de ocio como el tiempo en pantallas, para garantizar una mejor salud infantil, crecimiento y desarrollo (Fernández-Gómez & Díaz-Campo, 2014; OMS, 2021b).

Una dieta adecuada no solo debe proporcionar la cantidad de energía necesaria para que una persona desarrolle su actividad diaria, sino que debe ser equilibrada y sana, es decir su composición en macronutrientes y micronutrientes también debe ser el adecuado. La cantidad de dietas denominadas como "Dietas Saludables" es muy extensa, pero realmente evidencia científica en el papel que éstas pueden tener en la prevención y/o tratamiento de enfermedades es muy limitado. Actualmente, la DM está considerada como una de las dietas en las que existe más evidencia científica sobre su papel protector frente a determinadas enfermedades.

La DM es un término utilizado para identificar un patrón dietético de los habitantes de la cuenca mediterránea. Además del comportamiento nutricional incluye características sociales, culturales, económicas y medioambientales, como la preferencia por los productos locales y frescos, la actividad física, un momento de intercambio social, diálogo intercultural, por lo que se considera un estilo de vida saludable más que sólo un patrón dietético (Organización de las Naciones Unidas para la Educación, la Ciencia y la Cultura [UNESCO], 2013; Ministerio de Cultura y Deporte-Gobierno de España, 2013; Lăcătușu *et al.*, 2019). Entre los componentes principales tenemos el aceite de oliva, las legumbres, los cereales no refinados, las frutas, verduras, frutos secos, una proporción moderada de pescado y productos lácteos bajo en grasa, bajo consumo de carne y sus derivados (Rodríguez-Palmero, 2000). En el año 2010 la UNESCO reconoció a la DM como patrimonio cultural inmaterial de la humanidad (UNESCO, 2013).

Una buena adherencia a la DM tradicional se ha asociado a bajas tasas de enfermedades crónicas, eficaz para reducir el riesgo de síndrome metabólico, a una elevada

esperanza de vida entre la población (Kesse-Guyot *et al.*, 2013; Lăcătușu *et al.*, 2019), con propiedades inmunomoduladoras y disminución en la incidencia de la inflamación, debido a los efectos positivos por algunos de sus componentes (ácidos grasos monoinsaturados MUFA y poliinsaturados PUFA , vitaminas, polifenoles, minerales, fibra) (Barrea *et al.*, 2021; Finicelli *et al.*, 2022). Los PUFA regulan a la baja la expresión de factores proinflamatorios, como IL-1β, IL-6, TNF-α, VCAM-1, MCP-1, reducen los niveles de ROS y especies nitrogenadas (Lordan *et al.*, 2017; Pauls *et al.*, 2018). El elevado consumo de fibra aporta beneficios a la microbiota intestinal ya que puede modular su composición y favorecer la liberación de ácidos grasos de cadena corta como el acetato, butirato y propianato (Barrea *et al.*, 2021). Por desgracia, la adherencia a la DM se está deteriorando (Grao-Cruces *et al.*, 2013), debido al incremento generalizado en la era moderna y globalizada de dietas más occidentales caracterizadas por consumo de alimentos pobres en nutrientes y los cambios conductuales cada vez más sedentarios, menos sociales y familiares (Da Silva *et al.*, 2009).

Con la finalidad de tener un cuestionario de fácil uso e interpretación que permita valorar si existe o no una adecuada adherencia a la DM por parte de la población infantil y juvenil, Serra-Majem *et al.* desarrollaron y publicaron en el 2004 el cuestionario KidMed (Serra-Majem *et al.*, 2004). La muestra analizada fue de 3,850 niños, niñas y jóvenes de edades entre los 2-24 años que se extrajo de la población del estudio EnKid (Serra-Majem *et al.*, 2001). Este cuestionario consta de 16 preguntas (12 son favorables al patrón de DM y 4 son desfavorables a la misma) que es posible autoadministrarse o a través de una entrevista (tabla 2); las preguntas que denotaba una respuesta negativa con respecto a la DM se le asigna un valor de -1 y las positivas +1. Las sumas de todos los valores se clasificaron en tres niveles (Serra-Majem *et al.*, 2004):

- >8 Alta Adherencia a la DM (AADM)
- 4–7 Media Adherencia a la DM (MADM)
- ≤3 Baja Adherencia a la DM (BADM) (Serra-Majem *et al.*, 2004)

Tabla 2. Cuestionario KidMed (adaptado de Serra-Majem *et al.*, 2004).

PUNTUACIÓN	
Toma una fruta o un zumo natural todos los días.	+1
Toma una 2ª pieza de fruta todos los días.	+1
Toma verduras frescas (ensaladas) o cocinadas regularmente una +1 vez al día.	+1
Toma verduras frescas o cocinadas de forma regular más de una +1 vez al día.	+1
Consume pescado con regularidad (por lo menos 2-3 veces al a +1 semana).	+1
Acude una vez o más a la semana a un centro de comida rápida (fast food) tipo hamburguesería.	-1
Le gustan las legumbres y la toma más de 1 vez a la semana.	+1
Toma pasta o arroz casi a diario (5 días o más a la semana).	+1
Desayuna un cereal o derivado (pan, etc.).	+1
Toma frutos secos con regularidad (al menos 2-3 veces a la +1 semana).	+1
Se utiliza aceite de oliva en casa.	+1
No desayuna.	-1
Desayuna un lácteo (yogurt, leche, etc…).	+1
Desayuna bollería industrial, galletas o pastelitos.	-1
Toma 2 yogures y/o 40 g queso cada día.	+1
Toma golosinas y/o caramelos varias veces al día.	-1

El CFCA es un tipo de encuesta dietética/alimentaria ampliamente utilizada desde los años 90 en estudios epidemiológicos (Pérez *et al.*, 2015). Son cuestionarios que pueden ser autoadministrados o por algún entrevistador o entrevistadora que no necesita ser experto o experta en el tema, es decir, son de fáciles de aplicar, de bajo coste y tienen la capacidad para clasificar a las personas según su ingesta dietética (Zhuang *et al.*, 2012; Zazpe *et al.*, 2020). En sus inicios se diseñó para proporcionar información del tipo descriptiva-cualitativa de patrones de ingesta, evolucionando hasta aquellos más complejos que incluyen el tamaño de la ración habitual para un cálculo cuantitativo de nutrientes como vitaminas y minerales (Pérez *et al.*, 2015). De acuerdo al interés de la investigación, estos cuestionarios pueden focalizarse por ejemplo en exposiciones alimentarias relacionadas a

alguna patología, el consumo específico de algún nutriente, calcular una media de las calorías diarias consumidas en un grupo poblacional o determinar su calidad de la dieta (Martin-Moreno *et al.*, 2007; Pérez *et al.*, 2015). Los CFCA pueden variar entre ellos de acuerdo al número de ítems poseen, los de > 100 y que preguntan por el tamaño de la porción suelen aportar información más amplia como el tipo de alimentos, los macro y micronutrientes y energía; pero al requerir más tiempo para cumplimentarlo la fatiga por parte la persona encuestada es mayor, y por tanto tiende a reducir la calidad de las respuestas. Los CFCA de ≤ 50 ítems al ser más cortos tienen la ventaja de ser más sencillos y rápidos de rellenar, pero podrían limitar recabar información como el consumo energético y nutricional (Flood *et al.*, 2014; Esteban-Figuerola *et al.*, 2020). Por tanto, es importante que previo a la recolección de datos, el CFCA electo esté previamente validado, y se adapte a la población seleccionada, en donde se tenga en cuenta variables importantes como la edad, cultura, zona y tipo de información a evaluar (Goni *et al.*, 2016).

La cantidad de calorías que una persona necesita a diario para vivir y desarrollarse adecuadamente depende de su nivel de actividad física, teniendo que existir un balance entre las calorías consumidas y gastadas, para que se garantice un estado nutricional y de salud óptimo. De acuerdo a la OMS la actividad física es: "cualquier movimiento corporal producido por los músculos esqueléticos, con el consiguiente consumo de energía" (OMS, 2022b). Los humanos hemos evolucionado para ser físicamente activos, nuestra supervivencia dependía de ello, por ejemplo, la recolección de alimentos que requería una actividad física prolongada e intensa. Lamentablemente en las últimas décadas factores como los cambios ocupacionales, globalización, urbanización rápida, revolución industrial y tecnológica, han provocado tanto en países de ingresos bajos, medio y altos, que las sociedades sean cada vez más sedentarias y directamente afecten su salud, hasta el punto de convertirse en un problema de salud pública por todas las repercusiones provoca (Ministerio de Educación y Ciencia-Ministerio de Sanidad y Consumo, 2006; OMS, 2010).

La inactividad física es el cuarto factor de riesgo más relevante de mortalidad a nivel mundial. La OMS estima que su inactividad corresponde la razón principal de

aproximadamente un 21 a 25% de los cánceres de mama y colon, 27% de la diabetes, y 30% de las cardiopatías isquémicas (OMS, 2009). El realizar insuficiente o nula actividad física contribuye al desequilibrio energético, existe evidencia que esta inactividad durante los primeros años de vida es un factor determinante en el aumento de los niveles de sobrepeso y obesidad, así como el desarrollo de trastornos graves en la salud infantil y juvenil, que frecuentemente se arrastran hasta la adultez (Speiser *et al.*, 2005; Ministerio de Educación y Ciencia-Ministerio de Sanidad y Consumo, 2006).

La práctica de actividad física regular a una intensidad moderada a vigorosa durante la niñez se asocia favorablemente con indicadores como la adiposidad, la salud ósea y esquelética, la salud cardiometabólica (tensión arterial, dislipidemia, hiperglucemia, resistencia a la insulina), el desarrollo cognitivo y motor (OMS, 2019b). Mejora el estado cardiorrespiratorio y muscular, el desempeño académico, y reduce los síntomas de depresión (OMS, 2022b).

Entre las razones del incremento de sedentarismo en los niños y niñas destaca el mayor tiempo dedican al uso de pantallas móviles, ordenadores, video juegos y televisión (Lisak *et al.*, 2018), esto repercute negativamente en su composición corporal con un aumento de la masa grasa, el riesgo cardiometabólico, la visión, desregulación del cortisol, el comportamiento, la forma física, sueño y autoestima (Morin-Major *et al.*, 2016; Lisak *et al.*, 2018; Nguyen *et al.*, 2020). La radiación electromagnética que emanan los dispositivos puede ser percibida como luz por la glándula pineal que es la productora de melatonina, y con ello afectar al sueño (Halgamuge, 2013). La existente relación entre la obesidad y el tiempo frente a pantallas pueden explicarse por la inactividad física, la disminución del sueño y la exposición a la publicidad que afecta negativamente a las elecciones alimentarias de los niños y niñas (Mihrshahi *et al.*, 2017).

Las recomendaciones de actividad física diaria acorde a la OMS para los niños, niñas, adolescentes entre 3 a 17 años son de al menos 60 minutos de intensidad moderada a vigorosa. Diariamente, para las edades comprendidas entre 3 a 4 años además deberían realizar diversas actividades físicas de variada intensidad durante como mínimo 180

minutos, ser repartidas a lo largo del día y cuantas más mejor; no deberían pasar más de una hora al día frente a pantallas. Entre 5 a 17 años las actividades recomendadas son primordialmente aeróbicas a lo largo de la semana, y las de alta intensidad al menos tres días por semana; el tiempo diario frente a pantallas debería ser inferior a dos horas (OMS, 2022b). Dichas recomendaciones a nivel real no se cumplen en su mayoría.

Una gran proporción de niños y niñas no cumple las recomendaciones, en el 2016 más del 80% de la población mundial era muy poco activa físicamente (Guthold *et al.*, 2020). En el estudio español, PASOS 2019, realizado en 38,887 personas de 8 a 16 años, el 63,3% y el 45,6% no cumplían las recomendaciones de actividad física y tiempo en pantallas, respectivamente (Gasol Foundation, 2019).

1.3. Asociación del parasitismo intestinal con el riesgo de malnutrición

En los apartados anteriores se han comentado los factores "clásicos" (y más estudiados) que están asociados a la malnutrición infantil, en el caso de este apartado tratará sobre el "parasitismo intestinal" (PI). Se detallará su definición, clasificación, se profundizará brevemente en ciertas especies relevantes y cómo pueden afectar a la salud de las personas, la epidemiología descriptiva a nivel mundial y en países como España y Ecuador (1.3.1). Además, se abordará cómo y cuáles especies podría afectar al estado nutricional (1.3.2), y por último los factores de riesgos asociados a la presencia de parasitismo intestinal (1.3.3).

El PI se define como una asociación biológica entre dos seres vivos de diferentes especies: el parásito y el hospedador; se da un beneficio unilateral, en donde el parásito vive en el intestino delgado/grueso del hospedador y a expensas de éste. Cuando se altera el equilibrio biológico establecido y éste favorece al parásito es cuando se pueden presentar manifestaciones clínicas para el hospedador; esto pasaría a definirse como: parasitosis intestinales, enfermedades parasitarias intestinales o infecciones parasitarias intestinales (Herbosa & Gutiérrez, 2011). Las manifestaciones de las infecciones parasitarias varían en

función de la especie, la edad e inmunidad del hospedador, la presencia de factores de riesgo y la carga parasitaria (Ojha *et al.*, 2014). Entre los mecanismos de transmisión tenemos la penetración intradérmica de la larva desde el suelo, por contacto directo de persona-persona o de animales-persona, y la ingestión de alimentos o de agua contaminada por materia fecal que contiene quistes, ooquistes, esporas viables, huevos o larvas de algún parásito (Medina *et al.*, 2010; Vidal-Anzardo *et al.*, 2020).

El PI está causado por helmintos intestinales y parásitos protozoarios, que conforman unos de los principales problemas de salud pública, sus prevalencias destacan en países de ingreso bajos y medios, sobre todo por sus deficientes sistemas de agua y saneamiento ambiental (Mohammed *et al.*, 2015; Feleke *et al.*, 2021).

Los helmintos (deriva de la palabra griega "*helmis*" que significa "gusano") pertenecen a un grupo taxonómico diverso denominado metazoos (Kupritz *et al.*, 2021). Son organismos multicelulares de tamaño grande, por ello en su etapa adulta se observan a simple vista (Centro para el Control y la Prevención de Enfermedades [CDC], 2022). Entre los helmintos más frecuentes a nivel mundial encontramos a los geohelmintos y *Enterobius vermicularis*. Lo que respecta a los geohelmintos (del inglés: *Soil-Transmitted Helminths*, STH), sus principales especies que infestan a las personas son la ascáride, *Ascaris lumbricoides*; el tricocéfalo, *Trichuris trichiura*; y los anquilostomas *Necator americanus* y *Ancylostoma duodenale*. Estas especies suelen ser abordadas de manera conjunta por la similitud en su diagnóstico y tratamiento (Ojha *et al.*, 2014; OMS, 2022c). Causan infección en los humanos a través del contacto con huevos o larvas de parásitos que se desarrollan en suelos cálidos y húmedos. Los adultos residen en el intestino delgado o grueso dependiendo de la especie, y sus miles de huevos son eliminados cada día por la materia fecal de personas infestadas (Auer & Aspöck, 2014; OMS, 2022c). Aquellas zonas donde los sistemas de saneamiento ambiental son inadecuados los huevos excretados pueden contaminar el suelo por varias vías: ingestión de agua contaminada, o de hortalizas poco cocidas y sin lavar, y en población infantil por jugar en suelo contaminado sin tener un lavado de manos previo a llevárselo a la boca. La infección de *A. lumbricoides* y *T. trichiura* se da por la ingestión de los huevos

embrionados, y se denominan ascariosis y tricuriosis respectivamente (OMS, 2022c). En el caso de los anquilostomas a través de penetración transcutánea (Fernández-Rivas *et al.*, 2019). Las infecciones leves por *A. lumbricoides* pueden ser asintomáticas, la clínica en personas sintomáticas es proporcional a la carga de gusanos. Los mecanismos lesivos de la infección por gusanos en las personas derivan del daño tisular directo; respuesta inmunológica frente a larvas, huevos o gusanos adultos; obstrucción intestinal, biliar o pancreática que causa dolor abdominal cólico, náuseas y vómitos; además puede provocar secuelas en el estado nutricional (Timón *et al.*, 2014; Manual Merck Sharp and Dohme [MSD], 2022a). La tricuriasis crónica provoca dolor abdominal, anorexia, anemia, pérdida peso y por tanto desnutrición sobre todo en niños y niñas. En presencia de más de 200 gusanos, es posible se presente colitis con tenesmo rectal, diarrea con moco y sangre, y prolapso rectal (Timón *et al.*, 2014; Manual MSD, 2022b). La enterobiasis es provocada por la especie parásita *E. vermicularis,* frecuentemente llamado oxiuro. Es uno de los principales parásitos en los niños y niñas, pero es posible su hallazgo en población adulta. Es un parásito de tipo antroponótico, cuya transmisión es directa o indirecta por la ingestión de sus huevos; es bastante frecuente que exista una autoinfección. Las hembras que residen en el intestino grueso del hospedador migran analmente para depositar hasta 10 000 huevos en la región perianal, que al cabo de un par de horas están listos para infectar y/o reinfectar (Auer & Aspöck, 2014; Bharti *et al.*, 2018). La enfermedad secundaria a *E. vermicularis* es relativamente inocua; la sustancia pegajosa y gelatinosa en la que se depositan los huevos junto a los movimientos que hace la hembra causa irritación perianal y vaginal con su característico prurito (Kucik *et al.*, 2004; Manual MSD, 2022c). El prurito perianal local puede provocar traumatismos cutáneos e infecciones bacterianas superpuestas; también, de forma menos frecuente causar vulvovaginitis en niñas prepúberes. Ocasionalmente, predisponer a una infección del tracto urinario inferior y enuresis secundaria, y raramente se han descrito granulomas peritoneales secundarios a oxiuros migratorios (Bharti *et al.*, 2018).

Los protozoos son organismos unicelulares microscópicos, pertenecientes el reino Protozoa (CDC, 2022). Su transmisión es de tipo fecal-oral, sobre todo a través del agua o alimentos contaminados y contacto de persona-persona; algunos animales actúan como

reservorio de estos parásitos que pueden ser infectantes para las personas, por tanto se consideran parásitos zoonóticos (Barros *et al.*, 2023). Son microorganismos ubicuos, abundantes y gran parte de ellos se presentan en de formas quistes o esporas que son muy resistentes en el medio ambiente. Se encuentran en aguas de superficie como lagos, ríos, manantiales, pozos y piscinas, también en el agua potable, aguas residuales y alimentos contaminados por estas aguas, por la utilización de abonos orgánicos o durante su manipulación. Algunos tipos de quistes/ooquistes de ciertas especies son resistentes a la cloración (*Giardia duodenalis*, *Entamoeba* spp. y *Cryptosporidium* spp.). *Entamoeba histolytica* es un tipo de ameba considerada patógena, pero también pueden colonizar el intestino delgado especies comensales como *Entamoeba dispar*, *Entamoeba coli* o *Endolimax nana*. En el caso de *Blastocystis* sp. aún es controversial las manifestaciones clínica que puede provocar (Fletcher *et al.*, 2012; Rojo-Marcosa & Cuadros-González, 2016); y con respecto a los microsporidios (el más frecuente *Enterocytozoon bieneusi)* son considerados parásitos emergentes, y se ha relacionado su patogenicidad sobre todo en personas inmucomprometidas (Dengjel *et al.*, 2001).

G. duodenalis es una especie parásita flagelada intestinal de distribución mundial (CDC, 2017b). Los trofozoítos de este parásito viven en las criptas glandulares y la submucosa del intestino delgado (duodeno y yeyuno proximal), mientras que sus quistes se forman en el duodeno de manera intermitente y se excretan por la materia fecal (Barros *et al.*, 2023). Puede infectar a las personas y mamíferos (entre ellos animales de producción y compañía) provocando la enfermedad que se denomina giardiosis. Es el parásito infectante más común entre los protozoos enteropatógenos humanos (Kucik *et al.*, 2004); es una causa importante de diarrea transmitida por el agua y los alimentos, brotes en guarderías y la diarrea del viajero (Leung, 2011). El periodo de incubación va entre una a tres semanas. Aproximadamente entre el 50 al 75% de las personas infectados son asintomáticos (Lebwohl *et al.*, 2003; Leug *et al.*, 2019). A pesar de que es un parásito que afecta a personas de cualquier edad, su incidencia y manifestaciones se presenta especialmente en niños y niñas. *G. duodenalis* es un complejo de especies que consta de ocho *assemblages* (A-H), siendo los *assemblages* A y B (considerados zoonóticos) los dominantes en humanos, C y D en cánidos,

E en ungulados, F en félidos, G en roedores y H en mamíferos marinos (Ryan *et al.*, 2021). Las infecciones poco frecuentes y el aislamiento de genotipos zoonóticos de gatos y perros sugieren que son una fuente potencial de infección humana que puede adquirirse a través de la manipulación, el dormir juntos, los besos y lametones (Overgaauw *et al.*, 2009; Fletcher *et al.*, 2012).

Las amebas son organismos unicelulares y móviles mediante pseudópodos (Barros *et al.*, 2023). Se han descrito diferentes especies que pueden colonizar/infectar el intestino humano: *Entamoeba moshkovskii, Entamoeba bangladeshi, Entamoeba dispar, Entamoeba histolytica, Entamoeba polecki, Entamoeba nuttalli, E. coli, Entamoeba hartmanni, Iodamoeba bütschlii, Diantamoeba fragilis* y *E. nana* (Fleta *et al.*, 2000). *E. histolytica*/*E. dispar*/*E. moshkovskii*/*E. bangladeshi* son morfológicamente indistinguibles y constituyen el denominado "Complejo *Entamoeba*", por ello para determinar la especie se requiere de otras técnicas como las moleculares (CDC, 2019a). De todas las amebas descritas, *E. histolytica* es considerada la principal especie patógena, asociada a infecciones intestinales y extraintestinales, esta especie es la causante de la enfermedad denominada amebiasis, que incluye la colitis o disentería amebiana, y abscesos hepáticos; también se han observado abscesos pleuropulmonares, cerebrales y lesiones necróticas en la piel perianal y los genitales (CDC, 2019a; Manual MSD, 2022c). El 90% de los casos de *E. histolytica* son asintomáticos (Barro *et al.*, 2023). Al igual que ocurre con *G. duodenalis*, la población infantil resulta especialmente vulnerable (Mondal *et al.*, 2006). La colonización/infección por amebas se produce al ingerir agua o alimentos contaminados que contienen los quistes del parásito (Stanley, 2003), éstos pueden ser resistentes a bajas temperaturas, cloración del agua y a los ácidos gástricos y enzimas digestivas, viajan por el intestino delgado y, en la parte terminal del íleon o el colon se excretan para formar el estadio de trozofoíto (Barros *et al.*, 2023; Stanley, 2003). En el caso de la especie patógena, *E. histolytica*, sus trofozoítos al colonizar la luz del colon, pueden adherirse a sus células epiteliales y a los leucocitos polimorfonucleares y destruirlos, provocando una disentería mucosanguinolenta; estos trofozoítos también secretan proteasas que degradan la matriz extracelular, originando pequeñas úlceras en la mucosa y permitiendo la invasión de la submucosa, y al entrar en

zonas profundas, pueden diseminarse por la circulación portal y causar abscesos en otros órganos, sobre todo a nivel hepático (Stanley, 2003; Manual MSD, 2022c).

Blastocystis sp. es un protozoo unicelular genéticamente diverso y de distribución mundial, coloniza el tracto intestinal de las personas y de una gran cantidad de animales domésticos, en cautiverio y salvajes; por ello considerado una especie zoonótica (Wawrzyniak *et al.*, 2013; CDC, 2019d). Es uno de los parásitos intestinales más frecuentemente identificados en humanos. Puede transmitirse por la ingestión de agua o alimentos contaminados, o por contacto directo con otros humanos o animales (del Coco *et al.*, 2017). Los niños y niñas, sobre todo de países con ingresos bajos y medios, y los adultos inmunodeprimidos podrían ser más susceptibles a contraerlo (Wawrzyniak *et al.*, 2013). Morfológicamente se han descrito al menos seis formas diferentes: vacuolar, multivacuolar, avacuolar, granular, ameboide y quística (Tan, 2008). Su potencial patógeno sigue siendo poco claro y aún se debate. En ocasiones, se ha descrito y asociado a *Blastocystis* sp. con enfermedades intestinales (como diarrea, enfermedad inflamatoria intestinal, síndrome de intestino irritable, colitis ulcerosa) y extraintestinales (urticaria, anemia ferropénica); y en otras como comensal al no encontrar una relación entre su presencia y manifestaciones clínicas (Clark *et al.*, 2013; del Coco *et al.*, 2017). Los mecanismos por los cuales este parásito provocaría daño en el hospedador, sus posibles factores involucrados en la virulencia y riesgo de infección, siguen siendo poco conocidos (Scanlan, 2012). Se ha observado una amplia diversidad genética entre numerosos *Blastocystis* sp. aislados de diferentes hospedadores. Un total de 38 subtipos (STs) de *Blastocystis* han sido descritos hasta la actualidad (ST1-ST38), de los cuales ST1-ST10, ST12, ST14, ST16, ST23 y ST35 se han descrito en humanos (Stensvold & Clark, 2020; Baek *et al.*, 2022; Maloney *et al.*, 2023). Las investigaciones han descubierto que ST1, ST2, ST3, y ST4 parecen ser los subtipos predominantes en personas, y los otros son hallazgos esporádicos, aunque se detectan con frecuencia en varios grupos de animales, incluyendo aves y ungulados, y pueden estar relacionados con la transmisión zoonótica (Darwish *et al.*, 2023).

Los microsporidios conforman un grupo de parásitos unicelulares e intracelulares obligados que se han encontrado en una gran variedad de vertebrados e invertebrados incluidos los humanos (Stentiford *et al.*, 2016). Están estrechamente relacionados con el reino Fungi, pero dicha relación no está confirmada; su posición taxonómica ha sido debatida y revisada en varias ocasiones. Se caracterizan por la producción de esporas resistentes que varían de tamaño (entre 1-4 μm para las especies de importancia médica) (CDC, 2019c). Las esporas que pueden ser inhaladas, penetrar a través de los tejidos que rodean el ojo, por contacto persona-persona o contacto con animales infectados, se localizan en el intestino humano y pueden eliminarse por las heces, aunque la vía de transmisión sigue siendo incierta para muchas especies (CDC, 2019c; Manual MSD, 2022d). En el caso de la especie *Enterocytozoon bieneusi*, se considera que gran parte de las infecciones humanas se dan a través de una transmisión fecal-oral de esporas a través de alimentos o agua contaminados (Qiu *et al.*, 2019). La infección por microsporidios se denomina microsporidiosis; y su sintomatología dependerá de los órganos infectados, puede provocar síntomas intestinales como la diarrea, dolor abdominal o síntomas oculares (Manual MSD, 2022d). La microsporidiosis se considera una infección emergente debido al incremento de identificación de nuevas especies parásitas que infectan a personas y animales (Stentiford *et al.*, 2016). Hasta la actualidad se han descrito más de 1 500 especies pertenecientes a más de 200 géneros, y se han identificado unas 17 especies en personas, donde *E. bieneusi* es el más frecuente, responsable del 90% de las microsporidiosis humanas a nivel mundial (Shen *et al.*, 2020), seguido de algunas especies de *Encephalitozoon* (*E. intestinalis, E. cuniculi, E. hellem*) (CDC, 2019e). La microsporidiosis ocurre más frecuentemente en personas inmunocomprometidas (Lobo *et al.*, 2012) pero también se ha descrito en poblaciones aparentemente sanas que podrían actuar como diseminadores inadvertidos (Sak *et al.*, 2011). El número de casos asintomáticos en el que se ha identificado *E. bieneusi* en personas inmunocompetentes deja en manifiesto su importante epidemiológica y la transcendencia que posee en la transmisión del parásito (Shen *et al.*, 2020); además su potencial zoonótico es evidente debido a la identificación en numerosas especies de mamíferos y aves (Santín *et al.*, 2011). Hasta el momento se han descrito más de 600 genotipos de *E. bieneusi* distribuidos

en 11 grupos filogenéticos distintos, de los cuales el Grupo 1 y el Grupo 2 comprenden los que tienen potencial zoonótico (Li *et al.*, 2019).

1.3.1. Epidemiología

Las infecciones parasitarias intestinales suponen una alta carga de morbilidad humana; alrededor de 4.500 millones de personas corren el riesgo de desarrollarlas, más de 2000 millones ya están infectadas, y 300 millones presentan manifestaciones clínicas graves (Pazmiño *et al.*, 2022). Las causadas por helmintos a escala global afectan aproximadamente a 1.500 millones de personas (24% de la población mundial), la mayoría residentes en países de ingresos bajos y medios donde destacan aquellas zonas endémicas: Asia, América Latina, el Caribe y el África subsahariana (Hotez *et al.*, 2008; OMS, 2022c). Más de 260 millones de niños y niñas en edad preescolar y 654 millones en edad escolar, residen en zonas donde la transmisión de los STH es intensa, y por tanto requieren intervenciones preventivas y tratamientos (OMS, 2022c). Aproximadamente 804 millones, 477 millones y más de 200 millones de personas en el mundo tienen *A. lumbricoides*, *T. trichiura* y *E. vermicularis* respectivamente. En cuantos a los protozoos intestinalis no se dispone de datos concretos sobre sus prevalencias, dada su distribución cosmopolita y la falta de obligatoriedad en su declaración. *G. duodenalis* anualmente se estima que infecta alrededor de 200 millones de personas y con registros de unas 500,000 muerta al año (Hajare *et al.*, 2022). Por causa de la amebiasis por *E. histolytica* se calcula que cada año fallecen entre 40.000 y 100.000 personas; en la actualidad se sabe que muchos de los 500 millones de personas infectadas con *Entamoeba* están colonizados por la especie no patógena *E. dispar* (Stanley, 2003). En el caso de *Blastocystis* sp. se estima es que infecta/coloniza a más de 1000 millones de personas en el mundo (del Coco *et al.*, 2017). Los estudios de *E. bieneusi* sobre todo se han basado en personas inmucomprometidas. Las cifras reportadas oscilan entre un 2,5% a 78% en personas seropositivas al VIH con diarrea, mientras que en aquellas sin diarrea eran mucho más bajas (1,4% a 4,6%) (Matos *et al.*, 2012)

El PI y las infecciones que ciertas especies son capaces de provocar, son un importante problema de salud infantil que históricamente ha afectado sobre todo a países de ingresos económicos bajos y medios, y se ha asociado además a zonas consideradas endémicas como las tropicales y subtropicales. De acuerdo a investigaciones de los últimos años el PI en países de ingresos altos como España es cada vez más frecuente, debido a cambios demográficos, climáticos y de comportamiento. Entre las razones se han descrito la emigración desde países endémicos, el aumento de los viajes internacionales, el consumo de alimentos frescos y la acogida/adopción de niños y niñas de estas zonas, el mayor consumo de alimentos de cultivo ecológico y la crisis climática (López-Vélez *et al.*, 2005; Chover *et al.*, 2010; Fuentes-Corripio *et al.*, 2010; Werner, 2014; Horton *et al.*, 2019; Trelis *et al.*, 2022). Las investigaciones de prevalencia de PI en población escolar en España son escasos; estudios previos reportaron cifras de PI entre el 10,7% y el 28,0% (Chover *et al.*, 2010; González-Moreno *et al.*, 2011; Reh *et al.*, 2019; Koster *et al.*, 2021). La prevalencia de los geohelmintos, *A. lumbricoides* y *T. trichiura* en la actualidad en territorio español ha disminuido siendo casi que inexistente gracias a las mejoras higiénico sanitarias; se reportan casos sobre todo en personas migrantes procedentes de zonas endémicas (África subsahariana, Latinoamérica y Asia) (Carranza-Rodríguez *et al.*, 2018). *E. vermicularis* es el nematodo más frecuente entre los niños y niñas españolas, con unas prevalencias aproximadas del 10% (Chover *et al.*, 2010; Baéz *et al.*, 2013). En cuanto a los protozoos, se han reportado cifras de *G. duodenalis* en niños y niñas de este país entre 6,1% al 18% (Chover *et al.*, 2010; Reh *et al.*, 2019; Köster *et al.*, 2021; Mormeneo *et al.*, 2022). Con *E. histolytica* ocurre algo similar a los geohelmintos respecto a sus bajas prevalencias y a la población en la que se ha identificado (migrantes o viajeros provenientes de zonas endémicas) (López-Vélez *et al.*, 2003; Cobo *et al.*, 2016; Roure *et al.*, 2019). De *Blastocystis* sp. en población pediátrica de España se han descrito prevalencias entre 10% al 28,2% (Chover *et al.*, 2010; Reh *et al.*, 2019; Köster *et al.*, 2021; Mormeneo *et al.*, 2022). La identificación de *E. bieneusi* en este país se ha dado principalmente en personas inmunodeficientes y viajeros que provienen de zonas endémicas; en el estudio de Köster *et al.* en niños y niñas asintomáticos en Madrid, la prevalencia de este microsporidio fue inexistente (Köster *et al.*, 2021).

América Latina es una de las zonas consideradas endémicas por sus altas prevalencias de PI, las desigualdades sociales padecen llevan como consecuencias deficientes sistemas de saneamiento ambiental, y con ello mayor riesgo de contraer alguna o varias especies parásitas; Ecuador lamentablemente no es un país ajeno a esta realidad, y pesar de ello los estudios son insuficientes para conocer las prevalencias a nivel local y nacional (Puente-Agostini *et al.*, 2011; Cajamarca *et al.*, 2017). El PI en Ecuador afecta aproximadamente al 80% de su población rural y al 40% de su población urbana marginal (Cajamarca *et al.*, 2017), las infecciones parasitarias de acuerdo al Ministerio de Salud Pública del Ecuador (MSP) en 2014, ocuparon el segundo lugar en la lista de las principales causas de morbilidad ambulatoria, y se encontraban dentro de las diez primeras causas de consulta pediátrica (Gildner *et al.*, 2016; Abad-Sojos *et al.*, 2017; Cajamarca *et al.*, 2017). Los helmintos *A. lumbricoides*, *T. trichiura* y *E. vermicularis*, son especies prevalentes en este país, estudios reportar cifras que van del 7-45%, del 3-25%, y 0,5-10,6%respectivamente (Sackey *et al.*, 2003; Jacobsen *et al.*, 2007; Levecke *et al.*, 2011; Mejía *et al.*, 2013; Abad-Sojos *et al.*, 2017; Weatherhead *et al.*, 2017; Calvopina *et al.*, 2019; Guevara *et al.*, 2019). En cuanto los protozoos intestinales las cifras varían por especie parásita, *G. duodenalis* (4-40%), los miembros del complejo *Entamoeba* (12-34%) y *Blastocystis* sp. (7-81%) (Sackey *et al.*, 2003; Jacobsen *et al.*, 2007; Levecke *et al.*, 2011; Atherton *et al.*, 2013; Mejia *et al.*, 2013; Pazmiño *et al.*, 2014; Helenbrook *et al.*, 2015; Vasco *et al.*, 2016; Abad-Sojos *et al.*, 2017; Cajamarca *et al.*, 2017; Palacios-Ordóñez, 2017; Weatherhead *et al.*, 2017; Sarzosa *et al.*, 2018; Calvopina *et al.*, 2019; Guevara *et al.*, 2019; Lowenstein *et al.*, 2020). Lo referente a prevalencias de microsporidios en este país sólo existe un estudio realizado en pacientes VIH positivos con síndrome diarreico, en donde de los 89 pacientes estudiados el 25% resultaron positivos para *Microsporidium* spp. (Pazmiño *et al.*, 2014).

1.3.2. Afectación del parasitismo intestinal al estado nutricional

Existen evidencia que el estado nutricional infantil puede verse alterado negativamente por la presencia de ciertas especies parásitas intestinales, entre ellas destacan

A. lumbricoides, *T. trichiura*, *G. duodenalis* y *E. histolytica*. Las infecciones provocadas por estos parásitos intestinales, contribuyen en gran medida a la carga mundial de morbilidad, sobre todo en los países de bajos ingresos y escasos recursos de las regiones tropicales y subtropicales (Checkey *et al.*, 2015; Kotloff *et al.*, 2017; Montresor *et al.*, 2020). En estas zonas endémicas, las infecciones concomitantes son frecuentes, y su efecto sinérgico podría exacerbar los resultados sanitarios perjudiciales en las personas infectadas (Petney *et al.*, 1998). Las infecciones crónicas o repetidas por geohelmintos y protozoos durante la infancia están estrechamente relacionadas con el retraso del crecimiento (UNICEF, 2021). En las poblaciones pediátricas, la desnutrición crónica se refiere tanto a la reducción del crecimiento físico como al deterioro cognitivo, y representa un importante problema de salud pública que conlleva consecuencias adversas para la salud, la educación y la economía a lo largo de toda la vida (Black *et al.*, 2013). Debido a su carácter más crónico que agudo, las infecciones por geohelmintos sólo se abordan parcialmente en los estudios de la Carga Mundial de Morbilidad (Global Burden of Disease: GBD) (GBD 2019; Diseases and Injuries Collaborators, 2020), mientras que las infecciones por protozoos intestinales causantes de diarrea no se consideran formalmente enfermedades tropicales desatendidas (OMS, 2023b).

Los geohelmintos, *A. lumbricoides* y *T. trichiura*, causan tasas de crecimiento reducidas, malnutrición proteico-energética, discapacidad intelectual, déficit cognitivo, disminución de la ingesta de alimentos, deficiencia de hierro y malabsorción de nutrientes (Crompton & Nesheim, 2002; Cooper *et al.*, 2003). La escasez en el aumento de peso que se da durante las infecciones por los STH puede estar relacionado a que estos parásitos viven en el intestino, lo cual interferiría en la nutrición del hospedador e induciría daño en la mucosa intestinal, provocando una reducción en la capacidad del hospedador para extraer y absorber los nutrientes provenientes de la alimentación (Bethony *et al.*, 2006; Ojha *et al.*, 2013). La respuesta inflamatoria por parte del hospedador puede llevar a una producción de monocinas proinflamatorias (Interleuquina-1, Interleuquina-6 y el Factor de Necrosis Tumoral-alpha), éstas reducen el apetito, provocan pérdidas de proteínas e incrementan el gasto energético en reposo (Crompton & Nesheim, 2002). Además estos helmintos pueden alterar las funciones inmunitarias, alterando la respuesta a antígenos y/o patógenos; las

infecciones de tipo crónicas inducen una hiporrespueta de las células T (Maizels & Yazdanbakhsh, 2003; Borkow & Bentwich, 2008; Ojha *et al.*, 2013). *A. lumbricoides* puede causar problemas de malabsorción de proteínas, lactosa y algunas vitaminas liposolubles, porque provoca un acortamiento de las vellosidades intestinales, alargamiento de criptas, disminución de la relación vellosidad/cripta, e infiltración celular de la lámina propia (Ojha *et al.*, 2013). La existencia de gusanos adultos de esta especie en el intestino, también pueden provocar síntomas gastrointestinales, obstrucción o perforación del intestino, colecistitis, y ocasionalmente obstrucción del conducto pancreático; los gusanos migratorios pueden causar abscesos hepáticos (Khuroo *et al.*, 1990; Ojha *et al.*, 2013). Los gusanos adultos de *T. trichiura* son capaces de alterar la arquitectura normal de la mucosa colónica (Ojha *et al.*, 2013). Este parásito también puede provocar pérdidas de sangre en heces, que contribuye a la pérdida de hierro y por tanto desarrollo de anemia. Según estimaciones una infección de unas 200 lombrices, podrían aumentar las necesidades diarias de hierro en un niño o niña en 4,25mg/día. Además, es común en muchas zonas endémicas la existencia de deficiencias de hierro por situaciones como el déficit alimentario, y si a esta situación se le suma las pérdidas de sangre por la tricurosis, el estado nutricional del hospedador se vería agravado (Crompton & Nesheim, 2002). Con respecto al protozoo *G. duodenalis*, este puede ser un factor desencadenante de intolerancia y/o malabsorción alimentaria secundaria, dispepsia o síndrome de intestino irritable (Al-Mekhlafi *et al.*, 2005; Cifre *et al.*, 2018; Trelis *et al.*, 2019). Se sabe que provoca la activación de linfocitos CD8 en las vellosidades intestinales de la mucosa absortiva, una disminución de la proporción entre vellosidades y criptas, y deficiencia de disacaridasas (Cotton *et al.*, 2011; Halliez *et al.*, 2013; Bartelt & Sartor, 2015; Gozalbo *et al.*, 2020). Los estudios han asociado la presencia de *G. duodenalis* con alteraciones en la absorción de hierro, disminución del hierro sérico, bajos niveles de Hb en sangre y anemia ferropénica (Al-Mekhlafi *et al.*, 2005; Gozalbo *et al.*, 2020). La giardiosis también podría ser un factor de riesgo de deficiencia de zinc, vitaminas A y B12, en población en edad escolar (Fletcher *et al.*, 2012). Estudios han identificado la infección temprana por *G. duodenalis* como un factor de riesgo de retraso en el crecimiento entre los niños y niñas (Berkman *et al.*, 2002; Al-Mekhlafi *et al.*, 2005), y que aquellos y aquellas con múltiples

infecciones al año durante los dos primeros años de vida, presentaban puntuaciones de función cognitiva más bajas a los nueve años que los niños o niñas con una o ninguna infección por este parásito (Berkman *et al.*, 2002; Prado *et al.*, 2005; Donowitz *et al.*, 2016; Pickering *et al.*, 2019).

1.3.3. Factores de riesgo

La epidemiología de los parásitos intestinales a nivel mundial es dependiente a factores ambientales, climáticos, sociodemográficos, socioeconómicos, educativos, culturales y de comportamiento (Cardozo & Samudio, 2017). Los países de ingresos bajos y medios son los que mayores cifras acumulan, y que más repercusiones presentan en su salud por las inadecuadas condiciones en la que viven; cabe destacar que las poblaciones infantiles de estos países debido a su inmadurez inmunitaria, y la frecuente exposición a parásitos por el entorno poco higiénico en el que crecen, presentan un alto riesgo de contraer infecciones parasitarias (Wasihun *et al.*, 2020). Unos correctas prácticas higiénico-sanitarias a nivel personal, doméstica y ambiental son eslabones importantes para prevenir el PI, por ello aquellos países donde existe mayor nivel de estas tres variables acumulan cifras muy bajas de infecciones por helmintos como *A. lumbricoides* y *T. trichiura*. Por ejemplo, en el caso de Japón las lombrices son prácticamente inexistentes durante las décadas siguientes post Segunda Guerra Mundial, esto debido a un programa integrado de reestructuración sanitaria combinado con el cribado y el tratamiento de los casos positivos (Bharti *et al.*, 2018). El saneamiento ambiental deficiente e incluso inexistente, lamentablemente sigue siendo un problema grande en países de ingresos bajos y medios, y esto conlleva a que las cifras de PI no disminuyan; la fuente de agua de una comunidad es un factor predictor de infecciones parasitarias, que la mayoría de veces en países es escasos recursos no es apta para el consumo, por motivos como la contaminación fecal; en el estudio de Al-Saad y colaboradores de 2008 las personas que utilizaban agua en tanques tenían mayor riesgo de múltiples infecciones (Al-Saad *et al.*, 2018). La disponibilidad de letrinas y defecar al aire libre son otras de las causas potenciales, aquellas zonas que presentan ambientes poco limpios provocados por la defecación al aire libre son más propensas a transmitir parásitos

a través del suelo, agua, fómites, alimentos u otros medios (Assemie *et al.*, 2021). El lavado de manos y uñas frecuente presenta una asociación con el riesgo de contraer parásitos; Gelaw y colaboradores (2013) encontraron una correlación significativa entre las prácticas de higiene de las manos y las tasas de infección parasitaria entre escolares. Una higiene deficiente de manos y uñas también tiene relación con un nivel socioeconómico bajo, ya sea por la falta de materiales de higiene o de instalaciones adecuadas; de acuerdo a una investigación los y las estudiantes de madres sin estudios presentaban un mayor riesgo de infecciones parasitarias, lo que podría reflejar que las madres con estudios tienen mayor conocimiento preventivo, y mejor situación económica que les permitiría practicar y tener acceso a materiales como agua y jabón (Assemie *et al.*, 2021). El lavado y desinfección de productos frescos como las frutas y verduras, es otro factor protector frente al PI, sobre todo cuando el suelo y agua de la zona están contaminados (Bethony *et al.*, 2006). El contacto con animales entre ellos los domésticos, a los cuales no se les realiza un control parasitológico y desparasitación, conforman una fuente importante de riesgo de PI, porque actúan como reservorios y vehículo de transmisión hacia las personas, además, resultan ser contaminantes ambientales especialmente de fuentes de agua (Rodríguez-Sáenz, 2014). La zona de residencia también puede ser un predictor de riesgo, vivir en una zona rural aumenta las posibilidades de contraer PI, por razones como mayor contacto con el suelo, acceso limitado al agua potable y letrinas, menor nivel educativo y económico, son lugares que continúan siendo desatendidos por los gobiernos (Assemie *et al.*, 2021; Rodríguez-Sáenz, 2014).

1.4. Prevención y promoción de la salud

Este cuarto y último apartado abordará el tema de promoción de la salud, se resaltará el valor que tiene el sector educativo para combatir y prevenir la malnutrición infantil, la importancia de generar alianzas entre diversos sectores. Se describirá el enfoque preventivo de la malnutrición que se está llevando a cabo en países como España y Ecuador; Además, se detallará ciertos factores importantes a tener en cuenta en el desarrollo de programas de

intervención de la malnutrición, entre ellos la perspectiva "One Health", y por último el enfoque de interculturalidad en salud y su innegable relevancia.

La Declaración de Yakarta sobre la Promoción de la Salud en el Siglo XXI, menciona lo valioso e importante que es invertir en promocionar la salud. Permite a las personas tener un control sobre su salud para mejorarla, contribuye significativamente a reducir las desigualdades, asegurar los derechos humanos y construir un capital social. También, manifiesta la necesidad clara de romper las fronteras tradicionales, se requiere generar/crear redes de apoyo y cooperación entre sectores no gubernamentales, gubernamentales, privados y públicos (OMS, 1998).

Los Objetivos de Desarrollo Sostenible (ODS) adoptados por los miembros de las Naciones Unidas hacen hincapié en la necesidad de acabar con el hambre y todas las formas de malnutrición, así como de lograr la seguridad alimentaria para el año 2030. (Cavagnari *et al.*, 2023), además, señalan la importancia de los enfoques multisectoriales para abordar la malnutrición (Bezanson *et al.*, 2010; International Food Policy Research Institute [IFPRI], 2011; Banco Mundial, 2013). El sector educativo es una piedra angular en la batalla contra ella (Bundy *et al.*, 2009), por este motivo, en los países de ingresos bajos y medios se han puesto en marcha programas que abordan la doble carga de la malnutrición (Hawkes *et al.*, 2020) dentro del sector educativo (principalmente en las escuelas primarias) (Bundy *et al.*, 2017; United Nations System Standing Committee on Nutrition [UNSCN], 2017). La Iniciativa de Escuelas Amigables con la Nutrición (NFSI, por sus siglas en inglés) de la OMS es una de estas iniciativas que proporciona un marco para garantizar programas escolares integrados que aborden esta doble carga, teniendo en cuenta los cinco componentes generales: políticas de nutrición escolar, concienciación y capacitación de la comunidad escolar, planes de estudio que promuevan la nutrición y la salud, entorno escolar propicio para una buena nutrición y servicios escolares de apoyo nutricional y sanitario (Bundy *et al.*, 2017; UNSCN, 2017; OMS, 2021c). A pesar de estos esfuerzos, no existen protocolos consolidados con estrategias sobre cómo pueden aplicarse los programas de nutrición en los países de ingresos bajos y medios a través del sector educativo.

En el caso de los países de ingresos altos como España, los programas actualmente están enfocados al problema del sobrepeso y obesidad, por ser un grave problema de salud pública que los aqueja cada vez más. En el contexto de Europa, el Plan de Acción Europeo de la OMS para la Prevención y el Control de las ENT también reconoce la importancia de la prevención como la opción más factible para frenar la epidemia del sobrepeso y la obesidad (OMS, 2021b). Al igual que sucede en los países de ingreso bajos y medios, el sector educativo y de promoción de salud es primordial para prevenir y revertir las cifras actuales.

Ecuador, lamentablemente, a pesar de las políticas, estrategias y programas implementados en los últimos años, como el Plan Nacional del Buen Vivir, el Plan Intersectorial de Alimentación y Nutrición, la Intervención Nutricional Territorial Integral (INTI), el Programa Acción Nutrición (PAN) (CEPAL, PMA, 2017; Gutiérrez *et al.*, 2018; MSP, 2018), y la incorporación de hábitos saludables en el currículo y el incremento de dos a cinco horas de educación física para escuelas y colegios públicos (MSP, 2018; Ministerio de Educación del Ecuador, 2023), no ha logrado una mejora sustancial en las cifras de malnutrición escolar. Este hecho evidencia que hay mucho trabajo por delante, y que Ecuador debe enfocarse en la malnutrición en las formas de déficit y exceso, tomando en cuenta la transición epidemiológica nutricional que se está dando.

De acuerdo a Hawkes *et al.* (2020) la evidencia demuestra que las acciones que promueven un crecimiento sano durante la infancia y hábitos dietéticos saludables en lo largo de la vida, combinados con entornos alimentarios sanos, ingresos y educación adecuados, y los conocimientos y habilidades, tienen el potencial de beneficiar las diversas formas de malnutrición tanto por exceso como déficit. En la Figura 4, se aprecia lo que proponen estos investigadores, se puede ver una descripción sencilla de cómo las intervenciones podrían aprovechar estos factores comunes para actuar sobre la malnutrición.

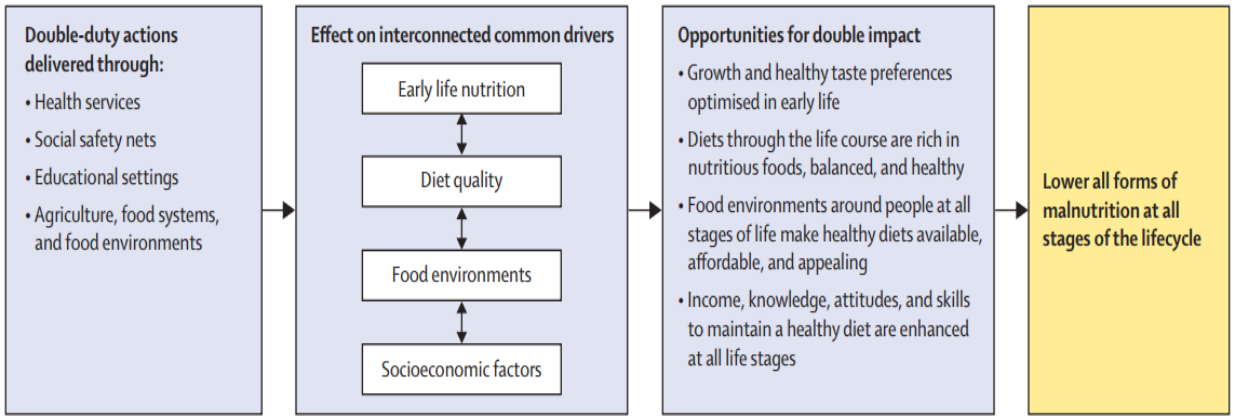

Figura 4. Marco lógico para evaluar las oportunidades de doble effecto de las medidas de nutrición (Hawkes *et al.*, 2020).

Los enfoques preventivos de malnutrición infantil además de todo lo mencionado, es importante no dejen a un lado el parasitismo intestinal y sus factores de riesgo asociados, por estar directamente relacionados en la calidad de vida y estado nutricional de los niños y niñas. Todas las medidas a tener en cuenta para reducir el riesgo de PI en la población deben ser desde una perspectiva "One Health", en donde la salud animal, humana y medioambiental están estrechamente relacionados y son interdependientes (FAO, 2023). "One Health" además, es un enfoque para diseñar y aplicar programas, políticas, legislación e investigación en el que múltiples sectores se comunican y colaboran para lograr mejores resultados de salud pública. Puede aplicarse a nivel comunitario, subnacional, nacional, regional y mundial, y se basa en una gobernanza, comunicación, colaboración y coordinación compartidas y eficaces; facilitaría a las personas una mejor comprensión de los beneficios indirectos, así como de los riesgos, equilibrios y oportunidades para promover soluciones equitativas y holísticas. (OMS, 2017, 2021d, 2023c). Desde el enfoque "One Health", es primordial el factor educativo, preventivo y de promoción de la salud. Conocer las vías de transmisión de los parásitos, las medidas higiénico-sanitarias a practicar, mejorar el saneamiento ambiental y los servicios sanitarios de las zonas, control y desparasitación de los animales domésticos, son algunas de las variables esenciales a tener en cuenta. En zonas endémicas también es necesario campañas de desparasitación masivas encaminadas a cortar el ciclo epidemiológico de los parásitos. La política pública nacional de los países

así como organismos internaciones, deben promover y garantizar un acceso igualitario en la educación y salud de las personas (Mascarini-Serra, 2011; Rodríguez-Sáenz *et al.*, 2015).

De acuerdo a la Organización Panamericana de la Salud (OPS): "La incorporación del enfoque intercultural de la salud en los programas de capacitación y desarrollo de los recursos humanos se presenta como una estrategia en el logro de la equidad. La interculturalidad será entendido como un proceso social interactivo, de reconocimiento y respeto de las diferencias existentes en una y entre varias culturas, en un espacio determinado, indispensable para construir una sociedad justa en el ámbito político, económico, social, cultural, etario, lingüístico, de género y generacional" (OPS, 1999). Teniendo en cuenta la importancia de este concepto, es imprescindible que previo a cualquier programa de intervención se tenga un correcto y completo diagnóstico del colectivo a intervenir, con una participación activa de quienes formar el grupo poblacional en todo el proceso, así como una intervención con un enfoque intercultural de la salud, adaptada y respetuosa a sus creencias, costumbres, tradiciones, cultura y lengua, que tenga en cuenta aspectos como la equidad de género y el respeto al medioambiente (OPS, 1999; Rodríguez-Martín, 2015; Brenes *et al.*, 2019). La denominada Interculturalidad en Salud es una importante oportunidad para un enriquecimiento multidireccional, y una estrategia óptima y eficaz para lograr los objetivos, que debe ser parte de todo proceso de diagnóstico e intervención (OPS, 1999; Rodríguez-Martín, 2015).

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CAPÍTULO II:
OBJETIVOS

Objetivo General y Específicos

Objetivo General

Analizar el estado nutricional de muestras de poblaciones escolares en diferentes contextos y su asociación con variables clásicas (dieta, ejercicio físico y otros estilos de vida), así como con otras variables más novedosas como el parasitismo intestinal. Este análisis nos permitirá realizar un diagnóstico previo a la propuesta de una intervención nutricional ya que las características del contexto son de vital importancia para el éxito de la intervención. La propuesta de intervención que se realice contemplará diferentes ámbitos que no solo comprometerá al sector educativo.

Objetivos Específicos

1. Estimar el estado nutricional de muestras de población escolar de la Comunidad Valenciana (España) y de las provincias de Chimborazo y Guayas (Ecuador). Para este primer objetivo específico se aplicará un método denominado "Nutrimetría", que combina dos variables antropométricas sencillas y accesibles (denominadas como “clásicas”) con la intención de facilitar su interpretación conjunta y generar una visión más amplia del estado nutricional.

2. Conocer las características sociodemográficas (edad, sexo, localidad de residencia, número de convivientes), hábitos alimentarios, estilo de vida (ejercicio físico y uso de pantallas) y hábitos de comportamiento (uso de agua potable, lavado de manos antes/después comida y antes/después uso del váter, lavado de futas y verduras, contacto con animales domésticos, práctica de juegos al aire libre) de muestras de población escolar de la Comunidad Valenciana (España) y de las provincias de Chimborazo y Guayas (Ecuador).

3. Estimar la prevalencia y espectro de especies de parásitos intestinales en muestras de población escolar de la Comunidad Valenciana (España) y de las provincias de Chimborazo y Guayas (Ecuador).

4. Analizar las posibles asociaciones entre el estado nutricional de los niños y niñas escolares de la Comunidad Valenciana (España) y de las provincias de Chimborazo y Guayas (Ecuador) y las variables sociodemográficas, hábitos alimentarios, estilo de vida, hábitos de comportamiento y parasitismo intestinal.

5. Elaborar una propuesta de Marco Lógico de Intervención, extrapolable a cualquier sector educativo escolar, teniendo como base los problemas identificados, desde la perspectiva "One Health" que permita mejorar el estado nutricional de los y las niñas escolares de la Comunidad Valenciana (España) y de Chimborazo y Guayas (Ecuador).

CAPÍTULO III: ARTÍCULOS DE INVESTIGACIÓN

3.1. Assessment of the Health Status of Spanish Schoolchildren Based on Nutrimetry, Lifestyle and Intestinal Parasites

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Resumen

La malnutrición en población escolar española, y su relación con los estilos de vida, ha sido estudiada, pero nunca antes se habían tenido en cuenta la Nutrimetría (un indicador antropométrico del estado nutricional), y los datos sobre parasitismo intestinal y sus factores de riesgo. Participaron 206 niños y niñas de 3 a 11 años, de dos colegios de la Comunidad Valenciana. Se recogieron características demográficas, alimentarias, de estilos de vida, hábitos de comportamiento, datos antropométricos (peso, talla) y coproparasitológicos. Se utilizó Nutrimetría para determinar el estado nutricional. Se realizaron análisis estadísticos para establecer las asociaciones entre el estilo de vida, las especies parasitarias seleccionadas y el estado nutricional. Se hizo un análisis de regresión logística multivariante para evaluar la fuerza de la asociación de los factores de riesgo sospechosos con la presencia de parasitismo intestinal. La prevalencia de exceso de peso en los y las escolares fue del 32.6%. El 43,9% tenía una alta adherencia a la Dieta Mediterránea, cuya ingesta media diaria era de 2428,7 kcal. Se identificó parasitismo intestinal en el 49,5% de los niños y niñas (*Giardia duodenalis*: 28,6%). La procedencia del agua de bebida resultó ser un factor de riesgo de parasitismo intestinal. No se pudo confirmar ninguna asociación positiva entre las variables analizadas y el estado nutricional. La Nutrimetría es un buen indicador para un estudio completo del estado nutricional.

Palabras clave: malnutrición; nutrimetría; escolares; estilos de vida; parásitos intestinales; factores de riesgo

nutrients

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Article

Assessment of the Health Status of Spanish Schoolchildren Based on Nutrimetry, Lifestyle and Intestinal Parasites

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Abstract: Malnutrition in Spanish schoolchildren, and its relationship with lifestyles, has been studied, but Nutrimetry (a nutritional status indicator), and data on intestinal parasitism and its risk factors, have never before been taken into account. A total of 206 children aged 3–11 years, from two schools in the Valencian Community, participated. Demographic characteristics, diet, lifestyles, behavioural habits and anthropometric (weight, height) and coproparasitological data were collected. Nutrimetry was used to analyse nutritional status. Statistical analyses were performed to ascertain associations between lifestyle, selected parasite species and nutritional status. Multivariate logistic regression analysis was used to assess the strength of the association of the suspected risk factors with the presence of intestinal parasitism. The prevalence of overweight was 32.6%. A total of 43.9% had a high adherence to the Mediterranean Diet, for which mean daily intake was 2428.7 kcal. Intestinal parasitism was identified in 49.5% of the children (*Giardia duodenalis*: 28.6%). The source of drinking water was found to be a risk factor for intestinal parasitism. No positive association between the variables analysed and nutritional status could be confirmed. Nutrimetry is a good indicator for a complete analysis of nutritional status. It highlights the prevalence of overweight. Intestinal parasitism was identified in almost half of the participants and is a variable that should not be underestimated.

Keywords: malnutrition; nutrimetry; schoolchildren; lifestyles; intestinal parasites; risk factors

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1. Introduction

Child malnutrition continues to be a global health problem with multifactorial origins [1]. In Europe, there are epidemic proportions of overweight that constitute one of the main health issues; it is a predetermining factor for the future development of non-communicable diseases such as type 2 diabetes, hypertension and dyslipidaemia [2], with Spain being at the forefront in terms of childhood overweight and obesity [3]. Previous studies in Spanish children report overweight figures ranging from 21.2% to 45.2% [4–9]. The body-mass-index-for-age z-score (BMIZ) is an indicator used for the diagnosis of nutritional status; its use is easy, as well as its interpretation, and it is also the most widely used, but it is advisable to complement it with other indicators. For this reason, Selem-Solís et al., in 2017, developed the "Nutrimetry" method, which combines two accessible and easy anthropometric variables, the BMIZ and the height-for-age z-score (HAZ), whose intention is to facilitate a joint interpretation of these two indicators and generate a more complete diagnosis of nutritional status [10,11].

Traditional Mediterranean diets (characteristic of Euro-Mediterranean countries, based on traditional food and drink and often homemade) are associated with a low prevalence of chronic diseases and a longer life expectancy among populations with adherence to them [12]. Unfortunately, adherence to this type of diet is deteriorating [13], with a preference for high-calorie, ultra-processed products replacing vegetables and fruit [2,8,14,15]. The KidMed questionnaire is an easy and good tool to assess adherence to the Mediterranean diet (AMD) [16], but it does not take into account daily caloric and nutrient intake, a limitation that a food frequency questionnaire (FFQ) would provide. FFQs allow valid, accurate information to be obtained and are easy to administer without the need for a specialised interviewer [17–19]. Malnutrition problems, especially overnutrition, are directly related to sedentary lifestyles. The WHO recommends that children should spend at least one hour a day engaging in moderate- or vigorous-intensity physical activity (PA) and that screen time (ST) should be less than two hours a day [20]. However, many children do not meet these recommendations [21], similarly to what was found in the 2019 PASOS study in the Spanish population aged 8–16 years.

It is common for nutrition studies to be carried out with the variables described above, such as anthropometric, dietary and lifestyle values, leaving aside the possible causes of infectious and immunological origin that may be associated with these states of malnutrition, such as intestinal parasitism. These infections are an important child health problem that has historically affected mainly low-income countries, but is becoming more frequent in high-income countries, such as Spain, due to demographic, climatic and behavioural changes [22–27]. There are some intestinal parasite species associated with an altered nutritional status, such as the unicellulars *Giardia duodenalis* and *Cryptosporidium* spp. and the helminths *Ascaris lumbricoides* and *Trichuris trichiura* [28–30]. In the case of other parasites, such as amoebae, *Enterobius vermicularis* and *Blastocystis* sp., there is no evidence that they affect nutritional status and some of the species may even be considered commensal, but their presence will be related to inadequate hygienic sanitary measures [31,32] and, as they all share faecal–oral transmission, they can spread easily [33,34]. Studies carried out in Spain in recent years have estimated the prevalence of intestinal parasites in children ranging from 10.7% to 28.0% [27,32,35–39]. These results vary according to the study areas, as well as by the techniques used, where molecular techniques (PCR) increase the sensitivity of detection [40]. Specifically, in the school population of the Valencian Community, the study by Chover et al. in 2010 [27] is one of note, in which they found that 27.4% of the children had at least one species that was identified by light microscopy.

In the Valencian Community to date, there are studies of schoolchildren that have analysed nutritional status with the most frequent indicator (BIMZ), its relationship with the "typical variables" such as dietary habits and lifestyles, but not with intestinal parasitism, and the last existing study on the prevalence of intestinal parasitism in this type of population is from 2010 only, and was analysed with one faecal sample by optical microscopy and without analysing the risk factors of parasitism. In view of the above, the aim of this research is to evaluate the nutritional status of schoolchildren attending primary schools in the Valencian Community with a different indicator such as Nutrimetry, and the relationships with eating habits (KidMed and FFQ), lifestyles (PA and ST) and parasite species that may alter it, and to analyse the possible risk factors associated with the presence of intestinal parasitism.

2. Materials and Methods

2.1. Ethics Considerations

This research has the approval of the Human Research Ethics Committee of the University of Valencia (the procedure number is H1518738039128 (date: 1 March 2018)), in order to meet the principles of the Helsinki Declaration and obey Spain's legislations on biomedical research and protection of personal data.

2.2. Study Design and Setting

A prospective cross-sectional study was carried out involving 206 schoolchildren, aged between three and eleven years, in the Valencian Community who attended two schools, one in La Cañada (Valencia) and another in Vila-Real (Castellón). Data and samples were collected from participating schoolchildren during 2019–2020. The municipalities to which the public schools belonged were very similar in terms of socioeconomic and socio-demographic characteristics (Table S1, [41,42]).

2.3. Recruitment and Sampling

One school per zone was selected that met the following criteria: (1) the number of students enrolled had to be greater than 200, (2) the school had to be of a medium-high socioeconomic level and (3) there had to be commitment and involvement in all phases on the part of the teachers and directors of the institution. School principals were contacted and invited to participate on a voluntary basis in order to achieve the third point. A total of 113 schoolchildren were recruited in Vila-Real and 96 in La Cañada.

Informative meetings were personally held with teachers, who explained the goals and procedures of the project to schoolchildren. This phase lasted three months. Meanwhile, the school principal sent a voluntary invitation letter and information to all parents in their children's school diaries. Prior to the information and sample collection phase, informed consent forms were obtained and signed by parents for all participating children. No financial remuneration was given for being part of the research. Once all the weight and height data and samples were processed and analysed, a report was personally delivered to each parent in a sealed envelope.

2.4. Data Collection

Five nutritionists were trained to collect data and samples. The information collected followed previously standardized protocols. Anthropometric assessment, a standardised questionnaire and a coproparasitological assessment were carried out as indicated below:

(a) Anthropometric Assessment

At the time of weighing, the participants wore the least amount of clothing possible, and an Omrom® (Hoofddorp, The Netherlands) electronic scale with an accuracy of 100 g was used. Height was measured without shoes and socks and was taken with a Seca216® (Hamburgo, Germany) mechanical measuring rod with a precision of 1 mm.

Anthropometric data, date of birth and gender were entered into the computer programs WHO Anthro for under-fives and WHO AnthroPlus for over-fives (WHO 2009; Anthro for Personal Computers, Version 3.01: Software for Assessing Growth and Development of the World's Children). This obtained HAZ and BMIZ and classified participants into different types and degrees of malnutrition [43]. Nutrimetry combines these two indicators in a three-by-three table (Figure 1) and facilitates a final joint interpretation of nutritional status.

The nine nutricodes generated by the combination of HAZ and BMIZ can be seen in Figure 2, and represent the diagnosis of nutritional status [10].

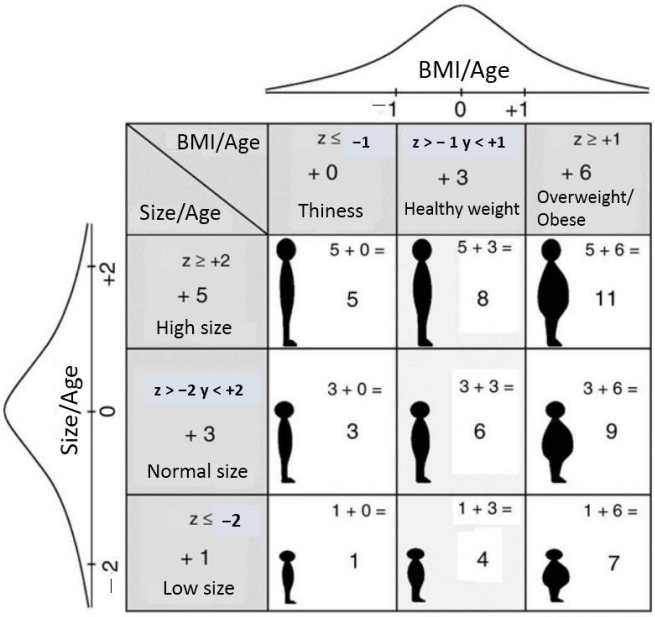

Figure 1. Nutrimetry information as the joint interpretation of HAZ and BMIZ (Tapia-Veloz et al., 2022) [44].

Nutricodes	Interpretation
1	Low HAZ + Low BMIZ
3	Normal HAZ + Low BMIZ
5	High HAZ + Low BMIZ
4	Low HAZ + Normal BMIZ
6	Normal HAZ + Normal BMIZ
8	High HAZ + Normal BMIZ
7	Low HAZ + High BMIZ
9	Normal HAZ + High BMIZ
11	High HAZ + High BMIZ

Figure 2. Nutricodes (Tapia-Veloz et al., 2022) [44].

(b) Questionnaire

Questions included: (b.1) demographic characteristics (age, gender, locality of residence, number of relatives residing at home); (b.2) eating habits (KidMed questionnaire [16], FFQ [45]); (b.3) lifestyles (physical activity, use of screens); and (b.4) behavioural habits (source of drinking water, hand and fruit/vegetable washing, contact with domestic animals, playing outdoors). The participants returned the completed questionnaires, together with the signed informed consent forms, for collection, as described above (Table S2).

(c) Coproparasitological Assessment

To collect stool samples, the participating children whose parents signed the informed consent were given uniquely labelled kits (10 mL polystyrene plastic tube with 5 mL 70% ethanol inside, disposable spatula, Graham tape and instructions). Parents assisted the children to collect them and delivered them to the school the next day. Collected stool samples were immediately transported to the Parasitology Laboratory of the University of Valencia and stored at 4 °C until further processing. A total of 206 samples were collected and processed. One stool sample per child was collected. A 3 g stool sample from each participant was concentrated and filtered for 5 min at 2500 rpm using Midi Parasep® filter devices (Apacor Ltd., Wokingham, UK). From the sediment obtained, a part was used for DNA extraction (see below) and, to the remaining part, a few drops of 10% formalin was added in a 1:3 ratio for microscopy analysis. With a small amount of the remaining

sediment, thin smears were also prepared and stained with the Modified Ziehl-Neelsen (MZN) for the detection and identification of *Cryptosporidium* spp. and other coccidian protozoa. Graham tapes were examined by 10× microscopy for detecting eggs of *Enterobius vermicularis*. All stool samples were observed microscopically at 10× and 40× in order to detect structures such as eggs, cysts, oocysts, trophozoites and larvae. If none were found, it was considered negative. DNA was extracted and purified from all samples in order to perform molecular techniques to complement the microscopic observation. Genomic DNA was isolated from 200 mg of each stool sample using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Extracted and purified DNA samples were eluted in 100 µL of PCR-grade water and kept at 4 °C until further molecular analysis. The presence of *G. duodenalis* was investigated using a real-time PCR (qPCR) targeting a 62-bp region of the small subunit of the rRNA (*ssu* rRNA) gene of the parasite [46]. The final mixing reaction volume was 15 µL, a commercial assay with the specific primers and probe (LightMix Modular Assays Giardia; Roche®, Basel, Switzerland), together with mastermix (dNTPS, buffer, thermostable Taq polymerase) (PerfeCTa qPCR ToughMix; Quanta Biosciences, Gaithersburg, MD, USA), was used. An amount of 5 µL of sample and positive control DNA was added to each well, to which the negative control water was added. The StepOnePlus thermcycler qPCR® (Applied Biosystems®, Foster City, CA, USA) was used. Samples that amplified before cycle 43 were considered positive. *Blastocystis* sp. was identified through a direct PCR protocol, targeting a 600 bp fragment of the *ssu* rRNA gene [47,48]. In the case of *Cryptosporidium* spp., a nested PCR protocol was used to amplify a 587 bp fragment of the *ssu* rRNA gene of this parasite [49]. Detailed information on the oligonucleotides used for the molecular identification of the unicellular parasites investigated in this study is presented in Table S3.

2.5. Data Analysis

Descriptive statistics were calculated, including measures of central tendency (mean and median), measures of dispersion (standard deviation, range and coefficient of variation) and measures of shape (asymmetry and pointing) for quantitative variables, as well as the absolute and relative frequencies for the qualitative variables. To see a possible heterogeneity of the results, an analysis of association, stratified by age and gender, was carried out. The Mann–Whitney U test and Fisher's exact test (non-parametric tests) were used when the data were stratified and their sample size was less than 15. To test the null hypotheses of no association between the risk of intestinal parasitism and the lifestyle habits controlled by confounders or effect modifiers, multiple logistic regression analyses, with dummy variables for categorical terms, were applied. Different models were tested. Analyses with odds intestinal parasitism as an outcome (yes/no) were always adjusted by primary school of origin. Covariates used in the models were demographic data and the traditional hygiene measures (washing hands, washing fruits and vegetables, contact with domestic animals and sources of drinking water such as bottled, tap and osmosis water). All the covariates were categorical variables. The magnitude of the association was expressed as adjusted odds ratios (AORs) with a 95% confidence interval (CI). A p-value ≤ 0.05 was considered significant. All the variables were analysed using SPSS software version 26.0 (Statistical Package for Social Sciences, Chicago, IL, USA).

3. Results

The participants were schoolchildren from two primary schools in the Valencian Community (Spain). A total of 206 schoolchildren (40.3% girls), aged three to eleven years (mean: 8.1 ± 2.1 years), participated.

Totals of 31.6% (65/206), 1.5% (3/206) and 1.0% (2/206) of the total children corresponded to nutricodes 9, 3 and 11 (associated with malnutrition), respectively, highlighting the high prevalence of nutricode 9, which corresponds to a normal height and overweight. Nutricodes 1, 4, 5 and 7 were non-existent. The nutritional status stratified by gender and age, and assessed by Nutrimetry, of the participants is detailed in Table 1. No statistical dif-

ferences were found between the two stratified variables and the distribution of nutricodes ($p \leq 0.05$).

Table 1. Frequencies (%) of the participating children according to the nutricodes stratified by gender and age.

	BOYS (n = 123)			* p-Value	GIRLS (n = 83)			* p-Value	** p-Value
Nutricode	**<5 (n = 5)**	**5–8 (n = 67)**	**≥9 (n = 51)**		**<5 (n = 6)**	**5–8 (n = 35)**	**≥9 (n = 42)**		
Thinness									
1	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)		
3	0 (0)	1.5 (1)	0 (0)		0 (0)	2.9 (1)	2.4 (1)		
5	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)		
Normal Weight									
4	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)		
6	60 (3)	64.2 (43)	66.7 (34)	0.918	66.7 (4)	62.8 (22)	69.0 (29)	0.807	0.883
8	0 (0)	0 (0)	0 (0)		0 (0)	2.9 (1)	0 (0)		
Overweight and Obesity									
7	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)		
9	40 (2)	34.3 (23)	33.3 (17)		33.3 (2)	25.7 (9)	28.6 (12)		
11	0 (0)	0 (0)	0 (0)		0 (0)	5.7 (2)	0 (0)		

The number of children is shown in brackets. * p-value for the comparison of the distribution of the frequencies of the codes established by Nutrimetry and age; ** p-value for the comparison of the distribution of the frequencies of the codes established by Nutrimetry and gender.

Regarding the AMD evaluated by the KidMed questionnaire, low AMD was found in 7.3% of all children, medium AMD in 48.8% and high AMD in 43.9%. When we stratified by gender, differences were found (p = 0.037) whereby boys presented higher numbers of high AMD compared to girls (70%, 63/90 vs. 30%, 28/90). The results of nutritional status by Nutrimetry and AMD are shown in Table 2; we did not find any statistical difference by nutricodes ($p \leq 0.05$).

Table 2. Frequencies (%) of the participating children according to the nutricodes stratified by the AMD (KidMed questionnaire).

Nutricode	**Low AMD (n = 15)**	**Medium AMD (n = 100)**	**High AMD (n = 90)**	* p-Value
Thinness				
1	0 (0)	0 (0)	0 (0)	
3	0 (0)	3.0 (3)	0 (0)	
5	0 (0)	0 (0)	0 (0)	
Normal weight				
4	0 (0)	0 (0)	0 (0)	0.611
6	66.7 (10)	69 (69)	62.2 (56)	
8	0 (0)	0 (0)	1.1 (1)	
Overweight and obesity				
7	0 (0)	0 (0)	0 (0)	
9	33.3 (5)	27 (27)	36.7 (33)	
11	0 (0)	1.0 (1)	1.1 (1)	

Number of children is shown in brackets. * p-value for the comparison of the distribution of the frequencies of the codes established by Nutrimetry and the AMD.

Regarding FFQ analysis, in the participating boys, the mean kcals consumed per day from their total diet, and kcals derived from carbohydrates (CHO), protein and fat were 2428.7 kcal (SD: 548.9 kcal), 1392.7 kcal (SD: 298.8 kcal), 113.3 kcal (SD: 30.4 kcal), and 923.1 kcal (SD: 248.7 kcal); for girls, these were 2372.1 kcal (SD: 528.1), 1365.2 kcal (SD: 281.4 kcal), 108.3 kcal (SD: 25.4 kcal) and 898.5 kcal (SD: 249.1 kcal). No differences were

found according to sex ($p \leq 0.05$). The frequency according to the Nutrimetry codes for daily intake in kcals of total diet, CHO, protein, and fat in the surveyed schoolchildren are shown in Table 3, and no statistical differences by nutricodes were obtained.

Table 3. Daily intake of total kcals, and CHO, protein and fat (kcals) of the participating children stratified by the nutricodes.

Nutricode	% (*n*)	TOTAL kcal			CHO (kcal)			PROTEIN (kcal)			FAT (kcal)		
		Mean	Sd	* *p*-Value	Mean	Sd	* *p*-Value	Mean	Sd	* *p*-Value	Mean	Sd	* *p*-Value
Thinness													
1	0 (0)												
3	1.5 (3)	2391.9	459.3		1354.4	264.6		108.1	21.5		929.4	228.1	
5	0 (0)												
Normal Weight													
4	0 (0)												
6	65.5 (135)	2357.5	480.7	0.45	1353.8	272.4	0.37	108.3	26.1	0.21	895.9	212.6	0.69
8	0.5 (1)	2849.8	-		1640.0	-		142.1			1067.8		
Overweight and Obesity													
7	0 (0)												
9	31.6 (65)	2498.8	651.9		1435.1	329.9		117.0	33.1		946.7	313.7	
11	0.9 (2)	2458.1	341.5		1436.6	82.9		125.9	9.4		895.5	249.2	

Number of children is shown in brackets. * *p*-value for the comparison of the distribution of the frequencies of the codes established by Nutrimetry and the total kcal intake, and kcal intake derived from CHO, protein and fat. CHO: carbohydrates.

When we analyse the prevalence of other lifestyle habits in the enrolled population, 72.3% and 30.6% of the participants did not comply with recommendations for PA (at least one hour per day) and ST (less than two hours per day), respectively. We did not find any differences between gender or age and also with nutritional status.

Intestinal parasitism was also evaluated in all the participants (Table 4). Overall, 49.5% (102/206) of the participating schoolchildren were infected/colonised by at least one intestinal parasite species and 14.6% (30/206) had two or more species. The four species identified were *G. duodenalis* (28.6%; 59/206), *Blastocystis* sp. (19.4%; 40/206), *E. vermicularis* (19.4%; 2/206) and *Entamoeba hartmanni* (1.0%; 2/206). No statistical differences in intestinal parasitism were found when stratifying by gender and age groups. Of the species identified, *G. duodenalis* may affect the nutritional status of children, so we analysed this parasite and the two most prevalent nutricodes (6 and 9). We found no statistical differences ($p = 0.369$) of the participants corresponding to nutricode 6 and 9 where 27.4% and 30.8% had *G. duodenalis*, respectively. Regarding the traditional risk factors for intestinal parasitism, the prevalences were as follows: 52.9% had contact with animals; 6.3% did not wash fruits/vegetables before consumption; 34.5% and 51.0% did not wash their hands before eating and after bathing, respectively; 64.1%, 9.7% and 26.2% consumed bottled, tap and osmosis water, respectively; and, in 12.6%, there were five or more relatives residing at their home.

Table 4. Frequencies (%) of the studied population according to the presence of total intestinal parasitism, multiparasitism and detected species analysed by optical microscopy and PCR.

	TOTAL
Detected Species	**(*n* = 206)**
G. duodenalis	28.6 (59)
Blastocystis sp.	19.4 (40)
E. vermicularis	19.4 (40)
E. hartmanni	1.0 (2)
Total intestinal parasitism	49.5 (102)
Total intestinal multiparasitism	14.6 (30)

Number of children is shown in brackets.

Finally, to assess the risk of intestinal parasitism as a function of possible risk factors (classic or traditional risk factors) in the studied population, a multivariate logistic regression analysis was carried out (Table 5).

Table 5. Multivariate logistic regression analysis to estimate the risk of intestinal parasitism in the participating children.

Variable	Category	*n*	Intestinal Parasitism		
			AOR	95% CI	* *p*-Value
Gender	Girl	83	1.352	0.738–2.479	0.329
	Boy	123	Ref.	-	-
Age group	3 to <5 years	11	2.495	0.572–10.882	0.224
	5 to <9 years	102	0.719	0.382–1.354	0.307
	9 to <12 years	93	Ref.	-	-
Contact with animals	Yes	97	1.111	0.609–2.028	0.731
	No	109	Ref.	-	-
Washing fruits/vegetables	Yes	193	Ref.	-	-
	No	13	0.853	0.246–2.963	0.802
Handwashing before eating	Yes	135	Ref.	-	-
	No	71	1.293	0.659–2.536	0.455
Handwashing after bathing	Yes	101	Ref.	-	-
	No	105	0.823	0.435–1.557	0.549
Source of drinking water	Bottled	132	Ref.	-	-
	Tap	20	3.263	1.076–9.897	0.037
	Osmosis	54	0.522	0.264–1.0320	0.061
Plays outdoors	Yes	147	0.965	0.481–1.939	0.921
	No	59	Ref.	-	-
Number of relatives residing at home	2–4 people	180	Ref.	-	-
	≥5	26	0.430	0.167–1.109	0.081

AOR: adjusted odds ratio. CI: confidential interval. * Statistical significance was set at $\alpha = 0.05$. Ref: category of reference.

The only variable that was significantly related to the risk of intestinal parasitism was the source of drinking water. The schoolchildren who consume tap water had significantly greater odds of having intestinal parasitism compared to those who consume bottled water (OR = 3.263; CI 95%: 1.076–9.987; $p = 0.037$). Surprisingly, those who consume water filtered by osmosis did not show significantly lower odds (OR = 0.522; CI 95%: 0.264–1.032; $p = 0.061$). When other multivariate logistic regression models using demographic and/or lifestyle variables (adjusted and non-adjusted) were tested no significant associations were found.

4. Discussion

We studied the nutritional status of schoolchildren using Nutrimetry, the first time this indicator has been used in the Spanish population. Previous research only used the BMIZ as an indicator [7,50–54]. The prevalence of overweight in our research (codes 7, 11) was 32.6% and the prevalence of thinness (code 3) was 1.5%. Previous studies in similar populations reported figures in line with those found, with overweight ranging from 21.2% to 45.2% [4–9] and underweight from 0.4% to 13.3% [7,54]. According to the National Health Survey (2017) of the Spanish Ministry of Health, in the Valencian Community, the prevalence of overweight was 28.2% [55], a number that, compared to the one we found (32.6%), confirms its increase. These data are also in accordance with the WHO, which states that, among children and adolescents (5 to 19 years of age), overweight and obesity have increased dramatically in recent years [56]. Excess weight in schoolchildren is clearly an urgent public health problem to be tackled.

Although the majority of participants had a nutritional status that was in codes 6 and 9, and while the remaining codes (1, 3, 4, 5, 7, 8 and 11) had very few or no children, this is a novel indicator because it would facilitate comparisons with other populations. Nutrimetry allows for a more detailed and in-depth description and analysis of the population distribution of malnutrition by combining two indicators, that are normally used separately, to obtain a single diagnosis.

It does not require specialised software to generate the nine subgroups. It uses the WHO growth curves as a reference and can therefore be considered a suitable anthropometric indicator. Furthermore, it uses neutral language (numbers) to report the results, which facilitates communication without an emotional semantic load by avoiding the use of words such as "fat" or "skinny" [10,11,43].

We found that 7.3%, 48.8% and 43.9% of the children had low, medium and high AMD, respectively. These percentages obtained are in agreement with other studies in similar populations [9,15,57]. There are studies that found statistical differences between AMD and nutritional status, where having high adherence is a protective factor against excess weight [58–61], but there are also investigations that did not find statistical differences [9,15,57,62,63]. In the case of our work, we found no differences. It is important to note that KidMed does not take into account calories consumed and, therefore, a high adherence does not guarantee a healthy nutritional status in its entirety, which is why we included the FFQ.

The mean age of the schoolchildren was 8.1 years (±2.1 years); EFSA recommends 1815 kcal for boys and 1696 kcal for girls per day at this age, with CHO being between 45 and 60% of the total, protein being between 0.75 and 0.92 g/kg and fat being between 20 and 35% of the total [64]. The results obtained from the FFQ analysis are higher than the recommended values, revealing a possible overestimation. The long FFQ, the one we used, has the disadvantage of overestimating the total kcals and nutrients consumed [64–67]. This is due to the greater effort and time required to answer the questionnaire, the sum of consumption frequencies due to the existence of many items, the errors in the estimation of the frequency and the perception of the size of portions may be greater than the real ones [68–72], especially considering that the respondents were the legal representatives. There was no association between nutritional status and mean total kcals and macronutrient concentrations; we also believe this is due to the overestimation of the FFQ. Healthy eating is an essential factor, but must be accompanied by lifestyles that include daily PA in children (of at least one hour of moderate/vigorous intensity) and less than two hours in front of screens in order to ensure proper health [20,73]. PA at this age is favourably associated with indicators such as adiposity, bone and skeletal health, cardiometabolic health and cognitive and motor development [20]. In the PASOS study (2019) carried out on 38,887 Spaniards aged 8 to 16 years, 63.3% and 45.6% did not comply with the recommendations for PA and ST, respectively [74]; these are similar data to those that we found (72.3% and 30.6%, respectively).

Although no association was found between nutritional status and the two methods used to assess dietary intake (FFQ and AMD), the prevalence of overweight is high and most participants did not meet the daily PA recommendations. For overweight/obesity to exist, there must be an incorrect imbalance between the energy that is spent and consumed for their age. Response bias could be an important factor that may have led to not finding an association, but this does not rule out it being a reality. In order to avoid this limitation for future studies, an explanation of portion size could be strengthened, an additional method, such as a 24 h reminder, could be included and a second FFQ could be used to allow a comparison.

The high prevalence of intestinal parasitism has been associated with tropical/subtropical countries in association with poverty [75]. This reality is changing in areas with different economic and climatic characteristics, such as Spain, where numbers are increasing. The reasons for this include migration from endemic countries, increased international travel, consumption of fresh food and fostering/adoption of children from these

areas, more consumption of organically grown food and the climate crisis [22–27]. In the present study, 49.5% of the participants had intestinal parasitism, a high number compared to other studies which reported between 10.7% and 28.0% [27,32,35–39]. Chover et al. (2010) [27], in their work on schoolchildren in Valencia, identified *Blastocystis* sp. (14.9%), *E. vermicularis* (9.6%) and *G. duodenalis* (6.1%) as the most prevalent species; in our case, these were found in 28.6%, 19.4% and 19.4% of participants, respectively. Our numbers are probably higher due to an increase in intestinal parasitism, but also due to the combination of light microscopy and molecular techniques that stand out as being more sensitive [40]. When analysing the relationship between Nutrimetry and *G. duodenalis*, we did not find a significant association, this is possibly because the impact towards nutritional status usually occurs when there is undernutrition and, in our research, this condition was very low. Even so, its presence should not be underestimated. The number of cases of *G. duodenalis* was high and the similarity of its prevalence with a study in children conducted in an endemic area such as Ecuador (30.8%) is striking. It is possible that some of these participants were asymptomatic, which facilitates the spread of the parasite when not treated [33,76]. This species can affect the intestinal barrier and can be a triggering factor for secondary food intolerance/malabsorption, dyspepsia or irritable bowel syndrome [30,34,77].

The factors we took into account as risk factors associated with intestinal parasitism in children are in line with those studied by other researchers, who found no association between these variables and being parasitized [32,36,78]. Our multivariate logistic regression analysis revealed that the only variable associated with intestinal parasitism was the source of drinking water. Schoolchildren who consumed tap water were significantly more likely to be parasitized compared to those who consumed bottled water. Those who consumed water filtered by osmosis did not show significantly lower odds. These data should be taken with caution, especially considering that the number of participants who consumed tap water was very low (9.7%), that some variable that we did not take into account could alter the results and the limitation of the low sample size.

Certain limitations may have compromised the accuracy of the results and conclusions of this study. The epidemiological data generated here might not be representative of the whole Valencian Community scenario, for example: the data collected in the questionnaire could lead to a certain recall/recording bias; the two questionnaires used to collect information on dietary habits should be complemented by some additional method; and, finally, some risk factors for intestinal parasitism not included could provide further analysis. Future studies should be carried out in larger samples so that they can be representative, where the analysis of intestinal parasitism should be an important variable to be taken into account together with its symptomatology. All this would provide a clearer picture in order to take measures to improve the nutrition, health, diet and lifestyle status of the school population of the Valencian Community.

5. Conclusions

Nutrimetry can be an interesting indicator, that is easy to use and interpret and is suitable for use at a clinical and epidemiological level, for a more complete analysis of the nutritional status of children because it allows the obtaining of a joint diagnosis of HAZ and BMIZ that are typically used separately.

This study shows the high prevalence of overweight in schoolchildren, which confirms the alarming figures worldwide and that urgent measures need to be taken to correct it at the family, school and governmental levels.

Lifestyles are a fundamental aspect to take into account because of their relationship with nutritional status, but it is necessary, above all at the level of dietary habits, to combine various methods that improve their analysis and facilitate the finding of associations with nutritional status.

Regarding intestinal parasitism, the high numbers found are striking, especially when compared with previous studies in similar populations. *G. duodenalis* was the most prevalent, which can cause digestive problems in addition to malnutrition, such as the malab-

sorption of certain nutrients. Therefore, intestinal parasites in Spain need more attention in order to understand the associated factors and their impact on children's health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu15122801/s1, Table S1: Socioeconomic and socio-demographic characteristics of the municipalities of the two participating public schools; Table S2: English version of the standardised epidemiological questionnaire used in this study; Table S3: Oligonucleotides used for the molecular identification and/or characterization of the intestinal protists parasites investigated in the present study.

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3.2. Prevalence and associated risk factors of intestinal parasites among schoolchildren in Ecuador, with emphasis on the molecular diversity of *Giardia duodenalis*, *Blastocystis* sp. and *Enterocytozoon bieneusi*

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Resumen

Los helmintos intestinales, incluidas las infecciones por helmintos transmitidos por el suelo y por protistas gastrointestinales (PG), contribuyen en gran medida a la carga mundial de morbilidad, especialmente en países de bajos ingresos como Ecuador. Su epidemiología en estos entornos es en gran parte desconocida. Este estudio prospectivo transversal investiga la portación de helmintos intestinales, incluyendo STH, y PG en escolares asintomáticos (3-11 años) en las provincias de Chimborazo y Guayas, Ecuador. Se recogieron muestras de heces individuales (n = 372) y cuestionarios epidemiológicos sobre datos demográficos y posibles factores de riesgo de los escolares participantes. Se utilizó el examen microscópico convencional como método de cribado, y ensayos moleculares (PCR y secuenciación de Sanger) para investigar más a fondo la epidemiología de algunos PG. Se utilizó un análisis de regresión logística multivariante para evaluar la fuerza de la asociación de los presuntos factores de riesgo con la presencia de helmintos y PG. Se observó al menos una especie de parásito intestinal mediante microscopía en el 63,2% (235/372) de los escolares participantes. *Enterobius vermicularis* (16,7%, 62/372; IC 95%: 13,0-20,9) y *Blastocystis* sp. (39,2%, 146/372; IC 95%: 34,2-44,2) fueron los más prevalentes entre los helmintos y PG, respectivamente. Se detectaron los genotipos A (50,0%), B (37,5%) y A+B (12,5%) en *Giardia duodenalis* y ST3 (28,6%), ST1 y ST2 (26,2% cada uno) y ST4 (14,3%) en *Blastocystis* sp. Se identificaron tres genotipos, dos conocidos (A: 66,7%; KB-1: 16,7%) y uno nuevo (HhEcEb1, 16,7%) en *Enterocytozoon bieneusi*. El municipio de origen, el hacinamiento en el hogar y los malos hábitos sanitarios y de higiene personal fueron factores de riesgo de colonización por parásitos intestinales en la infancia. A pesar de los programas gubernamentales masivos de administración de fármacos, las infecciones por STH y PG siguen siendo un problema de salud pública en las poblaciones pediátricas que viven en entornos de escasos recursos. Se necesitan métodos analíticos moleculares para comprender mejor la epidemiología de estos parásitos intestinales. Este estudio aporta información novedosa sobre la presencia de variantes genéticas de *Blastocystis* sp. y *E. bieneusi* circulantes en poblaciones humanas ecuatorianas.

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Prevalence and associated risk factors of intestinal parasites among schoolchildren in Ecuador, with emphasis on the molecular diversity of *Giardia duodenalis*, *Blastocystis* sp. and *Enterocytozoon bieneusi*

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Abstract

Background

Intestinal helminths, including Soil-Transmitted Helminth (STH), and Gastrointestinal Protist (GP) infections are major contributors to the global burden of disease, particularly in low-income countries such Ecuador. Their epidemiology in these settings is largely unknown.

Methodology

This prospective cross-sectional study investigates the carriage of intestinal helminths, including STH, and GP in asymptomatic schoolchildren (3–11 years) in the Chimborazo and Guayas provinces, Ecuador. Single stool samples ($n = 372$) and epidemiological questionnaires on demographics and potential risk factors were collected from participating schoolchildren. Conventional microscopy examination was used as screening method, and molecular (PCR and Sanger sequencing) assays were used to further investigate the epidemiology of some GP. A multivariate logistic regression analysis was used to evaluate the strength of the association of suspected risk factors with the presence of helminths and GP.

Principal findings

At least one intestinal parasite species was observed by microscopy in 63.2% (235/372) of the participating schoolchildren. *Enterobius vermicularis* (16.7%, 62/372; 95% CI: 13.0–

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20.9) and *Blastocystis* sp. (39.2%, 146/372; 95% CI: 34.2–44.2) were the most prevalent among helminths and GP, respectively. Assemblages A (50.0%), B (37.5%) and A+B (12.5%) were detected within *Giardia duodenalis* and ST3 (28.6%), ST1 and ST2 (26.2% each), and ST4 (14.3%) within *Blastocystis* sp. Three genotypes, two known (A: 66.7%; KB-1: 16.7%) and a novel (HhEcEb1, 16.7%) were identified within *Enterocytozoon bieneusi*. Municipality of origin, household overcrowding, and poor sanitation and personal hygiene habits were risk factors for childhood intestinal parasites colonization.

Conclusions/Significance

Despite massive government drug administration programs, STH and GP infection remain a public health concern in paediatric populations living in poor-resource settings. Molecular analytical methods are required to better understand the epidemiology of these intestinal parasites. This study provides novel information on the occurrence of *Blastocystis* sp. and *E. bieneusi* genetic variants circulating in Ecuadorian human populations.

Author summary

Intestinal parasitic infections remain a public health concern in poor-resource areas in Ecuador, primarily affecting paediatric populations. Our results indicate that mass drug administration programs targeting intestinal helminths, including STH, are insufficient to effectively control the spreading and perpetuation of these pathogens in deprived communities. These interventions must be complemented with measures directed to improve household quality and living conditions, sanitation, and health education for schoolchildren and their families. Together with access to safe drinking water, these measures are necessary to reduce infections with STH and GP in poor communities in Ecuador. Under the One Health approach, more research should be conducted to ascertain to which extent the animal reservoirs (livestock, wildlife) and contaminated environmental (water, soil, grass) contribute to the burden of human disease caused by intestinal parasites.

Introduction

Intestinal helminths, including Soil-Transmitted Helminth (STH), and Gastrointestinal Protist (GP) infections are major contributors to the global burden of disease, particularly in resource-deprived, low-income countries from tropical and subtropical regions [1–3]. In these endemic areas, concomitant infections are common, and their synergistic effect might exacerbate detrimental health outcomes in infected individuals [4]. Chronic or repeated STH and GP infections during childhood have a strong link with stunting, the most common form of malnutrition in children aged under five years [5]. In paediatric populations, stunting or chronic malnutrition refers to both reduced physical growth and cognitive impairment, representing a major public health concern leading to lifelong adverse health, education, and economic outcomes [6]. Because of their chronic rather than acute nature, STH infections are only partially addressed in the Global Burden of Disease (GBD) studies [7], whereas diarrhoea-causing GP infections are not formally considered as neglected tropical diseases [8]. These limitations impair our understanding of the epidemiology of STH and GP in endemic areas.

STH including hookworms (*Ancylostoma duodenale*, *Necator americanus*), roundworms (*Ascaris lumbricoides*, *Strongyloides stercoralis*), and whipworms (*Trichuris trichiura*) are parasitic nematodes that infect humans through contact with soil contaminated with parasite eggs

or infective larval stages [9]. It is estimated that 820 million people are infected with *A. lumbricoides*, 460 million with *T. trichiura*, 460 million with *N. americanus* and *A. duodenale*, and 386 million with *S. stercoralis* in 102 countries worldwide [10, 11]. These infections contributed to an estimated 3.4-million Disability-Adjusted Life-Years (DALYs) and 6,000 deaths [12].

Among GP, the protozoa *Cryptosporidium* spp., *Giardia duodenalis*, and *Entamoeba histolytica* are regarded as relevant diarrhoea-causing pathogens globally. They are faecal-orally transmitted either directly through contact with infected humans and other animals, or indirectly via ingestion of contaminated food or water. Cryptosporidiosis is the second cause of childhood mortality after rotaviral enteritis in sub-Saharan African and southwest Asian countries [13] causing an estimated 48 million DALYs [14]. Although rarely mortal, giardiasis affects 200 million people globally every year, mainly children aged between two and five years [15]. *Entamoeba histolytica* has been linked to an increased risk of death in infants and toddlers with moderate-to-severe diarrhoea in sub-Saharan Africa [16]. Other less frequent GP, but still of public health relevance, include the microsporidia *Enterocytozoon bieneusi* and the Stramenopile *Blastocystis* sp. The former is an opportunistic pathogen primarily infecting immunocompromised individuals [17], whereas the latter has been increasingly linked to a variety of intestinal (diarrhoea, irritable bowel syndrome) and extra-intestinal (urticaria) clinical manifestations [18]. All the GPs mentioned above are featured by a large intra-species molecular diversity that influence their host range and specificity, pathogenicity, virulence, and zoonotic potential. Therefore, assessing the species/subtypes/genotypes involved is essential to characterise the epidemiology of these parasites [19–21].

As a shared feature, intestinal helminths and GP infections are highly endemic amongst people who are poor with little or no access to basic services and infrastructures. Under these circumstances, environmental improvements including access to safe drinking water, basic sanitation, and hygiene have been demonstrated effective to reduce the burden of those infections [12, 22–24].

In Ecuador, intestinal parasitic infections are widespread. *A. lumbricoides* (7–45%) and *T. trichiura* (3–25%) are the most common STH (S1 Table) [25–32]. Regarding GP, the most prevalent species are *G. duodenalis* (4–40%) and members of the *Entamoeba* complex (12–34%). *Cryptosporidium* spp. (3–14%) and *Blastocystis* sp. (7–81%) are less commonly documented, although sometimes at high prevalence rates (S2 Table) [25–40]. When considered together, these data indicate that 80% of the rural and 40% of the urban-marginal populations in Ecuador are affected by intestinal parasites. They rank second in the list of the main causes of ambulatory morbidity and within the top ten causes of paediatric [25, 28, 38]. Most of the helminths and GP colonization rates reported in these studies were found in apparently healthy children and using microscopy examination as screening test. Molecular genotyping data is available from few studies and only for *G. duodenalis* and *Blastocystis* sp. [34, 37, 39].

This aim of this study was to investigate the prevalence and associated risk factors of intestinal helminths and GP in schoolchildren from the Chimborazo and Guayas provinces, two regions were little or no information at all was available on the epidemiology of these pathogens. Microscopy was conducted for all samples collected and PCR-based methods for molecular characterization of some relevant GPs.

Methods

Ethics statement

This study was approved by the Ethics Committee for Human Research of the University of Valencia (procedure number: H1518738039128, 1 March 2018), by the Ethical Subcommittee

for Research on Human Subjects of the Central University of Ecuador-SEISH-UCE (procedure number: 001-UV-2019, 22 January 2019), by the National Directorate of Prevention and Control Strategies and the National Directorate of Health Promotion of the Ministry of Public Health-MSP of Ecuador (procedure number: MSP-DIS-2020-0219-O, 18 May 2020) to meet the principles established by the Helsinki Declaration and by the Spanish/Ecuadorian legislations regarding biomedical research and personal data protection. Formal written consent was obtained from each parent/guardian of the participants.

Study design and setting

A prospective cross-sectional study was conducted in asymptomatic schoolchildren (3–11 years) in the Ecuadorian municipalities of Penipe and Pallatanga (in the Sierra region of the Chimborazo province) and General Antonio Elizalde (GAE, in the Coast region of the Guayas province). Stool samples were collected from participating schoolchildren from June to August 2020 and analysed for the presence of intestinal parasites by microscopy. In parallel, molecular (PCR and Sanger sequencing) methods were used to detect and genotype the main diarrhoea-causing protists species (*Entamoeba* spp., *G. duodenalis*, *E. bieneusi*, *Blastocystis* sp., and *Cryptosporidium* spp.).

The three surveyed municipalities differ from each other in geographic, climatic, demographic, and socioeconomic factors, providing a representative picture of the life conditions in the country (Fig 1). Penipe [meters above sea level (MASL): 2,500–5,424] has a cold [average annual temperature (AAT): 14˚C], mountainous climate; Pallatanga (MASL: 1,200–1,462) has a temperate sub-humid mountainous climate (AAT: 20˚C) and GAE (MASL: 300–700) has a humid tropical climate (AAT: 24˚C).

In order to contextualise the reality of each municipality analysed and according to the available data, it should be noted that Pallatanga has a Gini coefficient, which is a measure to represent income inequality within a social group, of 0.37; Penipe of 0.35; and GAE of 0.30 [41]. The mean number of years of schooling of the population in Pallatanga is 6.2 years; in Penipe, it is 7.7 years; and in GAE, it is 8.3 years [41, 42]. The percentage of households with inadequate sanitation facilities in Pallatanga is 14.9%; in Penipe 11.4%, and in GAE 5.7%. The percentage of homes with a public water supply in Pallatanga is of 44.8%, in Penipe of 76.6%, and in GAE of 63.2% [43].

Sample collection

Schools were approached in collaboration with the local university (Escuela Superior Politécnica de Chimborazo, ESPOCH). School heads were contacted and invited to participate in the study. Three public primary schools agreed to participate each with 355–510 schoolchildren. There were 372 schoolchildren enrolled, aged 3–11 years (median: 8.0 years), of which 115 were enrolled in GAE, 161 in Pallatanga, and 96 in Penipe. The rate ratio of boys/girls was 0.86. All recruited schoolchildren were from communities of low-to-medium socioeconomic status.

Informative meetings were personally held with teachers, who explained the goals and procedures of the project to schoolchildren. Parents/legal guardians were informed by formal letter. Participating schoolchildren were provided with uniquely labelled sampling kits (10 mL polystyrene plastic tube, disposable spatula, Graham tape, and instructions) to obtain individual samples. Parents/legal guardians assisted in the collection of samples from consenting schoolchildren and brought the samples to school the next day. A group of 10 students of the ESPOCH were trained to assist in the sample collection. Collected samples were immediately transported at the Chemistry Laboratory of ESPOCH and stored at 4˚C until further processing.

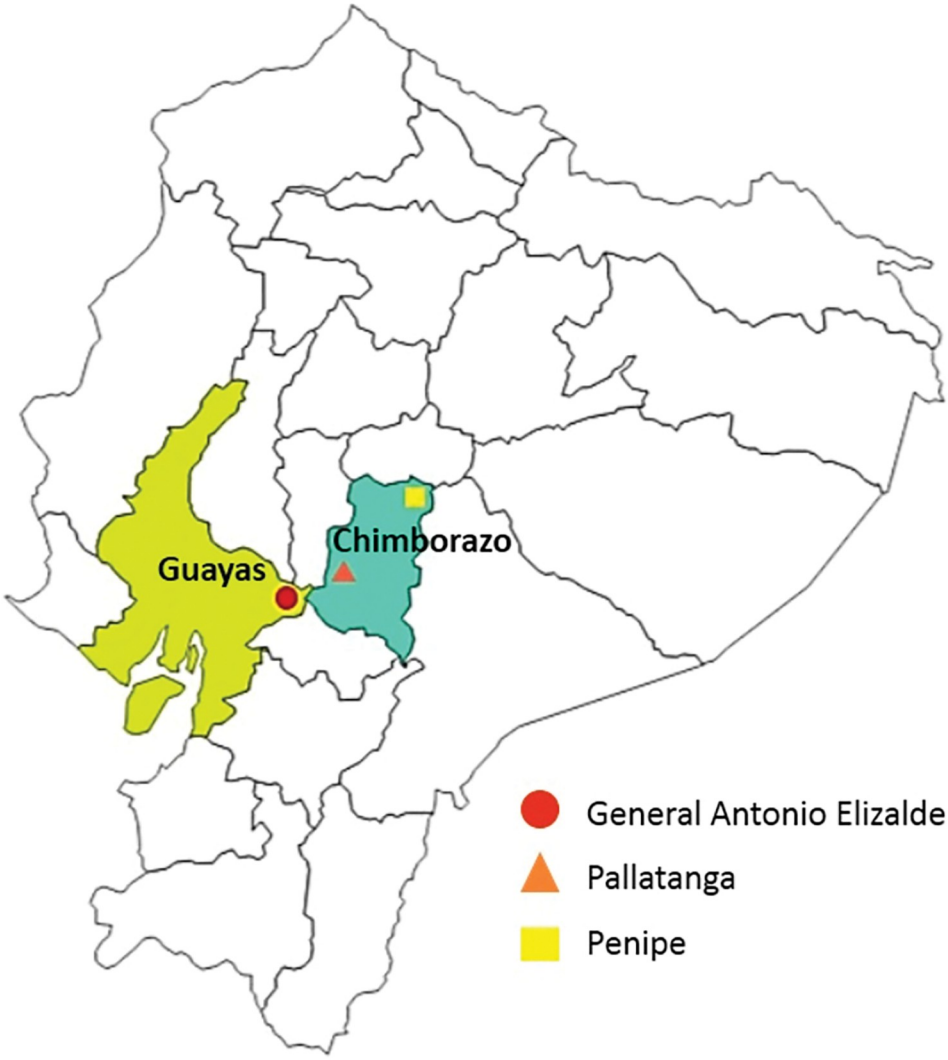

Fig 1. Map of Ecuador. The geographical localization of the three municipalities surveyed in the present study in Chimborazo and Guayas provinces are indicated.

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Epidemiological questionnaire

A standardized questionnaire (S3 Table) was provided as part of the sampling kit to be completed by the children's parents/legal guardians. Questions included: (i) demographic characteristics (age, gender, municipality of residence, number of siblings, number of relatives residing at home); (ii) behavioural habits (consumption of unsafe water, hand and fruit/vegetable washing, contact with domestic animals, playing outdoors); and (iii) clinical manifestations (diarrhoea, constipation, abdominal pain, vomiting, weight loss, allergic manifestations, teeth grinding, anal itching, abdominal distention, and flatulence). Completed questionnaires and written informed consents signed by the parent/guardian were returned for collection by each participating schoolchild as described above.

Parasitological assessment

An aliquot of fresh stool samples from each participant was analysed at the Chemistry Laboratory of ESPOCH using the Kato-Katz technique for the detection, identification, and

quantitation of intestinal helminths. The remaining faecal material was preserved in 70% ethanol and, together with the Graham tapes, shipped to the Parasitology Laboratory of the Faculty of Pharmacy, University of Valencia (Spain) for further analyses.

A 3-gram stool sample from each participant was concentrated and filtered for 5 min at 2,500 rpm using Midi Parasep filter devices (Apacor Ltd., Wokingham, UK). The sediment obtained was divided in two aliquots. One was used for DNA extraction (see below); the other was fixed with 10% formalin in a 1:3 proportion for microscopic examination; and with a small amount of the remaining sediment thin smears were also prepared and stained with the Modified Ziehl-Neelsen (MZN) for the detection and identification of *Cryptosporidium* spp. and other coccidian. Graham tapes were examined by 10x microscopy for detecting eggs of *Enterobius vermicularis*. A sample was considered negative when no parasite structures (eggs, larvae cysts, oocysts or trophozoites) were observed.

DNA extraction and purification

Genomic DNA was isolated from 200 mg of each stool sample using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Extracted and purified DNA was eluted in 100 μL of PCR-grade water and kept at 4˚C until further molecular analysis.

Molecular detection and characterization of intestinal protists

Molecular studies focused on the most prominent GP species studied as a cause of diarrhoea and those under discussion or emergency. The presence of *G. duodenalis* was investigated using a real-time PCR (qPCR) method targeting a 62-bp region of the small subunit of the rRNA (*ssu* rRNA) gene of the parasite as the initial screening method [44]. For assessing the molecular diversity of this protozoa at the assemblage and sub-assemblage levels, a sequence-based multilocus genotyping (MLST) scheme targeting the genes encoding for the glutamate dehydrogenase (*gdh*), β-giardin (*bg*), and triose phosphate isomerase (*tpi*) proteins was adopted. Only samples that yielded cycle threshold (C_T) values <32 in qPCR were assessed under this MLST scheme. A semi-nested PCR was used to amplify a 432-bp fragment of the *gdh* gene [45], and nested PCRs were used to amplify 511 and 530 bp fragments of the *bg* and *tpi* genes, respectively [46, 47]. Detection and differential diagnosis between pathogenic *E. histolytica* and non-pathogenic *E. dispar* was carried out by a qPCR method targeting a 172-bp fragment of the *ssu* rRNA gene of the *E. histolytica*/*E. dispar* complex [48, 49]. The presence of *Cryptosporidium* spp. was assessed using a nested-PCR protocol to amplify a 587 bp fragment of the *ssu* rRNA gene of the parasite [50]. Identification of *Blastocystis* sp. was achieved by a direct PCR protocol targeting a 600 bp fragment of the *ssu* rRNA gene of the protist [51]. Detection of *E. bieneusi* was conducted by a nested PCR protocol to amplify the internal transcribed spacer (ITS) region as well as portions of the flanking large and small subunit of the ribosomal RNA gene as previously described [52]. Detailed information on the oligonucleotides and the PCR conditions used for the molecular identification and/or characterisation of the unicellular parasites investigated in the present study is presented in S4 and S5 Tables, respectively.

All the qPCR protocols described above were conducted on a Corbett Rotor Gene 6000 real-time PCR system (Qiagen). Reaction mixes always included 2x TaqMan Gene Expression Master Mix (Applied Biosystems, CA, USA). All the direct, semi-nested, and nested PCR protocols described above were conducted on a 2720 Thermal Cycler (Applied Biosystems). Reaction mixes always included 2.5 units of MyTAQ DNA polymerase (Bioline GmbH, Luckenwalde, Germany), and 5x MyTAQ Reaction Buffer containing 5 mM dNTPs and 15

mM $MgCl_2$. Laboratory-confirmed positive and negative DNA samples of human origin for each parasitic species investigated were routinely used as controls and included in each round of PCR. PCR amplicons were visualized on 1.5–2% agarose gels (Conda, Madrid, Spain) stained with Pronasafe (Conda) nucleic acid staining solution. A 100 bp DNA ladder (Boehringer Mannheim GmbH, Baden-Wurttemberg, Germany) was used for the sizing of obtained amplicons.

Sequence analyses

PCR products of the expected size on agarose gel were directly sequenced in both directions using appropriate internal primer sets (S4 Table). DNA sequencing was conducted by capillary electrophoresis using the BigDye Terminator chemistry (Applied Biosystems) on an on ABI PRISM 3130 automated DNA sequencer.

Raw sequencing data in both forward and reverse directions were viewed using the Chromas Lite version 2.1 (Technelysium Pty Ltd., South Brisbane, Australia) sequence analysis program (https://technelysium.com.au/wp/chromas/). The BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare nucleotide sequences with sequences retrieved from the NCBI GenBank database. Generated DNA consensus sequences were aligned to appropriate reference sequences using the MEGA version 10 software [53] to identify *Giardia* species and assemblages/sub-assemblages, *Cryptosporidium* species and *E. bieneusi* genotypes.

Blastocystis sequences were submitted at the *Blastocystis* 18S database (https://pubmlst.org/organisms/blastocystis-spp) for subtype confirmation and allele identification. The sequences obtained in this study have been deposited in GenBank under accession numbers ON866725-ON866736 (*G. duodenalis*), ON858734-ON858742 (*Blastocystis* sp.), and OQ267689-OQ267691 (*E. bieneusi*).

Phylogenetic analysis

Nucleotide sequences obtained in this study and *E. bieneusi* nucleotide sequences for genotypes previously identified in humans and animals as well as appropriate reference sequences to include all *E. bieneusi* groups retrieved from GenBank were aligned with the Clustal W algorithm using MEGA X [53]. Phylogenetic inference was carried out by the Neighbor-Joining (NJ) method as previously described [54]. Genetic distance was calculated with the Kimura parameter-2 model using MEGA X [53].

Data analysis

Descriptive statistics were calculated, including measures of central tendency (mean and median), measures of dispersion (standard deviation, range, and coefficient of variation) and measures of shape (asymmetry and pointing) for quantitative variables, as well as the absolute and relative frequencies for the qualitative variables. Association analyses were performed, stratifying by gender and age to observe possible heterogeneity in the results according to these factors. When data were stratified and the sample size was small ($n < 15$), non-parametric tests were used (Fisher's exact test and Mann–Whitney U test). To test the null hypotheses of no association between intestinal parasitism and demographic and socioeconomic factors and lifestyle habits controlling by confounders or effect modifiers, multiple logistic regression analyses with dummy variables for categorical terms, were applied. The magnitude of the association was expressed as adjusted odds ratios (AORs) with a 95% confidence interval (CI). A P-value ≤ 0.05 was considered statistically significant. All the variables were analysed using SPSS software version 26.0 (Statistical Package for Social Sciences, Chicago, IL, USA).

Results

Occurrence of intestinal parasites by optical microscopy

Overall, 63.2% (235/372) of the participating schoolchildren were infected/colonized by at least one intestinal parasite. Global prevalence rates for each sampling site varied from 41.7% (48/115) in GAE, 71.9% (69/96) in Penipe and 73.3% (118/161) in Pallatanga. The frequency of intestinal helminths and protists by optical microscopy in the surveyed schoolchildren by municipalities is shown in Table 1.

The most prevalent helminths were nematodes such as the human pinworm, *E. vermicularis* (16.7%, 62/372; 95% CI: 13.0–20.9), followed by the STHs, *A. lumbricoides* (9.4%, 35/372; 95% CI: 6.6–12.8) and *T. trichiura* (5.1%, 19/372; 95% CI: 3.1–7.9). *Hymenolepis nana*, the only cestode reported in the study, was detected at very low (<1%) infection rates and only in one of the schools in Pallatanga. Among GP, *Blastocystis* sp. was the most common protist identified (39.2%, 146/372; 95% CI: 34.2–44.2), followed by *Entamoeba coli* (26.3%, 98/372; 95% CI: 21.9–31.1), *G. duodenalis* (12.6%, 47/372; 95% CI: 9.4–16.4), and the *Entamoeba* complex (5.9%, 22/372; 95% CI: 3.7–8.8). Other species were identified at low (<5%, *Endolimax nana* and *Entamoeba hartmanni*) or very low (<1%, *Chilomastix mesnili* and *Dientamoeba fragilis*) infection/carriage rates. None of the 372 samples tested positive for *Cryptosporidium* spp. Prevalence rates among sampling sites ranged between 9.4–26.1% for *E. vermicularis*, 4.2–16.1% for *A. lumbricoides*, 1.7–9.3% for *T. trichiura*, 28.7–47.9% for *Blastocystis* sp., 7.0–39.6% for *Entamoeba coli*, 7.0–18.8% for *G. duodenalis*, and 0.9–10.6% for the members of the *Entamoeba* complex (Table 1).

Of 372 samples, a single parasite species was detected in 113 (30.4%). Multiparasitism with two species were found in 43 samples (11.6%) of which, 14 (32.6%) combined *Blastocystis* sp. + *E. coli*, 4 (9.3%) *Blastocystis* sp. + *E. vermicularis*, and 4 (9.3%) *Blastocystis* sp. + *G. duodenalis*. Seventy-seven samples (20.7%) presented multiparasitism with more than three species per individual, being the most common combination *Blastocystis* sp. + *G. duodenalis* + *E. coli* (9.3%).

Schoolchildren in Pallatanga were more likely to harbour helminths including *E. vermicularis*, *H. nana*, *A. lumbricoides* and *T. trichiura*. Schoolchildren in GAE were significantly less

Table 1. Frequencies (%) of infection/colonization by helminthic and protist species detected by microscopy according to the municipality of origin. P-values in bold indicate statistical significance (P≤0.05).

Species	GAE (*n* = 115)	Penipe (*n* = 96)	Pallatanga (*n* = 161)	Total (*n* = 372)	*P*-value
E. vermicularis	9.6	9.4	26.1	16.7	**0.001**
A. lumbricoides	4.3	4.2	16.1	9.4	**0.001**
T. trichiura	1.7	2.1	9.3	5.1	**0.006**
H. nana	0.0	0.0	1.9	0.8	0.136
Blastocystis sp.	28.7	47.9	41.6	39.2	**0.012**
E. coli	7.0	39.6	32.3	26.3	0.000
G. duodenalis	7.0	18.8	13.0	12.6	**0.036**
Entamoeba complex[a]	0.9	4.2	10.6	5.9	**0.002**
E. nana	1.7	8.3	5.0	4.8	0.084
E. hartmanni	1.7	4.2	6.2	4.3	0.195
C. mesnili	0.0	2.1	0.0	0.5	0.056
D. fragilis	0.0	1.0	0.0	0.3	0.237
Cryptosporidium spp.	0.0	0.0	0.0	0.0	–

[a]*Entamoeba* complex: *E. histolytica*/*E. dispar*/*E. moshkovskii*/*E. bangladeshi*.

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infected by GP including *Blastocystis* sp., *E. coli*, *G. duodenalis* and members of the *Entamoeba* complex than their counterparts in Pallatanga and Penipe (Table 1).

Occurrence of intestinal protists by molecular analysis techniques

In addition to microscopy, faecal DNAs from all 372 stool samples collected were investigated by PCR for the presence of protists *Cryptosporidium* spp., *E. histolytica*, *G. duodenalis*; Stramenopile *Blastocystis* sp., and Microsporidia *E. bieneusi*. The frequency of the unicellular parasites analysed by molecular techniques in the surveyed population is shown in Table 2. With the new molecular results, and for the selected species, 50.5% (188/372) of participants were harbouring at least one GP. In this case, global prevalence rates for each sampling site varied from 43.5% (50/115) in GAE, 50.9% (82/161) in Pallatanga, and 58.3% (56/96) in Penipe.

PCR-based data, for the selected species, showed marked differences with microscopy-based data. Thus, *G. duodenalis* (26.6%, 99/372; 95% CI: 22.2–31.4) was the most prevalent species found, followed by *Blastocystis* sp. (22.6%, 84/372; 95% CI: 18.4–27.2), *E. dispar* (14.5%, 54/372; 95% CI: 11.1–18.5), and *E. bieneusi* (1.6%, 6/372; 95% CI: 0.6–3.5). Neither *Cryptosporidium* spp. nor *E. histolytica* were detected in the surveyed population. Prevalence rates among sampling sites ranged between 20.5–31.3% for *G. duodenalis*, 11.3–37.5% for *Blastocystis* sp., 4.3–24.8% for *E. dispar*, and 0.0–2.5% for *E. bieneusi* (Table 2). Remarkably, *G. duodenalis* infection rates were two-fold higher by PCR than by conventional microscopy (26.6% *vs*.12.6%), but the opposite result was observed for *Blastocystis* sp. (22.6% *vs*. 39.2%).

Molecular characterization of *Giardia duodenalis*

A total of 99 samples tested positive for *G. duodenalis* by qPCR. Generated C_T values had a median value of 35.3 (range: 19.0–44.0). Genotyping analyses were attempted in the 17.2% (17/99) of samples that yielded qPCR C_T values <32. Out of the 17 DNA isolates investigated, 35.3% (6/17), 23.5% (4/17), and 41.2% (7/17) yielded amplicons of the expected sizes at the *gdh*, *bg*, and *tpi* loci, respectively. Of them, 58.8% (10/17) were amplified at least at one single locus, whereas multi-locus genotyping data at the three loci were available for 17.6% (3/17) (Table 3).

Assemblage/sub-assemblage assignment was conducted by direct comparison of the sequencing results obtained at the three loci (*gdh*, *bg*, and *tpi*) investigated. Overall, assemblage A (50.0%, 4/8) was more prevalent than assemblage B (37.5%, 3/8). A mixed A+B infections was detected in one sample (12.5%, 1/8) (Table 3).

Out of the six *gdh* sequences, one (16.7%) and two (33.3%) were assigned to the sub-assemblages AI and AII, respectively, showing 100% identity with reference sequences L40509 and L40510. Assemblages BIII and BIV were identified in two (33.3%) and one (16.7%) isolates, respectively. The two BIII sequences differed by 3–7 SNPs with reference sequence AF069059.

Table 2. Frequencies (%) of infection/colonization by protist species investigated by PCR in the studied population according to the municipality of origin.

Species	GAE (*n* = 115)	Penipe (*n* = 96)	Pallatanga (*n* = 161)	Total (*n* = 372)	*P*-value
G. duodenalis	31.3	31.3	20.5	26.6	0.066
Blastocystis sp.	11.3	37.5	21.7	22.6	0.000
E. dispar	4.3	9.4	24.8	14.5	0.000
E. bieneusi	1.7	0.0	2.5	1.6	0.308
Cryptosporidium spp.	0.0	0.0	0.0	0.0	–
E. histolytica	0.0	0.0	0.0	0.0	–

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Table 3. Multilocus genotyping results of the eight *G. duodenalis*-positive samples successfully genotyped.

Patient ID	*gdh*	*tpi*	*bg*	Assigned assemblage	Assigned sub-assemblage
EC1048	–	AII	–	A	AII
EC1092	–	AII	B	A+B	AII+B
EC1116	BIII	B	B	B	BIII
EC1117	BIII	BIII	B	B	BIII
EC1488	BIV	BIII	–	B	BIII+BIV
EC1559	AII	AII	–	A	AII
EC1653	AII	AII	AII	A	AII
EC1668	AI	–	–	A	AI

gdh: Glutamate dehydrogenase; *tpi*: Triose phosphate isomerase; *bg*: β-giardin.

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The only BIV sequence identified varied by a single SNP from reference sequence L40508 (Table 4).

Out of the four *bg* sequences, one (25.0%) was identified as sub-assemblage AII and was identical to reference sequence AY072723. The remaining three sequences (75.0%) were identified as assemblage B and differed from 2–3 SNPs from reference sequence AY072727. Because *bg* is unsuitable for intra-assemblage discrimination for assemblage B, no information at the sub-assemblage level was available at this locus (Table 4).

Finally, out of the seven *tpi* sequences, four (57.1%) were identified as sub-assemblage AII and were identical to reference sequence U57897. The remaining three sequences (42.9%) differed by 1–5 SNPs with reference sequence AF069561 (Table 4).

Molecular characterization of *Blastocystis* sp

A total of 92 faecal DNA samples yielded amplicons of the expected size on agarose gels compatible with *Blastocystis* sp. Of them, 91.3% (84/92) were successfully subtyped. The remaining eight isolates produced unreadable or poor-quality Sanger sequences (associated to faint bands on agarose gels) and were conservatively not considered as true *Blastocystis*-positive samples.

The frequency and molecular diversity of *Blastocystis* sp. isolates at the *ssu* rRNA locus is described in Table 5. Four distinct *Blastocystis* subtypes (ST) were identified, being the most

Table 4. Diversity, frequency, and main molecular features of *Giardia duodenalis* sequences generated.

Assemblage	Sub-assemblage	No. isolates	Locus	Reference sequence	Stretch	SNPs	GenBank ID
A	AI	1	*gdh*	L40509	88—416	None	ON866725
	AII	2	*gdh*	L40510	64—491	None	ON866726
B	BIII	1	*gdh*	AF069059	77—460	T147Y, G150A, C309Y, C336Y, G372R, C375Y, T456C	ON866727
		1	*gdh*	AF069059	40—460	C309Y, G372R, T456C	ON866728
	BIV	1	*gdh*	L40508	76—479	C255T	ON866729
A	AII	1	*bg*	AY072723	1—594	None	ON866730
B	B	2	*bg*	AY072727	102—550	C165T, C309T, G421A	ON866731
		1	*bg*	AY072727	102—590	C165Y, C309Y	ON866732
A	AII	4	*tpi*	U57897	294—805	None	ON866733
B	BIII	1	*tpi*	AF069561	1—456	G48A, G105A, G267A	ON866734
		1	*tpi*	AF069561	1—456	G48R, G105R, C186Y, G267R, C447T	ON866735
		1	*tpi*	AF069561	1—456	G399A	ON866736

bg: beta-giardin; *gdh*: glutamate dehydrogenase; R: A/G; *tpi*: triose phosphate isomerase; Y: C/T. SNP: Single nucleotide polymorphisms.

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Table 5. Diversity, frequency, and main molecular features of *Blastocystis* sp. *ssu* rRNA sequences (*ca*. 600 bp) generated in the present study.

Subtype	Allele	No. isolates	Reference sequence	GenBank ID
ST1	4	22	MN836826	ON858734
ST2	9	2	OL623671	ON858735
ST2	12	11	OK285237	ON858736
ST2	11+12	8	MF669067	ON858737
ST2	12+68	1	OK285237	ON858738
ST3	34	20	MT160370	ON858739
ST3	34+36	3	MN836837	ON858740
ST3	57	1	ON796017	ON858741
ST4	42	12	OK285228	ON858742

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prevalent ST3 (28.6%, 24/84), followed by ST1 and ST2 (26.2%, 22/84 each), and ST4 (14.3%, 12/84). Mixed infections samples involving different STs of the protists were also identified in four samples (4.8%, 4/84), although the exact identity of the STs involved could not be determined due to the complexity of sequence trace chromatograms with mixed patterns.

Regarding intra-subtype diversity, no diversity was observed for ST1 and ST4, all isolates belonged to alleles 4 and 42, respectively. In contrast, isolates assigned to ST2 and ST3 displayed a much larger genetic diversity. Thus, allele 12 was the most frequent genetic variant (50.0%, 11/22) found within ST2, followed by mixed infections involving alleles 11+12 (36.4%, 8/22), allele 9 (9.1%, 2/22) and a rare mixed infection by alleles 12+68 (4.5%, 1/22). Similarly, allele 34 accounted for most (83.3%, 20/24) of the genetic variants found within ST3, followed by mixed infections by alleles 34+36 (12.5%, 3/24), and allele 57 (4.2%, 1/24).

Molecular characterization of *Enterocytozoon bieneusi*

In the case of microsporidia, detection was performed by nested-PCR and only for *Enterocytozoon bieneusi* that is the most prevalent species causing human microsporidiosis [17]. Out of the six *Enterocytozoon*-positive isolates, four (66.7%, 4/6) were identified as genotype A, and one (16.7%, 1/6) as genotype KB-1, both showing 100% identity with reference sequences AF101197 and MN136778, respectively. The remaining isolate (16.7%, 1/6) corresponded to a novel genotype named HhEcEb1. The identity of this novel *E. bieneusi* genotype were confirmed in two independent PCR reactions. Novel HhEcEb1 differed from known genotype A by a single SNP (T to C) in position 158 of reference sequence AF101197. Phylogenetic analysis revealed that novel HhEcEb1clustered within zoonotic Group 1 (Fig 2).

Risk factors of intestinal parasites

Logistic regression analyses were conducted to assess the risk of intestinal parasitism based on the demographic, socioeconomic, and behavioural features of the surveyed schoolchildren population. Multivariate logistic regression analysis computing the whole study population showed that residing in a particular municipality was the only variable determining the presence of intestinal parasites (S6 Table). Schoolchildren living in Pallatanga or Penipe were 2.2-fold more likely to harbour helminths and/or GP infections than those living in GAE (AOR = 2.296; 95% CI: 1.226–4.301, $P = 0.009$; AOR = 2.231; 95% CI: 1.067–4.665, $P = 0.033$).

When data were stratified by municipality of origin, other variables reached statistical significance and they could be identified as risk factors for intestinal parasites, including more than five persons residing in the same house in GAE (AOR = 22.634; 95% CI: 1.778–288.093, $P = 0.016$); and no handwashing after bathing (AOR = 0.54, 95% CI = 0.005–0.541, $P = 0.013$)

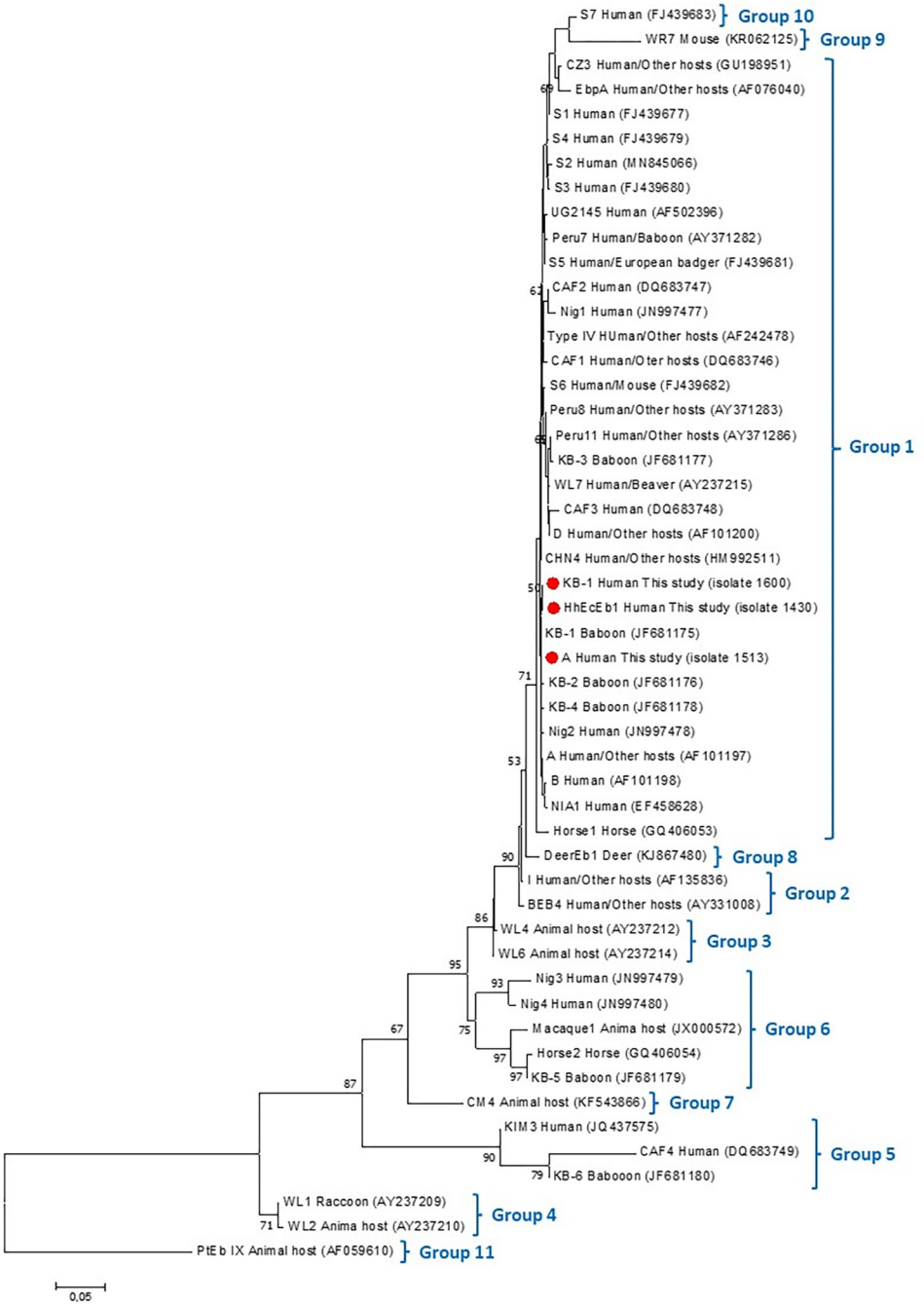

Fig 2. Phylogenetic relationships among *Enterocytozoon bieneusi* genotypes identified in this study. All genotypes identified in humans and animals, and genotypes to cover all groups of *E. bieneusi* were included for comparative purposes. Analyses were inferred by a Neighbor-Joining method of the entire ITS of rRNA gene based on genetic distances calculated by the Kimura two-parameter model (MEGA X software). All nucleotide sequences include host information with the GenBank accession number in parenthesis. Nucleotide sequences determined in this study are identified with red circles before the genotype name.

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and consumption of unsafe water (AOR = 0.127, 95% CI: 0.220–0.743, $P = 0.022$) in Penipe (S6 Table). None of the variables considered in the study increased the likelihood of being infected by intestinal parasites in Pallatanga.

Despite the fact that the children are school-going and apparently asymptomatic, the most prevalent clinical manifestations reported by the participating children were abdominal pain (40.6%, 151/372), flatulence (29.0%, 108/372), and diarrhoea (26.1%, 97/372).

Discussion

We studied the epidemiology and risk factors for helminths and GP infections in schoolchildren from the Chimborazo and Guayas provinces, Ecuador. Although microscopy was used as screening test, molecular (PCR and Sanger sequencing) methods were also used for in-depth investigation of the main parasitic intestinal protist species. Six out of 10 children had at least one intestinal parasite species. *E. vermicularis*, followed by *A. lumbricoides* and *T. trichiura* were the most common species among helminths, and *Blastocystis* sp., *E. coli* and *G. duodenalis* (12.6%) among GP. Regarding molecular diversity, assemblages A and B were detected within *G. duodenalis*, and subtypes ST1-ST4 within *Blastocystis* sp. Three genotypes, two known (A, KB-1) and a novel (HhEcEb1) were identified within *E. bieneusi*. Several household and behavioural factors indicative of greater poverty and social marginalisation were associated with helminths and GP infection risk. Household overcrowding, consumption of unsafe water, and poor personal hygiene habits were risk factors for childhood intestinal parasitic diseases.

Strengths of this survey include a large sample size representative of three epidemiological scenarios with distinct environmental, climatic and socioeconomic conditions. The use of PCR-based methods for GP detection and genotyping allowed for i) improved prevalence estimates compared to more traditional (microscopy) methods, and ii) assessing potential sources of infection and transmission pathways. This is one of the main contributions of the study, as GP molecular data is particularly scarce in Ecuador. This study provides also the first description of microsporidiosis by *E. bieneusi* in the country.

The roundworm *A. lumbricoides* and the whipworm *T. trichiura* have been consistently reported as the most dominant STH present in Ecuadorian human populations, with infection varying from 7–45% (*A. lumbricoides*) and 0.5–25% (*T. trichiura*) (S1 Table). In contrast, we identified the presence of *A. lumbricoides* and *T. trichiura* at comparatively lower (9.4% and 5.1%, respectively) infection rates. This result is probably associated to Mass Drug Administration (MDA) campaigns with albendazol by the Ministry of Health. Remarkably, the pinworm *E. vermicularis* was the most prevalent nematode detected by the Graham test in the surveyed paediatric population (16.7%), a much higher rate than that (0.5%) identified in indigenous communities in Santo Domingo de los Tsáchilas province by conventional coproparasitological procedures [31]. The Graham's perianal swab method is the recommended procedure for the detection of *E. vermicularis* in young children [55], these data suggest that the true prevalence of this helminth is likely much higher than initially anticipated.

Recent studies investigating the epidemiology of STH in Ecuador have provided interesting data. A longitudinal birth cohort study (new-borns to 8 years of age) has shown that infections with *A. lumbricoides* and *T. trichiura* peak between 3 and 5 years [56]. Marked differences in STH prevalence rates have also been documented nationwide. Thus, comparatively fewer *A. lumbricoides* and *T. trichiura* infections occur in coastal and highland areas than in the Amazon region where greater poverty and inadequate sanitation likely favour transmission [57, 58]. Local spatial clustering of STH infection has been observed among indigenous Shuar inhabiting two regions of Amazonian Ecuador [59]. This seems to be also the case of our study, where schoolchildren in Pallatanga or Penipe were at higher risk of infection by STH and/or GP than their counterparts in GAE.

Several environmental, sociodemographic, and behavioural factors have been linked to an increased risk of STH infection. *T. trichiura* infection intensity were lower among Shuar

indigenous people living in houses with wood floors than those with dirt floors [60]. Indeed, household dust samples have been shown to contain DNA for a variety of STH including *S. stercoralis* (52%), *A. lumbricoides* (39%), *Toxocara* spp. (42%), hookworm (18%) and *T. trichiura* (8%) [61]. In the present study, determinants of infection risk included household overcrowded conditions, consumption of unsafe water, and inappropriate personal hygiene practices, highlighting the strong link between poverty and STH infections.

Regarding GPs, microscopy examination of faecal specimens allowed the identification of a well-recognized pathogen (*G. duodenalis*), two species of uncertain pathogenicity (*Blastocystis* sp. and *D. fragilis*), and commensals of the genera *Entamoeba* (*E. coli*, *E. dispar*, *E. hartmanni*), *Endolimax nana* and *Chilomastix mesnili*. *G. duodenalis* is widely present in Latin American countries, where microscopy-based prevalence rates in humans have been typically reported in the range of 10–50%, mostly in paediatric populations [62].

In Ecuador, *G. duodenalis* has been identified at prevalence rates of 26–33% in children with clinical manifestation, of 16–40% in asymptomatic children, and of 4–20% in rural and urban community-based surveys (S2 Table). In the present study, overall *G. duodenalis* prevalence was of 12.6% and of 26.6% by microscopy examination and qPCR, respectively. This finding confirm i) the superior diagnostic performance of molecular methods over conventional assays, and ii) the suitability of qPCR for the detection of subclinical *Giardia* spp. infections in endemic areas. *G. duodenalis* is a species complex consisting of eight assemblages (A-H), with assemblages A and B the dominant assemblages in humans, C and D in canids, E in hoofed animals, F in felids, G in rodents and H in marine mammals [21]. In the present study, most (82.8%, 82/99) of the *Giardia*-positive samples by qPCR yielded C_T values $\geq$32, indicative of moderate-to-low parasite burdens. This fact explains the low amplification rates obtained at the *gdh*, *bg*, and *tpi* markers, all of them single-copy genes with limited sensitivity compared with the multiple-copy *ssu* rRNA gene used in qPCR for detection purposes. Our genotyping analyses revealed that assemblage A was more prevalent than assemblage B (50.0% *vs*. 37.5%). Similar frequencies (60% *vs*. 20%) have been previously identified in apparently healthy children in Pichincha province [37], but the opposite trend (32% *vs*. 61%) was observed in children with and without clinical manifestations in Esmeralda's province [34]. Mixed A+B infections have been found in 7–12% of isolates (including this study), suggestive of epidemiological scenarios where infection/re-infection events are frequent. Overall, the vast majority of human cases of giardiasis characterised in Ecuador are due to assemblages A and B. This finding supports the notion that human *G. duodenalis* infections are primarily of anthropic nature. However, and unknown fraction of these infections might be of animal origin, as both assemblages A and B are zoonotic. Although probably infrequent, zoonotic transmission events are possible, as demonstrated by the occasional presence of canine-adapted assemblage C in two asymptomatic children in Pichincha province [37].

In the present study, *Blastocystis* sp. carriage rates were estimated at 39.2% by microscopy and at 22.6% by *ssu*-PCR. One explanation for this discrepancy could be an incorrect diagnosis, because microscopy detection and identification are challenging [63]. The epidemiology of *Blastocystis* sp. in Ecuador is largely unknown. The protist has been identified in 7–20% of asymptomatic individuals of all age groups both in urban and rural settings in Esmeraldas, Pichincha, and Santo Domingo de los Tsáchilas provinces by conventional microscopy, but a carriage rate as high as 81.5% was identified by PCR in similar populations in Esmeraldas and Manabí provinces (S2 Table). These data seem to suggest that *Blastocystis* sp. is ubiquitous in the Ecuadorian population. A total of 34 *Blastocystis* sp. subtypes (STs) are currently recognized, of which ST1-ST10, ST12, ST14, ST16, ST23, and ST35 have been reported in humans [20, 64]. ST1 (14.4%), ST2 (10.1%), ST3(19.2%), and ST4 (2.3%) are the most frequent *Blastocystis* sp. subtypes identified in humans in the Americas [65]. Interestingly, in the present

study ST1-ST3 were found at very similar frequencies (27–30%), with ST4 being identified at a lower rate (15.0%). Of note, this is the first report of ST4 in Ecuador. In the only previous molecular epidemiological survey conducted in the country, ST1, ST2 and ST3 (alone or in combination) were identified in asymptomatic individuals in Esmeralda and Manabí provinces [39].

The fungi-related *E. bieneusi* is an emerging parasite responsible for 90% of human microsporidiosis reported worldwide [19]. It occurs most frequently in immunocompromised individuals [66], but it has also been reported in apparently healthy populations that might act as unnoticed disseminators [67]. More than 600 *E. bieneusi* genotypes distributed in 11 distinct phylogenetic groups have been described to date, which Group 1 and Group 2 comprising those with zoonotic potential [19]. Perhaps the most important contribution of this survey is the detection of *E. bieneusi* for the first time in Ecuador. Three different genotypes of *E. bieneusi* were identified, two known genotypes (A, KB-1) and a novel genotype (HhEcEb1). This finding demonstrated that an unexpected high diversity of *E. bieneusi* genetic variants were circulating in Ecuadorian asymptomatic children at low (but still significant) frequency rates. Remarkably, the identification of KB-1 represents also the second report of this genotype in humans globally after its initial description in a Chinese toddler attending kindergarten [68]. Previously, KB-1 was only described in a captive baboon in Kenya [69]. In contrast, the genotype A (syn. Peru1) of *E. bieneusi* is a common finding in human populations worldwide, particularly in immunocompromised patients [70], and has also been reported in other animals such non-human primates, dogs, or birds [19]. In a previous microscopy-based study conducted in Guayas province, microsporidial spores (of unknown species) were detected in 25% of HIV+ patients presenting with diarrhoea [35]. Our data contribute to improve our knowledge on the epidemiology of *E. bieneusi* in South America, a geographical region where this information is particularly scarce.

Previous studies conducted in Ecuador have reported the presence of members of the *Entamoeba* complex (*E. histolytica*/*E. dispar*/*E. moshkovskii*/*E. bangladeshi*) in 13–57% of the faecal samples examined by microscopy in different human populations (S2 Table). Because the transmission forms of these protists are morphologically indistinguishable, diagnostic differentiation of pathogenic *E. histolytica* and non-pathogenic *Entamoeba* species must be based on molecular methods. When PCR was used for this purpose, *E. histolytica* was detected at a prevalence rate of 2.8% (compared with the 69.8% of *E. dispar*) in rural and urban dwellers in Esmeraldas and Pichincha provinces [32]. In the present survey *E. histolytica* was not detected, whereas 14.5% of faecal DNA samples tested positive for *E. dispar* by qPCR.

Remarkably, no *Cryptosporidium* oocysts were detected using specific MZN staining. This apicomplexan protist has been identified at prevalence rates of 3–14% using microscopy as detection method in several Ecuadorian provinces including Azuay, Chimborazo, Esmeraldas, Pichincha, and Santo Domingo de los Tsáchilas provinces (S2 Table). As far as we know, no molecular-based studies have addressed the genetic diversity of *Cryptosporidium* spp. of human origin in Ecuador, this being a pending task that should be completed in future epidemiological surveys.

Our multivariate logistic regression analysis revealed marked differences of helminths and GP infection according to the municipality of origin, likely reflecting differences in socioeconomic status and sanitary conditions. When epidemiological data were stratified by municipality, variables associated with an increased risk of infection by intestinal parasite included overcrowded conditions (more than five people per household), limited or no access to safe drinking water, and inappropriate personal hygiene practices. These results are in line with those obtained in previous community surveys carried out in Ecuador [56, 59, 60]. Taken together, these findings demonstrate the strong link present between poverty and intestinal

parasitic infections. Interventions directed to improve drinking water quality, household flooring and toilet facilities and personal hygiene practices are greatly needed to minimize helminths and GPs transmission to humans.

Several limitations might have compromised the accuracy of the results obtained and the conclusions reached in this study. First, epidemiological data generated here might not be representative of the whole Ecuadorian scenario; second, microscopy examination of a single stool sample per participating child was used as screening method, likely sub-estimating the true prevalence rates of helminths and GP and, in the case of the latter, reducing the chances of genotyping analyses; third, concentration devices involving centrifugation steps for the recovery of labile parasitic forms (e.g., trophozoites) might compromise the quality/quantity of template DNA used in PCR testing; fourth, the detection of *D. fragilis* trophozoites by direct microscopy would have required faecal smears stained with trichrome or another permanent stain and not, as in our case, directly on droplets of the sediment, therefore our microscopic data may be underestimating the real situation; fifth, insufficient number of participants in some locations might have compromised the robustness of the statistical analyses conducted, particularly when available data were stratified by municipality of origin; sixth, questionnaire data may have resulted in some recall/recording bias; and seventh, this surveys has focused on the human reservoir of helminths and GP, but the animal and environmental reservoirs have not been considered.

To conclude, this study shows that helminths and protists remain at high prevalence rates in Ecuadorian schoolchildren living in poor-resource settings, despite the MDA campaigns regularly conducted on this specific population. Our results indicate that albendazol mass administration, specifically designed to target STH, is insufficient to effectively control these pathogens and must be complemented with interventions directed to improve household quality and living conditions, sanitation, and health education for schoolchildren and families. Together with access to safe drinking water, these measures would contribute to the reduction of intestinal parasites in poor communities in Ecuador. This study also provided relevant molecular data, including the first description in Ecuadorian human populations of i) the genetic diversity of *E. bieneusi*, including a novel genotype, and ii) the presence of *Blastocystis* ST4, for the first time described in Ecuador.

Molecular analytical methods are therefore powerful complementary tools to microscopy, and essential to unravel the epidemiology of intestinal parasites (particularly GP) including sources of infection, transmission routes, zoonotic potential, and diagnostic differentiation between pathogenic and commensal species. More research should be conducted to ascertain the role of domestic and wild animals and the environment (source waters, soil, grass and fresh produce) in the epidemiology of STH and GP in endemic areas.

Supporting information

S1 Table. Occurrence of parasitic intestinal helminths in human populations, Ecuador, 2002–2022.
(DOCX)

S2 Table. Occurrence and molecular diversity of parasitic intestinal protists in human populations, Ecuador, 2002–2022.
(DOCX)

S3 Table. English version of the standardized epidemiological questionnaire used in the present study.
(DOCX)

S4 Table. Oligonucleotides used for the molecular identification and/or characterization of the parasitic intestinal protists.
(DOCX)

S5 Table. PCR cycling conditions used for the molecular identification and/or characterization of the parasitic intestinal protists.
(DOCX)

S6 Table. Multivariate logistic regression analysis for intestinal parasitism in the surveyed population according to the municipality of origin. Adjusted Odds Ratios (AORs) and 95% Confidential Intervals (95% CI) are indicated.
(DOCX)

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3.3. Evaluation of School Children Nutritional Status in Ecuador Using Nutrimetry: A Proposal of an Education Protocol to Address the Determinants of Malnutrition

Estephany Tapia-Veloz, Mónica Gozalbo, Gabriela Tapia-Veloz, Tannia Valeria Carpio-Arias, María Trelis y Marisa Guillén

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Resumen

El sector educativo es una piedra angular en la batalla contra la malnutrición infantil. Sin embargo, aún no existen protocolos consolidados que delineen estrategias sobre cómo los programas de nutrición en países de ingresos bajos y medios pueden ser ejecutados a través del sector educativo. Establecer el diagnóstico comunitario correcto es esencial antes de elaborar un plan de intervención para una población escolar que tenga en cuenta algo más que las variables tradicionales relacionadas con el estado nutricional. En el estudio participaron 574 niños y niñas de 3 a 11 años de tres instituciones educativas de diferentes municipios de Ecuador. Se obtuvieron datos sociodemográficos, antropométricos (peso y talla) y coproparasitológicos. Para el análisis del estado nutricional se utilizó la Nutrimetría, que es una combinación de dos indicadores antropométricos clásicos, y las frecuencias de los indicadores variaron entre las escuelas. Para mejorar el estado nutricional de los niños, se propuso un marco de trabajo centrado principalmente en el establecimiento de alianzas con el sector educativo y teniendo en cuenta la igualdad de género; el respeto al medio ambiente; y las costumbres, creencias y tradiciones de cada población. Los resultados obtenidos de los análisis de otras variables demostraron la importancia de un adecuado diagnóstico previo a cualquier tipo de intervención a nivel nutricional, ya que las características podrían variar por área local e incidir en el éxito de la intervención.

Palabras clave: malnutrición; intervención nutricional; nutrimetría; escolares; parásitos intestinales.

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Evaluation of School Children Nutritional Status in Ecuador Using Nutrimetry: A Proposal of an Education Protocol to Address the Determinants of Malnutrition

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Abstract: The education sector is a cornerstone in the battle against malnutrition in children. However, there are still no consolidated protocols that outline strategies for how nutrition programs in low- and middle-income countries can be delivered through the education sector. Establishing the correct community diagnosis is essential prior to the elaboration of an intervention plan for a school population that takes into account more than just traditional variables related to the nutritional status. A total of 574 boys and girls aged 3–11 years from three educational institutions in different municipalities in Ecuador participated in the study. Sociodemographic, anthropometric (weight and height) and coproparasitological data were obtained. Nutrimetry, which is a combination of two classical anthropometrics indicators, was used for the analysis of the nutritional status, and the indicators' frequencies varied among the schools. In order to improve the nutritional status of children, we proposed a framework mainly focusing on establishing alliances with the education sector and taking into account gender equality; respect for the environment; and the customs, beliefs and traditions of each population. The results obtained from the analyses of other variables demonstrated the importance of an adequate diagnosis prior to any type of intervention at the nutritional level, since characteristics could vary by local area and have an impact on the successfulness of the intervention.

Keywords: malnutrition; nutrition intervention; Nutrimetry; schoolchildren; intestinal parasites

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1. Introduction

Malnutrition in childhood has an immediate impact on a child's survival and development, and later on, it has an impact on their productivity and economic contribution to society [1,2]. The current agendas (the 2030 agenda, the global nutrition agenda, etc.) point out the importance of multisectoral approaches to addressing malnutrition [3–5]. The education sector is a cornerstone in the battle against malnutrition [6]. For this reason, programs that address the double burden of malnutrition (i.e., the existence of both undernutrition and overweight/obesity within the same population) [7] have been launched in low- and middle-income countries (LMICs) within the education sector (mainly in primary schools) [8,9]. The WHO's Nutrition-Friendly Schools Initiative (NFSI) is one such initiatives that provides a framework for ensuring integrated school-based programs that address this double burden, taking into account the five broad components: school nutrition policies, awareness and capacity building of the school community, nutrition-

and health-promoting curricula, supportive school environment for good nutrition, and supportive school nutritional and health services [8–10].

Despite these efforts, there are no consolidated protocols that outline strategies for how nutrition programs in LMICs can be delivered through the education sector.

In Latin America, the coexistence of chronic undernutrition (stunting), and overweight and obesity in children is increasingly evident. It is estimated that the prevalence of chronic undernutrition in this region at the school stage is 34.4% [11], while the prevalence of overweight is 25% [12]. According to the 2018 Health and Nutrition Survey (ENSANUT) of Ecuador, the chronic undernutrition rate in this country for children under five years of age decreased from 23.9% to 23.0% between 2014 and 2018; in rural areas, this indicator decreased from 31.9% to 28.7% in the same period. The rate of overweight and obesity in children aged 5 to 11 years increased from 29.9% to 35.4%. Differences by area of residence were also observed; values were 36.9% in urban areas and 32.6% in rural areas [13]. These data show that, although the prevalence of chronic undernutrition is slowly decreasing, the prevalence of overweight/obesity is alarmingly increasing among children in Ecuador [14].

It can be observed that the so-called double burden of malnutrition in the case of this country is not alien to its reality, and it is increasingly worrisome [15]. It is directly related to the various changes in economic, social and demographic factors that occur where inequity and poverty are present, such as a higher migration from rural to urban areas; globalization; changes in occupational structures; alterations in dietary patterns; and a lifestyle characterized by an increased production, distribution and consumption of high-energy processed foods, along with a decrease in physical activity. All these elements together are causing a nutritional transition in countries such as Ecuador [14–16].

However, intestinal parasitism constituted by species *Giardia intestinalis*, *Ascaris lumbricoides* and *Trichuris trichuria* presents scientific evidence that proves it to be a determinant factor in the nutritional status of children [17–19]. Its importance lies in its high prevalence and its effects on the health status of the child population, such as a loss of appetite, growth retardation, cognitive problems, the poor absorption of nutrients and poor immunity. It has a negative influence on the morbidity and mortality of this population and thus on the economic development of a country [11,20–22]. The least-favored populations of structurally impoverished countries, such as Ecuador, have the highest prevalence due to multiple factors, such as poor environmental sanitation conditions, socio-cultural conditions, inadequate hygienic–sanitary habits and a high rate of maternal and infant undernutrition [11,20–23]. It affects approximately 80% of the rural population and 40% of the urban marginal population [24]. Studies carried out in this country show the prevalence of *G. intestinalis* to be between 16.4% and 20.3% [25,26], the prevalence of *A. lumbricoides* to be between 9.8% and 43.8% [25,27] and the prevalence of *T. trichuria* to be between 10% and 46.4% [28–31]. It is in second place on the list of the main causes of ambulatory morbidity devised by the Ministry of Public Health of Ecuador in 2014, and it is within the top ten causes of pediatric consultation [24,25,32].

In this scenario, it seems absolutely compulsory to carry out an evaluation and assessment of the childhood nutritional status, taking into account other variables rather than the classical ones, such as Body Mass Index-for-Age Z-Score (BMIZ) and Height-for-Age Z-Score (HAZ), before implementing a nutrition protocol.

For the diagnosis of children's nutritional status at the population level, the BMIZ is an easy-to-use and -interpret indicator, but considering the nutritional transition that Ecuador is going through, it falls short. In view of this problem, the importance of a joint interpretation of the BMIZ with other indicators has been pointed out. This new approach has been implemented in individual care but not in epidemiological studies, which must be carried out before implementing a community intervention. For this reason, Solis and collaborators developed a method called "Nutrimetry", which combines two simple and accessible anthropometric variables, BMIZ and HAZ, with the intention of facilitating their joint interpretation and generating a broader view of the nutritional status [33,34].

We undertook this research study to evaluate the nutritional status of school children from three different areas in Ecuador as the first step in order to develop proper nutritional protocols within the education sector. In this paper, we first describe the nutritional status in three areas using a new epidemiological methodology, Nutrimetry, based on the combination of two classical variables. We also propose a theoretical framework to develop in the education sector taking in account all the specific characteristics of the studied area in Ecuador. Moreover, we suggest some key strategies for how nutrition programs can be properly delivered through the education sector and how they can be strengthened in Ecuador. This framework attempts a link to the "One Health" breakthrough approach. According to the WHO, "One Health" is an approach to designing and implementing programs, policies, legislation and research in which multiple sectors communicate and work together to achieve better public health outcomes. It can be applied at the community, subnational, national, regional and global levels and relies on shared and effective governance, communication, collaboration and coordination [35,36]. We also discuss gaps in research, as well as opportunities for intervention in other places in Ecuador.

2. Materials and Methods

2.1. Study Design and Description of the Studied Local Areas

This cross-sectional study was conducted in three Ecuadorian primary schools located in three different provinces: two of them were located in Chimborazo, which belongs to the Sierra region, municipality of Penipe and Pallatanga, and the other was located in Guayas, which belongs to the Coast region, municipality of General Antonio Elizalde (Figure 1). Penipe has a cold mountainous climate; Pallatanga has a temperate sub-humid mountainous climate; and General Antonio Elizalde has a humid tropical climate.

Figure 1. Map of Ecuador showing localization of the three municipalities in the Chimborazo and Guayas provinces.

According to the 2014 Map of Poverty and Inequality by Consumption in Ecuador, Pallatanga has a Gini coefficient of 0.37; Penipe has a Gini coefficient of 0.35; and General Antonio Elizalde has a Gini coefficient of 0.30 [37]. The years of schooling of the population in the Pallatanga municipality is 6.2 years; in Penipe, it is 7.7 years; and in General Antonio Elizalde, it is 8.3 years [38–40]. The percentage of households with inadequate housing characteristics in Pallatanga is 14.9%; in Penipe, it is 11.4%; and in General Antonio Elizalde, it is 5.7%. The percentage of homes with a public water supply in Pallatanga is 44.8%; in Penipe, it is 76.6%; and in General Antonio Elizalde, it is 63.2% [41]. These three

municipalities differ from each other due to their location, climate, and socio-demographic and socioeconomic characteristics, thus allowing diverse results to be obtained for each area.

2.2. Recruitment and Sample

A primary school for each of the three municipalities was selected according to the following criteria: (1) number of students attending the schools had to be more than 300 and (2) with a lower to medium socioeconomic status (SES) to be representative of the population of the area. Finally, (3) the school staff had to be committed to and engaged in this preliminary phase of the project. To achieve this last point, informative talks addressed to the staff were given in each educational center. The pupils in each grade were recruited through their leaders (main teacher assigned for the school year), who informed them about the project during their lessons and gave some examples about how important nutrition is for mental and physical development. This process took two months. Meanwhile, all the parents were also invited to participate by the schools' administrations, from whom they received an informative letter enclosed in the school diaries of their children. Informed consent forms signed by the parents of the participating students were obtained prior to data collection. No remuneration or incentive was provided for participation, but once the samples and anthropometric data were analyzed, a report with the results was delivered in a sealed envelope to their legal representatives, so that they could decide whether to proceed with medical assistance.

2.3. Ethics Approval

This study was approved by the Ethics Committee for Human Research of University of Valencia (procedure number: H1518738039128; 1 March 2018), by the Ethical Subcommittee for Research on Human Subjects of Central University of Ecuador (SEISH-UCE) (2019) and by National Directorate of Prevention and Control Strategies and National Directorate of Health Promotion of the Ministry of Public Health (MSP) (Ecuador, 2020) as part of the project Análisis de factores de riesgo y manejo de la malnutrición en edad escolar. Protocolo de actuación "aquí" para extrapolar "allí". We ensured that the fundamental principles established by the Declaration of Helsinki and Spanish/Ecuadorian legislation in the field of biomedical research, data protection and bioethics were respected.

2.4. Data Collection

A group of ten nutritionists were trained to obtain data. We used standardized protocols to gather information concerning (a) anthropometric variables (weight and height); (b) sociodemographic variables (age, sex, educational level of the father and mother, whether the father/mother worked, where the bathroom was and bottled water consumption); and (c) coproparasitological variables (1 stool sample).

(a) Anthropometric Assessment

An Omrom® electronic scale (accuracy, 100 g) and a Seca216® mechanical measuring rod (accuracy, 1 mm) were used to determine weight and height. Height was determined without shoes and socks, and body weight was determined with participants dressed only in their underwear. HAZ and BMIZ were calculated using World Health Organization (WHO) Anthro and AnthroPlus software (World Health Organization, 2009; Anthro for Personal Computers, Version 3.01: Software for Assessing Growth and Development of the World's Children), using the WHO child growth standard 2005 version for children aged 0–5 years and using the 2007 version for children and adolescents aged 5–19 years. These indicators classified children to varying degrees and types of malnutrition based on WHO reference data [42], which enable the analysis and interpretation of the growth patterns of children in any population in the world to be performed. For the final diagnosis of the nutritional status, Nutrimetry was used, where HAZ and BMIZ are combined in a 3×3 table (Figure 2) with the intention of facilitating their joint interpretation.

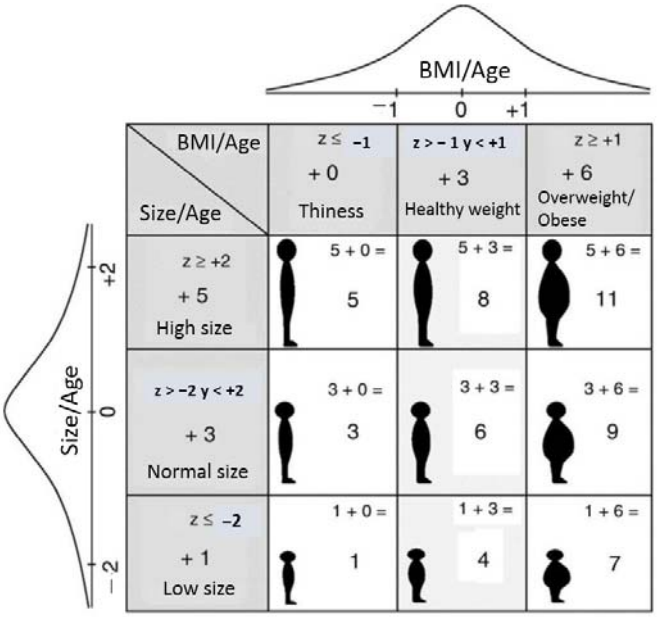


Figure 2. Nutrimetry (taken and translated from Selem-Solis et al. (2017).

The different combinations of HAZ and BMIZ result in nine categories, nutricodes, which are assigned nine-digit codes, 1, 3, 4, 5, 6, 7, 8, 9 and 11 (Table 1); these are responsible for representing the different diagnoses of the nutritional status [33].

Table 1. Nutricodes and their interpretations.

Nutricodes	Interpretation
1	Low HAZ + Low BMIZ
3	Normal HAZ + Low BMIZ
5	High HAZ + Low BMIZ
4	Low HAZ + Normal BMIZ
6	Normal HAZ + Normal BMIZ
8	High HAZ + Normal BMIZ
7	Low HAZ + High BMIZ
9	Normal HAZ + High BMIZ
11	High HAZ + High BMIZ

HAZ, Height-for-Age Z-Score; BMIZ, Body Mass Index-for-Age Z-Score.

(b) Sociodemographic variables

A questionnaire was used to collect personal data concerning the following:

(1) Date of birth, (2) sex, (3) education level of the father, (4) education level of the mother, (5) whether the father/mother worked, (6) where the bathroom was and (7) bottled water consumption. Items three to seven were used to assess the socioeconomic status level of the family.

(c) Coproparasitological Assessment

A total of 370 samples were collected and processed. One fresh stool sample was collected per participant. After the filtration and concentration of the samples, they were analyzed using the Kato–Katz technique and optical microscopy for the identification of forms of resistance of intestinal parasites in general (cysts and eggs); molecular diagnostic techniques (real-time polymerase chain reaction or qPCR) were also applied to identify one specific parasite (*Giardia intestinalis*).

Starting with 3 g per fecal sample, they were filtered and concentrated via centrifugation (2500 rpm, 5 min) in Midi Parasep tubes® (Apacor Ltd., Wokingham, UK). The sediment obtained was divided into two microtubes, one for the extraction of total stool DNA for qPCR and the other with 10% formalin for microscopic observation. QIAmp DNA

Stool Mini Kit (QIAGEN®, Hilden, Germany) was used for DNA extraction according to the manufacturer's instructions.

For the diagnosis of *G. intestinalis* DNA, qPCR was performed. This protocol specifically amplifies a fragment of the gene that encodes the parasite's small ribosomal subunit RNA (SSU rRNA) and shows high sensitivity [43]. A commercial assay with the specific primers and probe (LightMix Modular Assays Giardia; Roche®, Basel, Switzerland) was used, together with mastermix (dNTPS; thermostable Taq polymerase and buffer) (PerfeCTa qPCR ToughMix; Quanta Biosciences, Gaithersburg, MD, USA) for a mix reaction final volume of 15 µL. To each well, 5 µL of sample and positive control DNA was added, and water was added instead for the negative control. The analysis was performed with StepOnePlus real-time PCR thermocycler® (Applied Biosystems®, Foster City, CA, USA). Any sample that managed to amplify before 43 cycles was considered positive.

2.5. Data Analysis

Descriptive statistics were calculated, including measures of central tendency (mean and median), measures of dispersion (standard deviation, range and coefficient of variation) and measures of shape (asymmetry and pointing) for quantitative variables, as well as the absolute and relative frequencies for the qualitative variables. Association analyses were performed, stratifying by sex and age to observe possible heterogeneity in the results according to these factors. When data were stratified and the sample size was small ($n < 15$), non-parametric tests were used (Fisher's exact test and Mann–Whitney U test). Any *p*-value less than 0.05 was considered statistically significant. All the variables were analyzed using SPSS software (Statistical Package for Social Sciences for Windows, version 26.0; SPSS Inc., Chicago, IL, USA).

2.6. Theoretical Framework for the Implementation of Nutritional and Health Education in Ecuador

Figure 3 shows an overview of the main components of a comprehensive framework for the implementation of an adequate nutrition program in Ecuador taking in account the five broad components of the NFSI. This framework is based on there being accessible primary schools in the areas where an intervention must be carried out. Schools are the cornerstone, as they serve as the vehicle by which nutrition programs are delivered.

The development process consists of the following four phases:

Phase 1—Diagnosis: In this phase, Nutrimetry plays an important role, but it is not sufficient to improve the nutrition of vulnerable populations in food-insecure areas. For this reason, we propose six components on which information must be collected in order to establish a diagnosis. These six components are as follows: (1) school services, (2) socioeconomic status [44], (3) Nutrimetry, (4) agriculture resources [45], (5) water sanitation [11,20–23] and (6) myths, customs and traditions related to nutrition and/or health.

Phase 2—Identification of health-promoting resources at schools: Before implementing an intervention, it is important to identify any health-promoting resources. These resources promote health and facilitate coping with stressors, such as social relationships and dietary practices. To facilitate the mobilization of health resources, intervention strategies should be adjusted to real life in order to increase the chance of the successful implementation of newly adopted behaviors in everyday life [46].

Phase 3—Intervention: In Supplementary Table S1, an approach for an intervention to improve the nutritional status in Ecuador is shown. The six components mentioned in Phase 1 (Diagnoses) and some of the variables presented in Phase 2 (Identification of health-promoting resources at schools) are taken into account in order to establish intervention objectives, indicators, verification sources and activities. Phase 4—Evaluation outcomes: Nutrition indicators should be included in any intervention to assess the improvement of the nutritional and health status. However, we also must include other indicators related to the consumption of local and nutritious food.

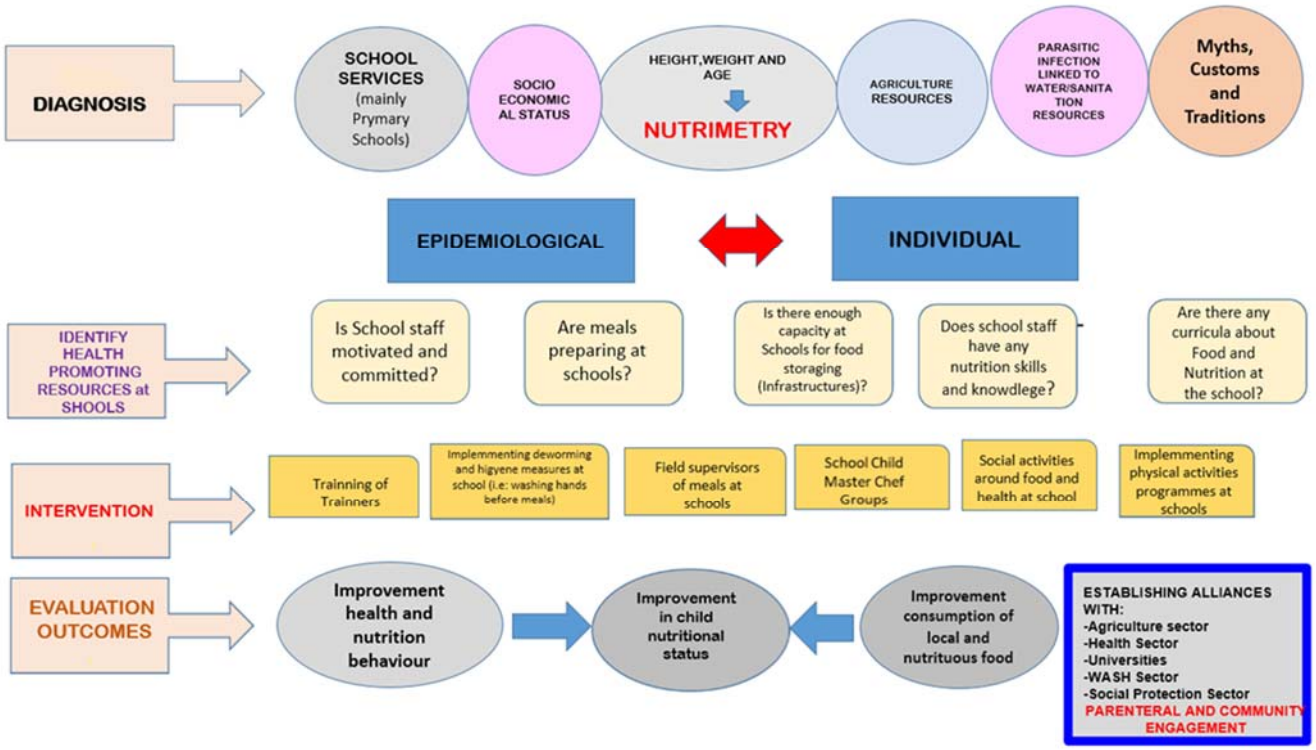


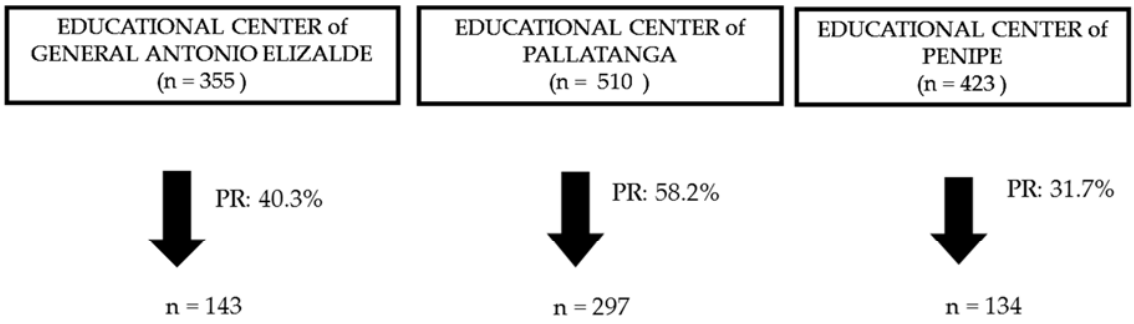
Figure 3. Theoretical framework for the implementation of nutritional and health education in Ecuador focused mainly on primary school environments.

3. Results

A total of 574 schoolchildren participated, of whom 304 were girls (53%) and 270 were boys (47%). The mean age was 7.7 ± 2.3 years. We recruited 297, 143 and 134 schoolchildren in Pallatanga, General Antonio Elizalde and Penipe, respectively.

The number of the total students of the three educational centers, the ratio of participation and the frequencies (%) of the studied population according to gender and the age group (<5 years, 5–8 years and ≥9 years) stratified by the three municipalities (General Antonio Elizalde, Pallatanga and Penipe) are shown in Figure 4.

Table 2 shows the following sociodemographic variables stratified by municipality: father's educational level, mother's educational level, whether the father or mother worked, indoor bathroom and bottled water consumption. We found statistically significant differences in the frequencies of all variables among the three municipalities ($p < 0.05$). General Antonio Elizalde presented figures that could be associated with an economic level that is higher than those of Penipe and Pallatanga, and this is consistent with the Gini coefficient described by the National Institute of Statistics and Census (INEC) in its document Map of Poverty and Inequality by Consumption in Ecuador 2014 [37]. These differences could especially be seen in the educational levels of the father and mother, bathing inside or outside of the house and bottled water consumption; in General Antonio Elizalde, the university level of education of the father and mother exceeded 30%, while in Pallatanga and Penipe, it did not even reach 9%. In General Antonio Elizalde, bathing inside the house occurred in almost all households (92.3%), while in Pallatanga and Penipe, it only occurred in approximately half of the population. There was a similar trend regarding bottled water consumption; in General Antonio Elizalde, 67.8% of participants said they consumed bottled water, while in Pallatanga and Penipe, this value was much lower, at 16.2% and 16.4%, respectively.



	Girls	Boys	Girls	Boys	Girls	Boys
Gender (%)	53.1 (76)	46.9 (67)	55.2 (164)	44.8 (133)	47.8 (64)	52.2 (70)
Age (years)						
<5	15.8 (12)	17.9 (12)	7.9 (13)	9.8 (13)	15.6 (10)	17.1 (12)
5-8	63.2 (48)	6.2 (41)	42.7 (70)	47.4 (63)	48.4 (31)	41.4 (29)
≥9	21.1 (16)	20.9 (14)	49.4 (81)	42.9 (57)	35.9 (23)	41.4 (29)

Figure 4. Participation rate (PR) and frequency (%) of the studied population according to gender and age group in the three municipalities. Data are shown as percentages of the total population stratified by municipality. The number of people is shown in brackets. There are no statistically significant differences ($p > 0.05$) for the distribution of the frequencies of participants by age and gender between the three municipalities.

Table 2. Frequencies (%) of the studied population according to education level of the father, education level of the mother, whether father or mother worked, indoor or outdoor bathroom and bottled water consumption stratified by municipality.

		General Antonio Elizalde	Pallatanga	Penipe	Total	* *p*-Value
		(n = 143)	(n = 297)	(n = 134)	(n = 574)	
Education level of the father	no studies	0 (0)	7.2 (17)	12.9 (16)	6.6 (33)	0.000
	elementary school	10.0 (14)	57.0 (135)	54.8 (68)	43.3 (217)	
	high school studies	55.7 (78)	31.2 (74)	24.2(30)	36.3 (182)	
	university studies	34.3 (48)	4.6 (11)	8.1 (10)	13.8 (69)	
Education level of the mother	no studies	0 (0)	6.3 (18)	12.7 (17)	6.2 (35)	0.000
	elementary school	8.4 (12)	51.0 (146)	50.7 (68)	40.2 (226)	
	high school studies	55.2 (79)	36.7 (105)	32.1(43)	40.3 (227)	
	university studies	36.4 (52)	5.9 (17)	4.5 (6)	13.3 (75)	
Father or mother worked	yes	94.2 (131)	89.7 (209)	96.8 (120)	92.7 (496)	0.036
Indoor or outdoor bathroom	indoor	92.3 (132)	53.9 (160)	43.3 (58)	61.0 (350)	0.000
Bottled water consumption	yes	67.8 (97)	16.2(48)	16.4 (22)	29.0(167)	0.000

Data are shown as percentages of the total population stratified by municipality. The number of people is shown in brackets. * *p*-value for the comparison of distribution of the frequencies among the three municipalities.

The results of the coproparasitological analysis are shown in Table 3. Overall, 38.6% of the participants presented intestinal parasites. *G. intestinalis* was the most prevalent parasite (30.8%), which was analyzed using light microscopy and qPCR to improve its sensitivity. *A. lumbricoides* was the second most prevalent parasite (9.5%), and *T. trichiura* was the third most prevalent parasite (5.2%); for both helminths, no molecular method was chosen because the Kato–Katz technique was optimal. Most of these parasitic species are ingested as cysts or eggs (infectious forms), and they are present in food or water as a

result of fecal contamination. When stratified by municipality, being parasitized or having *G. intestinalis* did not present a statistically significant difference ($p > 0.05$), but it did in the cases of *A. lumbricoides* and *T. trichiura*. Pallatanga had the highest figures of 16.1% and 9.3%, respectively, while the other municipalities presented lower data for the two helminths, which did not exceed 4.4% for *A. lumbricoides* or 2.1% for *T. trichiura*. The reasons for these differences are unknown and should be investigated in future research.

Table 3. Frequencies (%) of the studied population according to the presence of total parasitic infection and infection by *G. intestinalis*, *A. lumbricoides* and *T. trichiura* stratified by municipality.

	General Antonio Elizalde	Pallatanga	Penipe	Total	* *p*-Value
	(n = 114)	(n = 161)	(n = 95)	(n = 370)	
Parasitic Infection	37.7 (43)	41.6 (67)	34.7 (33)	38.6 (143)	0.478
G. intestinalis	34.2 (39)	27.3 (44)	32.6 (31)	30.8 (114)	0.452
A. lumbricoides	4.4 (5)	16.1 (26)	4.2 (4)	9.5 (35)	0.006
T. trichiura	1.8 (2)	9.3 (15)	2.1 (2)	5.2 (19)	0.001

Data are shown as percentages of the total analyzed samples of the population stratified by municipality. The number of people is shown in brackets. * *p*-value for the comparison of the distribution of the frequencies among three municipalities.

Regarding nutritional status, which was evaluated using Nutrimetry, the total data stratified by gender and age are shown in Table 4. The prevalence values of the nutricodes associated with malnutrition (nutricodes 9, 4 and 3) were 27.2%, 10.6% and 4.9%, respectively. Nutricode 5 was non-existent. We did not find any statistical difference ($p < 0.05$) between boys and girls stratified by age in the distribution of the nutricodes.

Table 4. Frequencies (%) of the studied population according to codes established for Nutrimetry stratified by gender and age group (<5 years, 5–8 years and ≥9 years).

	BOYS (n = 270)			* *p*-Value	GIRLS (n = 304)			* *p*-Value	** *p*-Value
	<5 (n = 37)	5–8 (n = 133)	≥9 n = (100)		<5 (n = 35)	5–8 (n = 149)	≥9 n = (120)		
Thinness Nutricode									
1	0 (0)	0.8 (1)	1.0 (1)		0 (0)	0.7 (1)	4.2 (5)		
3	2.7 (1)	8.3 (11)	4.0 (0)		11.4 (4)	2 (3)	4.2 (5)		
5	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)		
Normal Weight Nutricode				0.140				0.482	0.71
4	16.2 (6)	9.8 (13)	14 (14)		11.4 (4)	6.7 (10)	11.7 (14)		
6	62.2 (23)	55.6 (74)	50 (50)		54.3 (19)	53 (79)	49.2 (59)		
8	0 (0)	0 (0)	0 (0)		0 (0)	1.3 (2)	0.8 (1)		
Overweight and Obesity Nutricode									
7	2.7 (1)	1.5 (2)	6.0 (6)		2.9 (1)	0.7 (1)	1.7 (2)		
9	16.2 (6)	24.1 (32)	25.0 (25)		20 (7)	35.6 (53)	27.5 (33)		
11	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0.8 (1)		

Data are shown as percentages of the total population stratified by age and gender. The number of people is shown in brackets. * *p*-value for the comparison of the distribution of the frequencies of the codes established by Nutrimetry and age ** *p*-value for the comparison of the distribution of the frequencies of the codes established by Nutrimetry and sex.

We analyzed the distribution of the nutricodes of all of the schoolchildren in each municipality, so there were differences in some variables associated with socioeconomical status that could determine differences in nutritional status. The data are shown in Table 5.

Table 5. Frequencies (%) of the studied population according to codes established for Nutrimetry stratified by municipality.

	General Antonio Elizalde	Pallatanga	Penipe	Total	* *p*-Value
	(n = 143)	(n = 297)	(n = 134)	(n = 574)	
Thinness Nutricode					
1	0 (0)	1.3 (4)	3.0 (4)	1.4 (8)	
3	4.9 (7)	3.4 (10)	8.2 (11)	4.9 (28)	
5	0 (0)	0 (0)	0 (0)	0 (0)	
Normal Weight Nutricode					
4	2.8 (4)	11.1 (33)	17.9 (24)	10.6 (61)	0.000
6	49.7 (71)	52.2 (155)	58.2 (78)	52.9 (304)	
8	0 (0)	0.7 (2)	0.7 (1)	0.5 (3)	
Overweight and Obesity Nutricode					
7	0.7 (1)	3.4 (10)	1.5 (2)	2.3 (13)	
9	41.3 (59)	27.9 (83)	10.4 (14)	27.2 (156)	
11	0.7 (1)	0 (0)	0 (0)	0.2 (1)	

Data are shown as percentages of the total population stratified by municipality. The number of people is shown in brackets. * *p*-value for the comparison of the distribution of the frequencies of the codes established by Nutrimetry among the three municipalities.

4. Discussion

This paper attempts to establish the basis for a protocol to address the determinants of malnutrition in Ecuador using schools and education as the cornerstones. To our knowledge, this is the first time this approach has been used in the country. The data collected are part of a project that has just been awarded in the call for International Cooperation Projects 2022 of University of Valencia, where the intervention phase is planned to begin in January 2023; all the data presented in this article allowed us to have an overview of the reality and lay the groundwork for developing the proposed framework, which we are using with the matrix to prepare all the tools and materials to be used in the intervention.

We present the theoretical framework for the implementation of nutritional and health education in Ecuador and also the results of the analysis of some of the components of the theoretical framework. Although the authors are aware that only a small part of the components of the theoretical framework were analyzed, these preliminary results are really interesting and need to be analyzed before moving on to the implementation of the protocol, which is to be specific to each community.

We used Nutrimetry to assess the nutritional status of children in three areas of Ecuador. Nutrimetry, as well as the assessment of some other determinants, provides relevant epidemiological information to establish protocols for future nutritional interventions in schoolchildren. The prevalence of overweight (codes 7, 9 and 11) was 29.2%; the prevalence of thinness (codes 1, 3 and 5) was 6.3%; and the prevalence of short stature (codes 1, 4 and 7) was 14.3%. Similar figures were found in other studies in this Ecuadorian school population [25,47]. Our study analyzed three schools in three different municipalities, which differ due to their location, altitude, climate, population and economic characteristics. The latest published official data on the Gini coefficient for the municipalities are from 2014; there are more current studies but not at the municipal level [48,49]. According to this coefficient, which allows wage inequality to be measured, Pallatanga had the highest inequality (0.37), followed closely by Penipe (0.35) and finally by General Antonio Elizalde (0.30) [37]. When we determined whether there was an association between the area in which the schoolchildren lived and nutricodes, a statistically significant difference was observed ($p < 0.001$). Accordingly, Roche et al. (2016) stated that a high socioeconomic level decreases the risk of stunting [50] but also increases the rates of overweight [51]. This was

the case in General Antonio Elizalde, where overweight and obesity (codes 7, 9 and 11) reached 42.7% and were much more prevalent than some types of undernutrition; even code 1 (low height and thinness) was nonexistent. Pallatanga and Penipe, which have a lower socioeconomic level than General Antonio Elizalde, had lower figures for excess weight, but they were still high, at 31.3% and 11.9%, respectively. As for thinness (codes 1, 3 and 5), Pallatanga had a rate of 4.7%, and Penipe had a rate of 11.2%. According to these results, Penipe was the municipality with the lowest figures for excess weight and the highest figures for thinness; however, according to the Gini coefficient [37], its economic level is slightly higher than that of Pallatanga. This could be due to the fact that its socioeconomic growth may have changed in recent years, and Penipe is still a cold mountainous area at a high altitude with a large indigenous population; it has always had the highest percentages of malnutrition [50,52]. All the results presented show that a structurally impoverished country such as Ecuador, which is normally associated with high levels of child undernutrition, also currently presents alarming rates of excess weight. It is true that the trend of child undernutrition has been reduced, but this is still insufficient; thus, it is a latent problem that has not been eradicated. Therefore, there is an evident nutritional and epidemiological transition [14,53–55]. The problem is that any type of malnutrition during childhood is associated with adverse health consequences throughout life [56], affecting morbidity, mortality, the development of skills, educational performance, social inclusion, labor, the environment and productivity [57]. The reasons for the presence of an altered nutritional status are intimately linked to complex socioeconomic factors, such as changes in occupational structures; rapid urbanization; an increased penetration of the retail food industry, which has resulted in diets based on energy-dense and nutrient-poor foods; maternal education; and economic growth, which is often accompanied by persistent poverty and inequality, a reality not alien to that of Ecuador, where there is high economic inequity that is negatively affecting the nutritional status of its population [14,51,58,59]. To the best of our knowledge, this is the first time that Nutrimetry was used to assess the nutritional status in Ecuador. Previous studies only used the BMIZ indicator, the HAZ indicator or both indicators but analyzed them separately [13,14,50–53,59], so children suffering from malnutrition may not have been detected. In the case of the present study, 82 children with stunting (codes 1, 4 and 7) would have been left out, as well as 28 children who presented a recent alteration in nutritional status (code 3). Nutrimetry allows us to describe and analyze the population distribution of malnutrition in greater detail and depth. Nine subgroups are generated without the requirement of any specialized software for processing. It also complies with WHO criteria in order to be considered an anthropometric indicator, using its growth standards for interpretation. Furthermore, it discourages the stigmatization of individuals using neutral language since it reports results in numbers, avoiding the use of words such as "fat" and "skinny", thus facilitating the communication of the results and objectives to schoolchildren without an emotional semantic load [33,34].

Intestinal parasitism is an important factor for the correct epidemiological analysis in the case of countries such as Ecuador, as such countries are characterized by high numbers of intestinal parasitism, especially in children [60,61]. There is evidence that certain types of intestinal parasites, such as *G. intestinalis*, *A. lumbricoides* and *T. trichiura*, have an impact on nutritional status [17–19], and in the case of Ecuador, previous research has described significant prevalence of these parasites [25–30]. After an analysis of the samples collected, we found that 38.6% of the children were parasitized with any one of these three species. *G. intestinalis* was the most prevalent at 30.9%; this figure is higher than that reported in previous research [61–63], because we used, in addition to microscopy, real-time PCR, which is a more sensitive technique [64]. *A. lumbricoides* and *T. trichiura* had a prevalence of 9.5% and 5.1%, respectively, and other articles have reported similar figures [25,60,64]; to detect these helminths, we did not use any molecular technique, since optical microscopy together with the Kato–Katz technique is optimal for their detection. When analyzing the relationship between Nutrimetry and being parasitized, we did not find a statistically significant association, possibly because the nutritional impact is usually related to undernutrition,

and in our study, such prevalence was not high. However, it is still a problem, since the number of intestinal *G. intestinalis* cases was high; it is possible that many of these children were asymptomatic [65,66] and that it is a triggering factor for secondary food intolerance/malabsorption, dyspepsia or irritable bowel syndrome [19,67,68]. It is known that it causes the activation of CD8 lymphocytes in the intestinal villi of the absorptive mucosa, affecting the growth and cognitive development of children suffering from it [65,69,70]. Studies have also associated *G. intestinalis* with alterations in iron absorption, decreased serum iron, low blood Hb levels and iron-deficiency anemia [19,65]. As for helminths, these cause reduced growth rates, intellectual disabilities, cognitive deficits, decreased food intake, iron deficiency and the malabsorption of nutrients [17,27]. The relationship between the studied municipalities and being parasitized was not significant in the case of *G. intestinalis* ($p > 0.05$), but it was in the cases of *A. lumbricoides* and *T. trichiura* ($p < 0.05$), where Pallatanga had a higher prevalence than did Penipe and General Antonio Elizalde. These disparities may be due to the fact that the numbers of these two parasites vary greatly depending on the area studied; previous studies have reported values ranging from 10% to 46.8% [25,27–30]. It is necessary to further investigate the causes of these differences. The figures for the parasitic infections of *A. lumbricoides* and *T. trichiura* were low with respect to *G. intestinalis*, and this was probably due to the deworming campaigns carried out in schools for helminths but not for protozoa. The prevalence of these three pathogens may be associated with insufficient hygienic–sanitary habits, inadequate environmental conditions, and insufficient resources and education, which favor their development, maintenance and dissemination [71].

Ecuador, unfortunately, despite the policies, strategies and programs implemented in recent years, such as Plan Nacional del Buen Vivir, Plan Intersectorial de Alimentación y Nutrición, Intervención Nutricional Territorial Integral (INTI) and Programa Acción Nutrición (PAN) [57,72,73] and the incorporation of healthy habits in the curriculum and the increase from two to five hours of physical education for public schools and colleges [73,74], has not achieved a substantial improvement in school malnutrition figures. This fact is evidence that there is much work ahead and that Ecuador should be focused on malnutrition in the forms of deficit and excess, taking into account the nutritional epidemiological transition that is taking place. For the elaboration of the framework and for the initial evaluation of the school nutrition situation in our study, the NFSI tools [9] were taken into account because of their great relevance and usefulness in this population. The results of this study make it clear that there is an unquestionable need for intervention programs; therefore, the framework we propose as well as the intervention planning matrix (Table S1) could help in solving the current problems of schoolchildren malnutrition in Ecuador.

Finally, we would like to point out that given the volume and complexity of the information that researchers could obtain when this approach is implemented in other municipalities in Ecuador, it will likely be necessary to use informatics tools and integrated platforms. Nutritional Informatics (NI) could provide some valuable tools [75] and help to develop solutions to the health problems encountered within a transitioning food environment. The definition of NI expanded as an effective retrieval, organization, storage and optimum use of information, data and knowledge for food and nutrition-related problem solving; Informatics is supported using information standards, information processes and information technology. On the other hand, the importance of integrating social factors not only in nutritional and health education but also in medical care is hardly news [76]. Social and other "non-medical" determinants of health are also obtained and then digitally integrated. Acknowledging, measuring and addressing all these data into the mainstream community care context is essential to achieve true health and wellbeing among the populations living in our neighborhoods, regions and nations.

The present research study had some limitations; due to the non-representative study sample, the data obtained and analyzed from the three schools could not be extrapolated to the reality of the municipality or the country; a larger sample of participants is needed to be

representative, but in any case, we showed that school malnutrition is a reality that should be studied and solved. The leaders notified all the students enrolled to participate, but the rate of commitment varied for each educational center, being the lowest in Penipe, possibly due to the characteristics of its population, since many of the children lived in remote rural areas and for their parents to travel was to lose a day of work, which is something important to take into account in order to increase the degree of commitment as well as to investigate the reasons for low attendance. We only analyzed the nutritional status, parasitic infection and some items related to socioeconomic level, which are important, but it is necessary for future research to take into account the other variables proposed in the framework, as well as the collection of other health parameters to detect micronutrient deficiencies in order to have the best possible diagnosis prior to intervention. In addition, for an in-depth study of the socioeconomic level in Ecuador, there are validated questionnaires from the INEC [77] that collect many more data and that should be considered. The approximate costs derived from the implementation of the phases of the framework were not calculated; therefore, we do not know the necessary budget, and for the following studies, it would be relevant to establish this factor prior to its application and to confirm its feasibility.

5. Conclusions

The first step that must be taken before developing a nutritional intervention plan for the pediatric population is establishing the correct diagnosis of the current status of the area related to all the determinants of malnutrition. Nutrimetry can be used as a suitable indicator at the epidemiological and clinical levels, as it allows data to be obtained by combining two variables that are normally used separately, such as HAZ and BMIZ, and it is easy to use and interpret through the nutricodes generated, as well as being useful in the evaluation phase. It is important that the intervention to be carried out identifies the health-promoting resources adjusted and adapted to the reality of the population by taking into account the NFSI tools. The establishment of alliances with local centers, institutions and associations, whether public or private, would allow sustainability to be maintained, continuity to be ensured and self-sufficiency to be generated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14183686/s1, Table S1: Intervention planning matrix with intervention objectives, indicators, sources of verification and activities focused primarily on the elementary school setting.

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CAPÍTULO IV:
CONCLUSIONES

Conclusiones

1. La aplicación de la Nutrimetría ha permitido obtener información del estado nutricional en la infancia de manera fácil a través de la combinación de dos variables que normalmente se usan por separado BMIZ y HAZ. Este indicador, es recomendable a nivel epidemiológico y clínico para la estimación del estado nutricional.

2. La prevalencia de malnutrición infantil en la muestra analizada de población escolar española y ecuatoriana a través de la aplicación de la Nutrimetría, es elevada. En los dos países las cifras de sobrepeso/obesidad son altas, similares y alarmantes (32,6% España y 29,2% Ecuador). En España algún tipo de desnutrición es prácticamente inexistente, mientras que en Ecuador afecta a más del 20% de los niños y niñas. Las políticas, estrategias y programas implementados en los últimos años en los dos países, no han logrado controlar el incremento del exceso de peso, y en el caso de Ecuador tampoco la desnutrición, además, es evidente la transición nutricional que atraviesa este país y que podría hacer más crítica su malnutrición infantil.

3. Los estilos de vida analizados en los y las escolares de España revelan que casi la mitad tiene una alta ADM; las calorías totales y de macronutrientes calculadas a través del CFCA son superiores a los valores recomendados y evidencia una posible sobreestimación; un porcentaje mayoritario no cumple con las recomendaciones de actividad física y el 30,6% dedica más horas de las recomendadas al uso de pantallas.

4. Los estilos de vida son un aspecto fundamental a tener en cuenta por su relación con el estado nutricional, pero es necesario, sobre todo a nivel de hábitos dietéticos, combinar diversos métodos que mejoren su análisis y faciliten el hallazgo de asociaciones con el estado nutricional.

5. La prevalencia de malnutrición infantil (tanto por obesidad/sobrepeso como por desnutrición) está asociada con el nivel socioeconómico en Ecuador. El municipio con un mayor nivel socioeconómico, GAE, muestra cifras mayores de sobrepeso/obesidad y las más bajas de desnutrición, los municipios de Pallatanga y Penipe que tienen un nivel inferior, presentan cifras más bajas de exceso de peso, pero que siguen siendo alarmantes, y son quienes acumulan mayores prevalencias de desnutrición.

6. Las cifras de parasitismo intestinal en los niños y niñas de España llama la atención por su elevada prevalencia, sobre todo si se comparan con estudios previos en poblaciones similares; donde, la especie *G. duodenalis* es la más prevalente, siendo un patógeno reconocido y pudiendo relacionarse con episodios de diarrea, tanto aguda como crónica, y con malnutrición por las intolerancias alimentarias y los síndromes de malabsorción asociados a la parasitosis.

7. Los parásitos intestinales en España están desatendidos y olvidados, se necesita mayor sensibilización dirigida a los profesionales de la pediatría para comprender los factores asociados y su posible impacto en la salud, desarrollo físico y cognitivo, de los niños y niñas.

8. Los resultados del análisis coproparasitológico en Ecuador evidencian que más del 60% de los niños y niñas participantes presentan helmintos y/o protistas. Las especies identificadas más prevalentes son: *G. duodenalis*, *Blastocystis* sp., *E. coli* y *E. vermicularis*. Los escolares de Pallatanga tienen más probabilidades de albergar helmintos como *E. vermicularis*, *H. nana*, *A. lumbricoides* y *T. trichiura*. Los de GAE están significativamente menos infectados por PG, incluidos *Blastocystis* sp., *E. coli*, *G. duodenalis* y miembros del complejo *Entamoeba*, que sus homólogos de Pallatanga y Penipe.

9. Los datos moleculares obtenidos en la población escolar de Ecuador incluyen la primera descripción en poblaciones humanas ecuatorianas de la diversidad genética de *E. bieneusi*, incluyendo un nuevo genotipo, y la presencia descrita por primera vez de *Blastocystis* ST4.

10. Los métodos moleculares aplicados al diagnóstico parasitológico han resultado ser poderosas herramientas complementarias a la microscopía, y esenciales para aumentar y completar los datos de prevalencia y, con ello, aproximarnos de una forma más real a la epidemiología de los parásitos intestinales, incluidas las fuentes de infección, las vías de transmisión, el potencial zoonótico y la diferenciación diagnóstica entre especies patógenas y comensales.

11. Los resultados hallados en la población ecuatoriana demuestran el fuerte vínculo existente entre la pobreza, la falta de higiene y el parasitismo intestinal. La administración masiva de albendazol por parte del Gobierno de Ecuador, diseñada para combatir los geohelmintos, resulta ser insuficiente para controlar eficazmente estos patógenos, y debe complementarse con intervenciones dirigidas a mejorar la calidad y las condiciones de vida de los hogares, el saneamiento y la educación sanitaria.

12. El primer paso que se debe dar antes de desarrollar un plan de intervención nutricional para la población pediátrica es establecer el diagnóstico correcto del estado actual del área relacionado con todos los determinantes de la malnutrición.

13. Es importante que la intervención a realizar identifique los recursos promotores de salud ajustados y adaptados a la realidad local.

14. El establecimiento de alianzas con centros locales, instituciones y asociaciones, públicas o privadas, permitirá mantener la sostenibilidad y generar autosuficiencia.

15. Existe una necesidad incuestionable para generar programas de intervención, por lo que el marco que proponemos, así como la matriz de planificación de intervención podrían ayudar en la solución de los problemas actuales de malnutrición escolar tanto "aquí" como "allí".

ANEXOS

ANEXO I. Tablas suplementarias del artículo de investigación: *Assessment of the Health Status of Spanish Schoolchildren Based on Nutrimetry, Lifestyle and Intestinal Parasites*.

Table S1. Socioeconomic and socio-demographic characteristics of the municipalities of the two participating public schools.

Variable	Municipality		Reference
	Paterna (La Cañada)	**Vila-Real**	
Country	Spain	Spain	[41,42]
Autonomous community	Valencian Community	Valencian Community	
Province	Valencia	Castellón	
Population in relation to the community (%)	1.41	1.01	
Children under 16 years (%)	19.27	16.69	
Older than 64 years (%)	14.68	17.05	
Illiterate and not educated (%)	8.24	10.79	
Foreigners (number of people)	7314	6791	
Activity rate (%)	71.72	65.66	
Average budget per inhabitant (euros/inhabitant)	891.69	1052.07	
Telephone lines (no. telephone lines x 100 inhabitants)	45.30	37.44	
Passenger cars (no. of passenger cars x 100 inhabitants)	51.80	52.51	
Average cadastral value (euros)	81,585.32	76,623.49	
Total number of enterprises	5969	3277	
Surface area of the municipality (hectares)	3585	5512	
Urban Area (hectares)	1211.39	1183.23	

Table S2. English version of the standardised epidemiological questionnaire used in this study.

Variable	**Category and State Code**
School sample	String variable (free text)
Sampling kit ID	String variable (free text)
Sampling date	Date variable
Country of birth	Sampling date
Place of residence	Sampling date
Weight	Decimal variable (number in kilograms)
Height	Decimal variable (number in centimetres)
Sex	Girl (1) Boy (2)
Age	Integer variable (number)
Number of relatives residing at home	Integer variable (number)
Source of drinking water	Bottled (1) Tap (2) Osmosis (3)
Hand and fruit/vegetable washing	Yes (1) No (2)
Contact with domestic animals	Yes (1) No (2)
Playing outdoors	Yes (1) No (2)
Get at least one hour of physical activity every day	Yes (1) No (2)
Screen time is less than two hours per day	Yes (1) No (2)

KidMed Questionnaire [16]:

Adherence to the MEDITERRANEAN DIET in childhood	**yes/no**
Have a piece of fruit or natural juice every day.	
Have a 2nd piece of fruit every day.	
Eat fresh (salads) or cooked vegetables regularly once +1 time a day.	
Eat fresh or cooked vegetables regularly more than +1 time per day.	
Eat fish regularly (at least 2-3 times a +1 week).	
Goes once or more per week to a fast food centre (hamburger joint).	
Likes pulses and eats them more than once a week.	
Eats pasta or rice almost every day (5 days or more per week).	
Eats a cereal or derivative (bread, etc.) for breakfast.	
Eats nuts and dried fruit regularly (at least 2-3 times a week).	
Olive oil is used at home.	
No breakfast.	

Have a dairy product for breakfast (yoghurt, milk, etc.).	
Eat industrial pastries, biscuits or cakes for breakfast.	
Eat 2 yoghurts and/or 40 g cheese every day.	
Eat sweets and/or candies several times a day.	

FFQ (the original Spanish version is provided) [45]:

Indicate with an X which corresponds to the consumption of the foods listed for the child:

	7	6	5	4	3	2	1	0
I. LÁCTEOS	**Más 1/día**	**1 vez/día**	**5-6/ semana**	**3-4/ semana**	**1-2/ semana**	**< 1/semana**	**< 1 vez/mes**	**Nunca**
Leche entera (1 vaso/taza)								
Leche semi o descremada (1 vaso)								
Leche condensada (1 cuharada)								
Café con leche (1 vaso)								
Café cortado con leche								
Yogur entero (1=125g)								
Yogur semi o desnatado (1=125g)								
Requesón/cuajada/queso fresco (100g)								
Queso cremoso/porciones (50g)								
Queso curado tipo manchego (50g)								
Flan (uno)								
Natillas o similares (uno)								
Horchata (1 vaso)								
Batido de soja (1 vaso)								

	7	6	5	4	3	2	1	0
II. DULCES Y BOLLERÍA	**Más 1/día**	**1 vez/día**	**5-6/ semana**	**3-4/ semana**	**1-2/ semana**	**< 1/semana**	**< 1 vez/mes**	**Nunca**
Galletas tipo María (1 galleta)								
Galletas de chocolate (1 galleta)								
Ensaimada/croissant (uno)								
Donuts (uno)								
Magdalenas/bizcochos (uno)								
Tartas/pastelillos (1 ración)								
Chocolate con leche (1 trozo)								
Chocolate puro (1 trozo)								
Chocolate en polvo (una cucharada desayuno)								

	7	6	5	4	3	2	1	0
III. PAN Y CEREALES	**Más 1/día**	**1 vez/día**	**5-6/ semana**	**3-4/ semana**	**1-2/ semana**	**< 1/semana**	**< 1 vez/mes**	**Nunca**
Pan blanco (media barra. 125g)								
Pan integral (media barra 125g)								
Pan de molde (1 rebanada)								
Rosquilletas (3-4 unidades)								
Macarrones/espagueti (1 plato)								
Paella (1 plato)								
Arroz, no paella (1 plato)								
Sopa fideos/letras (1 plato)								

	7	6	5	4	3	2	1	0
IV. HUEVOS, CARNES, PESCADOS	**Más 1/día**	**1 vez/día**	**5-6/ semana**	**3-4/ semana**	**1-2/ semana**	**< 1/semana**	**< 1 vez/mes**	**Nunca**
Un huevo hervido								
Un huevo frito								
Un huevo en tortilla								
Una ración pollo plancha								
Una ración pollo frito								
Un filete ternera plancha								
Un filete ternera frito o guisado								
Carne de cordero								
Una ración carne cerdo (lomo, solomillo…)								
Una ración carne de conejo (130 g)								
Una ración hígado de cordero, cerdo, ternera								
Una ración de vísceras (callos, sesos…)								
Una hamburguesa de pollo/pavo								
Una hamburguesa ternera								
Una loncha de jamón serrano (25 g)								
Una loncha de jamón de york (25 g)								
Una ración de salchichón o chorizo								
Una salchicha/longaniza fresca								
Una morcilla								
Una ración de tocino,/bacon								
Foie-gras o patés (una ración)								
Calamares/sepia a la romana (1 ración)								
Un plato de pescado frito								
Un plato de pescado plancha o hervido								
Una lata de atún en conserva								
Una lata de sardinas en conserva								
Pescados en salazón o conserva								
Una ración de almejas o mejillones								
Una ración de marisco (gambas, cigalas...)								

	7	6	5	4	3	2	1	0
V. VERDURAS Y PATATAS	**Más 1/día**	**1 vez/día**	**5-6/ semana**	**3-4/ semana**	**1-2/ semana**	**< 1/semana**	**< 1 vez/mes**	**Nunca**
Lechuga/escarola en ensalada (125g)								
Un tomate solo o ensalada								
Zanahorias en ensalada								
Un plato de zanahorias guisadas								
Una cebolla en ensalada o asada								
Una patata hervida o asada								
Una ración de patatas fritas								
Una berenjena								
Un calabacín								
Un pimiento								
Plato de alcachofas/espinacas/acelgas								
Un plato de judias verdes								
Una ración de esparrágos								
Una ración de setas o champiñones								
Un plato de guisantes cocinados								
Col, coliflor o brécoles								
Un pepino								

	7	6	5	4	3	2	1	0
VI. LEGUMBRES	**Más 1/día**	**1 vez/día**	**5-6/ semana**	**3-4/ semana**	**1-2/ semana**	**< 1/semana**	**< 1 vez/mes**	**Nunca**
Un plato de lentejas								
Un plato de garbanzos								
Un plato de judias blancas								

	7	6	5	4	3	2	1	0
VII. FRUTAS Y FRUTOS SECOS	**Más 1/día**	**1 vez/día**	**5-6/ semana**	**3-4/ semana**	**1-2/ semana**	**< 1/semana**	**< 1 vez/mes**	**Nunca**
Una naranja o mandarina								
Un plátano								
Una manzana								
Una pera								
Un melocotón o similares								
Una ración de fresas								
Una ración de cerezas								
Un Kiwi								
Sandia o melón (1 tajada)								
Una ración de piña natural								
Melocotón o piña en almibar								
Una ración de aceitunas								
Una ración de almendras o avellanas								
Una ración de cacahuetes								
Una ración de nueces								

	7	6	5	4	3	2	1	0
VIII. ACEITES Y GRASAS	**Más 1/día**	**1 vez/día**	**5-6/ semana**	**3-4/ semana**	**1-2/ semana**	**< 1/semana**	**< 1 vez/mes**	**Nunca**
Aceite de oliva virgen extra (1 cucharada)								
Aceite de oliva refinado (1 cucharada)								
Aceites de girasol (1 cucharada)								
Aceite maíz (1 cucharada)								
Otros aceites vegetales (1cucharada)								
Mantequilla (1 cucharada o untada)								
Margarina (1 cucharada o untada)								
Manteca de cerdo (1 cucharada/untada)								

	7	6	5	4	3	2	1	0
IX. BEBIDAS Y REFRESCOS	**Más 1/día**	**1 vez/día**	**5-6/ semana**	**3-4/ semana**	**1-2/ semana**	**< 1/semana**	**< 1 vez/mes**	**Nunca**
Un vaso de zumo de naranja natural								
Un vaso de zumo de frutas envasado								
Un vaso de coca-cola								
Un vaso de gaseosa/fanta								
Un vaso de tónica o bitter								
Un vaso de vino blanco								
Un vaso de vino tinto o rosado								
Cerveza (1 caña)								
Café solo sin azucar (1 taza)								
Café con azucar/edulcorante (1 taza)								
Té u otras infusiones sin azucar								
Té u otras infusiones con azucar								

	7	6	5	4	3	2	1	0
X. OTROS ALIMENTOS	**Más 1/día**	**1 vez/día**	**5-6/ semana**	**3-4/ semana**	**1-2/ semana**	**< 1/semana**	**< 1 vez/mes**	**Nunca**
Sopas y cremas de sobre (1 plato)								
Palitos o delicias de pescado (1)								
Croquetas (1)								
Palomitas de maíz (1 paquete)								
Una bolsa de pipas de girasol								
Mayonesa (1 cucharada)								
Salsa de tomate (1 cucharada)								
Pizza								
Sal (una pizca a la comida)								
Azucar añadido (1 cucharada)								
Mermelada/miel (1 cucharada)								

Table S3. Oligonucleotides used for the molecular identification and/or characterization of the intestinal protists parasites investigated in the present study.

Target organism	Locus	Oligonucleotide	Sequence (5′–3′)	Reference
Giardia duodenalis	*ssu* rRNA	Probe	FAM–CCCGCGGCGGTCCCTGCTAG–BHQ1	[46]
		Gd-80F	GACGGCTCAGGACAACGGTT	[46]
		Gd-127R	TTGCCAGCGGTGTCCG	[46]
***Blastocystis* sp.**	*ssu* rRNA	BhRDr	GAGCTTTTTAACTGCAACAACG	[48]
		RD5	ATCTGGTTGATCCTGCCAGT	[48]
***Cryptosporidium* spp.**	*ssu* rRNA	CR-P1	CAGGGAGGTAGTGACAAGAA	[48]
		CR-P2	TCAGCCTTGCGACCATACTC	[49]
		CR-P3	ATTGGAGGGCAAGTCTGGTG	[49]
		CPB-DIAGR	TAAGGTGCTGAAGGAGTAAGG	[49]

ssu rRNA: Small subunit ribosomal RNA.

ANEXO II. Tablas suplementarias del artículo de investigación: *Prevalence and associated risk factors of intestinal parasites among schoolchildren in Ecuador, with emphasis on the molecular diversity of Giardia duodenalis, Blastocystis sp. and Enterocytozoon bieneusi.*

Table S1. Occurrence of parasitic intestinal helminths in human populations, Ecuador, 2002-2022.

Population	Province	Detection method	Samples (*n*)	Parasite species	Infection rate (%)	References
Asymptomatic children	Chimborazo	CM	203	*Ascaris lumbricoides*	35.5	[1]
				Hymenolepis nana	11.3	
				Hymenolepis diminuta	1.0	
				Strongyloides stercoralis	0.7	
				Trichuris trichiura	0.5	
Asymptomatic children	Esmeraldas	CM, qPCR	400	*Ascaris lumbricoides*	7.0	[2]
				Trichuris trichiura	3.0	
				Strongyloides stercoralis	0.8	
				Ancylostoma duodenale	0.5	
Asymptomatic children	Esmeraldas	ELISA	39	*Ascaris lumbricoides*	17.9	[3]
Asymptomatic children	Manabí	CM	112	*Ascaris lumbricoides*	9.8	[4]
				Hymenolepis nana	3.3	
Asymptomatic children	Pichincha	CM	244	*Ascaris lumbricoides*	39.7	[5]
				Trichuris trichiura	19.7	
Asymptomatic (all age groups)	St. Domingo de los Tsáchilas	CM	586	*Ascaris lumbricoides*	29.4	[6]
				Trichuris trichiura	11.4	
				Hookworm	1.0	
				Amphimerus sp.	0.5	
				Enterobius vermicularis	0.5	
				Hymenolepis	0.2	
				Paragonimus westermani	0.3	
				Strongyloides stercoralis	0.5	
Rural dwellers	Loja	CM	674	*Hymenolepis nana*	4.3	[7]
				Strongyloides stercoralis	4.3	
				Trichuris trichiura	4.3	
Rural and urban dwellers	Esmeraldas, Pichincha	CM, PCR	106	*Ascaris lumbricoides*	28.9–45.2	[8]
				Trichuris trichiura	10.9–24.5	
				Enterobius vermicularis	10.6	
				Strongyloides stercoralis	3.8–8.2	
				Hymenolepis nana	6.3	
				Taenia spp.	2.0	

CM: Conventional microscopy; ELISA: Enzyme-Linked Immunosorbent Assay; PCR: Polymerase Chain Reaction; qPCR: real-time PCR.

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Table S2. Occurrence and molecular diversity of parasitic intestinal protists in human populations, Ecuador, 2002-2022.

Population	Province	Detection method	Samples (*n*)	Parasite species	Infection rate (%)	Genotype (*n*)	References
Symptomatic children	Azuay	CM	42	*Giardia duodenalis*	33.3	ND	[1]
				Cryptosporidium spp.	14.3	ND	
Symptomatic and asymptomatic children	Esmeraldas	ELISA, PCR-RLFP	592	*Giardia duodenalis*	26.0	AI (3), AII (19), BIII (26), BIV (16), AII+BIII (5)	[2]
HIV+ patients with diarrhoea	Guayas	CM, DFA, SEM	89	Microsporidia	25.0	ND	[3]
Asymptomatic children	Chimborazo	CM	203	*Entamoeba* complex[a]	57.1	ND	[4]
				Entamoeba coli	34.0	ND	
				Giardia duodenalis	21.1	ND	
				Cryptosporidium spp.	8.9	ND	
				Chilomastix mesnili	1.7	ND	
Asymptomatic children	Esmeraldas	CM, qPCR	400	*Giardia duodenalis*	31.5	ND	[5]
				Cryptosporidium spp.	5.3	ND	
				Entamoeba. histolytica	1.0	ND	
Asymptomatic children	Esmeraldas	ELISA	39	*Giardia duodenalis*	28.2	ND	[6]
Asymptomatic children	Manabí	CM	112	*Entamoeba coli*	36.0	ND	[7]
				Entamoeba complex[a]	34.4	ND	
				Giardia duodenalis	16.4	ND	
Asymptomatic children	Pichincha	CM	244	*Giardia duodenalis*	39.7	ND	[8]
				Entamoeba complex[a]	18.5	ND	
Asymptomatic children	Pichincha	ELISA	64	*Giardia duodenalis*	34.4	ND	[9]
				Cryptosporidium spp.	3.1	ND	
Asymptomatic children	Pichincha	ELISA, PCR	316	*Giardia duodenalis*	20.0	A (6), B (2), C (2)	[10]
Asymptomatic (all age groups)	Azuay	CM	335	NS[b]	46.2	ND	[11]
Asymptomatic (all age groups)	Esmeraldas, Manabí	PCR-SSCP	55	*Blastocystis sp.*	81.5	ST1 (21), ST2 (10), ST3 (12), ST1+ST3 (1), ST1+ST2 (2)	[12]
Asymptomatic (all age groups)		CM	586	*Entamoeba coli*	27.5	ND	[13]
				Blastocystis sp.	19.6		

	St. Domingo de los Tsáchilas			*Entamoeba* complex[a]	12.5	ND	
				Entamoeba hartmanni	10.8		
				Endolimax nana	4.9		
				Giardia duodenalis	3.9	ND	
				Iodamoeba butschlii	3.6		
				Chilomastix mesnili	1.4	ND	
Asymptomatic (all age groups)	St. Domingo de los Tsáchilas	ELISA	306	*Giardia duodenalis*	20.3	ND	[14]
				Cryptosporidium spp.	4.3	ND	
Rural dwellers	Loja	CM, PCR-RLHB[c]	674	*Endolimax nana*	47.0		[15]
				Entamoeba coli	28.0	ND	
				Entamoeba complex[a]	16.2	ND	
				Chilomastix mesnili	<4.3	ND	
				Giardia duodenalis	<4.3	ND	
				Iodamoeba bütschlii	<4.3	ND	
Rural and urban dwellers	Esmeraldas, Pichincha	CM, PCR[d]	106	*Entamoeba dispar*	69.8	ND	[16]
				Entamoeba coli	60.4	ND	
				Giardia duodenalis	10.4	ND	
				Blastocystis sp.	6.6	ND	
				Enbadomonas duodenalis	6.6	ND	
				Enteromonas duodenalis	5.6	ND	
				Iodamoeba butschlii	5.6	ND	
				Endolimax nana	3.8	ND	
				Entamoeba histolytica	2.8	ND	

CM: Conventional microscopy; DFA: Direct Fluorescent Antibody assay; ELISA: Enzyme-Linked Immunosorbent Assay; ND: Not Determined; NS: Not Specified; PCR: Polymerase Chain Reaction; PCR-RFLP: Polymerase Chain Reaction-restriction Fragment Length Polymorphism; PCR-SSCP: Polymerase Chain Reaction-Single Strand Conformation Polymorphism, PCR-RLHB: Polymerase Chain Reaction-Reverse Line Hybridization Blot; SEM: Scanning Electron Microscopy.

[a] *Entamoeba* complex: *Entamoeba histolytica*/*Entamoeba dispar*/*Entamoeba moshkovskii*/ *E. bangladeshi*

[b] Study reporting general infection rates by any intestinal parasite.

[c] Only for the differential detection of members of the *Entamoeba* complex.

[d] Only for the differential detection of *E. histolytica* and *E. dispar*.

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Table S3. English version of the standardized epidemiological questionnaire employed.

Variable	**Category and State code**
School sample	String variable (free text)
Sampling kit ID	String variable (free text)
Sampling date	Date variable
Country of birth	Sampling date
Municipality of residence	Sampling date
Gender	Woman (1) Men (2)
Age	Integer variable (number)
Number of siblings	Integer variable (number)
Number of relatives residing at home	Integer variable (number)
Consumption of unsafe water	Yes (1) No (2)
Hand and fruit/vegetable washing	Yes (1) No (2)
Contact with domestic animals	Yes (1) No (2)
Playing outdoors	Yes (1) No (2)
Diarrhoea	Yes (1) No (2)
Constipation	Yes (1) No (2)
Abdominal pain	Yes (1) No (2)
Vomiting	Yes (1) No (2)
Weight loss	Yes (1) No (2)
Allergic manifestations	Yes (1) No (2)
Teeth grinding	Yes (1) No (2)
Anal itching	Yes (1) No (2)
Abdominal distention	Yes (1) No (2)
Flatulence	Yes (1) No (2)

Table S4. Oligonucleotides used for the molecular identification and/or characterization of the parasitic intestinal protist.

Target organism	Locus	Oligonucleotide	Sequence (5´–3´)	Reference
Giardia duodenalis	*ssu* rRNA	Probe	FAM–CCCGCGGCGGTCCCTGCTAG–BHQ1	[1]
		Gd-80F	GACGGCTCAGGACAACGGTT	[1]
		Gd-127R	TTGCCAGCGGTGTCCG	[1]
	gdh	GDHeF	TCAACGTYAAYCGYGGYTTCCGT	[2]
		GDHiF	CAGTACACCTCYGCTCTCGG	[2]
		GDHiR	GTTRTCCTTGCACATCTCC	[2]
	bg	G7_F	AAGCCCGACGACCTCACCCGCAGTGC	[3]
		G759_R	GAGGCCGCCCTGGATCTTCGAGACGAC	[3]
		G99_F	GAACGAACGAGATCGAGGTCCG	[3]
		G609_R	CTCGACGAGCTTCGTGTT	[3]
	tpi	AL3543	AAATIATGCCTGCTCGTCG	[4]
		AL3546	CAAACCTTITCCGCAAACC	[4]
		AL3544	CCCTTCATCGGIGGTAACTT	[4]
		AL3545	GTGGCCACCACICCCGTGCC	[4]
Entamoeba histolytica	*ssu* rRNA	Probe	FAM–TCATTGAATGAATTGGCCATTT–MGB	[5]
Entamoeba dispar		Probe	VIC–TTACTTACATAAATTGGCCACTTTG–MGB	[5]
Entamoeba histolytica/dispar		Ehd-239F	ATTGTCGTGGCATCCTAACTCA	[6]
		Ehd-88R	GCGGACGGCTCATTATAACA	[6]
Cryptosporidium spp.	*ssu* rRNA	CR-P1	CAGGGAGGTAGTGACAAGAA	[7]
		CR-P2	TCAGCCTTGCGACCATACTC	[7]
		CR-P3	ATTGGAGGGCAAGTCTGGTG	[7]
		CPB-DIAGR	TAAGGTGCTGAAGGAGTAAGG	[7]
Blastocystis sp.	*ssu* rRNA	BhRDr	GAGCTTTTTAACTGCAACAACG	[8]
		RD5	ATCTGGTTGATCCTGCCAGT	[8]
Enterocytozoon bieneusi	ITS	EBITS3	GGTCATAGGGATGAAGAG	[9]
		EBITS4	TTCGAGTTCTTTCGCGCTC	[9]
		EBITS1	GCTCTGAATATCTATGGCT	[9]
		EBITS2.4	ATCGCCGACGGATCCAAGTG	[9]

bg: β-giardin (bg); *gdh*: Glutamate dehydrogenase; ITS: Internal Transcribed Spacer; *ssu* rRNA: Small subunit ribosomal RNA; *tpi*: Triose Phosphate Isomerase. [1]Available in the Reference section of the main body of the manuscript.

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Table S5. PCR cycling conditions used for the molecular identification and/or characterization of the parasitic intestinal protist.

		Temperature and time						
Target organism	**Locus**	**Initial denaturation**	**Denaturation**	**Annealing**	**Extension**	**No. cycles**	**Final extension**	**Reference**
Giardia duodenalis	*ssu* rRNA	95°C 15 min	95°C 15 s	60°C 1 min	72°C 30 s	45	–	[1]
	gdh	95°C 3 min	95°C 30 s	55°C 30 s	72°C 1 min	35	72°C 7 min	[2]
	bg	95°C 7 min	95°C 30 s	65/55°C 30 s	72°C 1 min	35	72°C 7 min	[3]
	tpi	94°C 5 min	94°C 45 s	50°C 45 s	72°C 1 min	35	72°C 10 min	[4]
Entamoeba histolytica	*ssu* rRNA	95°C 15 min	95°C 15 s	60°C 1 min	72°C 30 s	45	–	[5]
Cryptosporidium spp.	*ssu* rRNA	94°C 3 min	94°C 40 s	50°C 40 s	72°C 1 min	35	72°C 10 min	[6]
Blastocystis sp.	*ssu* rRNA	95°C 3 min	94°C 1 min	59°C 1 min	72°C 1 min	30	72°C 2 min	[7]
Enterocytozoon bieneusi	ITS	94°C 3 min	94°C 30 s	55/57°C 30 s	72°C 40 s	35	72°C 10 min	[8]

bg: β-giardin (bg); *gdh*: Glutamate dehydrogenase; ITS: Internal transcribed spacer; *gp60*: 60 kDa glycoprotein; *ssu* rRNA: Small subunit ribosomal RNA; *tpi*: Triose phosphate isomerase.

References

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5. Verweij JJ, Oostvogel F, Brienen EA, Nang-Beifubah A, Ziem J, Polderman AM. Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* in northern Ghana. Trop Med Int Health. 2003; 8(12): 1153–1156. doi: 10.1046/j.1360-2276.2003.01145.x PMID: 14641852.
6. Tiangtip R, Jongwutiwes S. Molecular analysis of *Cryptosporidium* species isolated from HIV-infected patients in Thailand. Trop Med Int Health. 2002; 7(4): 357–364. doi: 10.1046/j.1365-3156.2002.00855.x PMID: 11952952.
7. Scicluna SM, Tawari B, Clark CG. DNA barcoding of *Blastocystis*. Protist. 2006; 157(1): 77–85. doi: 10.1016/j.protis.2005.12.001 PMID: 16431158.

8. Buckholt MA, Lee JH, Tzipori S. Prevalence of *Enterocytozoon bieneusi* in swine: an 18-month survey at a slaughterhouse in Massachusetts. Appl Environ Microbiol. 2002; 68(5): 2595–2599. doi: 10.1128/AEM.68.5.2595-2599.2002.

Table S6. Multivariate logistic regression analysis for intestinal parasitism in the surveyed population according to the municipality of origin. Adjusted Odds Ratios (AORs) and 95% Confidential Intervals (95% CI) are indicated.

		All				GAE (*n* = 115)				Penipe (*n* = 96)				Pallatanga (*n* = 161)			
	Category	***n***	**AOR**	**95% CI**	***P*-value**	***n***	**AOR**	**95% CI**	***P*-value**	***n***	**AOR**	**95% CI**	***P*-value**	***n***	**AOR**	**95% CI**	***P*-value**
Gender	Female	200	0.891	0.534–1.486	0.657	64	0.567	0.245–1.314	0.186	50	0.921	0.208–4.076	0.913	86	1451	0.592–3.557	0.416
	Male	172	–	–	–	51	–	–	–	46	–	–	–	75	–	–	–
Age group (years)	3–4	55	0.681	0.324–1.431	0.311	23	0.738	0.214–2.550	0.631	16	0.297	0.032–2.753	0.286	16	0.444	0.122–1.623	0.220
	5–8	174	1.241	0.697–2.209	0.464	63	1101	0.412–2.943	0.848	45	2115	0.459–9.734	0.336	66	1536	0.581–4.053	0.388
	9–11	143	–	–	–	29	–	–	–	35	–	–	–	79	–	–	–
Contact with animals	Yes	290	1.125	0.611–2.071	0.706	87	1538	0.572–4.132	0.393	83	0.859	0.111–6.625	0.884	120	0.821	0.290–2.327	0.711
	No	82	–	–	–	28	–	–	–	13	–	–	–	41	–	–	–
Washing fresh produce	Yes	254	–	–	–	98	–	–	–	58	–	–	–	98	–	–	–
	No	118	0.921	0.482–1.761	0.804	17	0.721	0.193–2.696	0.627	38	0.499	0.074–3.371	0.476	63	0.936	0.352–2.492	0.895
Handwashing before eating	Yes	261	–	–	–	92	–	–	–	64	–	–	–	105	–	–	–
	No	111	1.919	0.944–3.904	0.072	23	1665	0.511–5.420	0.398	32	7473	0.607–92.06	0.116	56	2179	0.646–7.351	0.209
Handwashing after bathing	Yes	292	–	–	–	97	–	–	–	78	–	–	–	117	–	–	–
	No	80	0.568	0.276–1.170	0.125	18	0.428	0.116–1.583	0.203	18	0.54	0.005–0.541	0.013	44	0.684	0.208–2.247	0.531
Safe water	Yes	163	–	–	–	81	–	–	–	36	–	–	–	46	–	–	–
	No	209	1.093	0.621–1.924	0.758	34	1279	0.491–3.330	0.614	60	0.127	0.22–0.743	0.022	115	1971	0.766–5.071	0.159
Plays outdoors	Yes	160	1.027	0.606–1.742	0.921	41	0.617	0.260–1.466	0.274	50	4212	0.832–21.33	0.082	69	1638	0.623–4.310	0.317
	No	212	–	–	–	74	–	–	–	46	–	–	–	92	–	–	–
No. of siblings	0	64	–	–	–	24	–	–	–	10	–	–	–	30	–	–	–
	1–2	205	1.099	0.567–2.127	0.78	80	0.734	0.257–2.094	0.563	38	0.336	0.024–4.811	0.422	87	1669	0.552–5.049	0.364
No. of relatives at home	1	103	1.788	0.669–4.779	0.247	11	0.304	0.036–2.255	0.273	48	3704	0.233–58.90	0.354	44	3034	0.583–15.795	0.187
	2–4	280	–	–	–	101	–	–	–	56	–	–	–	126	–	–	–
	>5	92	1.393	0.615–3.158	0.427	14	22.634	1.778–288.1	0.016	40	0.360	0.073–1.779	0.210	38	0.587	0.151–2.286	0.443

ANEXO III. Tabla suplementaria del artículo de investigación: *Evaluation of School Children Nutritional Status in Ecuador Using Nutrimetry: A Proposal of an Education Protocol to Address the Determinants of Malnutrition*

Table S1. Intervention planning matrix with intervention objectives, indicators, sources of verification and activities focused primarily on the elementary school setting.

Intervention objectives	Indicators	Verification sources	Activities
Involve the municipality's health center and town hall, a university education center, parents' associations, teachers and school authorities to guarantee autonomy and sustainability.	At least two meetings per year with and among the managers/representatives of all sectors that are to be involved.	Attendance record according to the established schedule.	- Generate written agreements with the local authorities stating that they will collaborate in order to guarantee their participation. - Take measures to improve sanitation in the area by the municipality and ensure equitable access to drinking water. - Ensure health assistance by the health center when necessary, in addition to its involvement in the promotion of health in the area. - Include incentives for collaborating with the community in one or more subjects (extra points or credits) in the university center to motivate student participation, as well as offering final degree or master's degree projects related to the topic.
Train university students, teachers and school authorities for all anthropometric data collection activities, educational activities, monitoring, evaluation and problem detection.	At least 70% of students, teachers and authorities will be properly qualified for all collection, intervention, monitoring, evaluation and problem detection activities.	- Record of attendance to scheduled training sessions. - Difference in knowledge between the two tests performed (one prior to the start of the training and the other at the end).	- Provide informative talks and interactive workshops adapted to each population according to their level of knowledge. - Train the trainer of trainers by establishing a circle to ensure autonomy and sustainability.

Improve knowledge and attitudes regarding healthy lifestyle habits (food, hygiene and health, and physical exercise) of schoolchildren, parents, teachers and school authorities.	Knowledge of healthy lifestyle habits will improve by at least 50% in the first year of the intervention.	- Record of attendance to programmed activities. - Difference in knowledge between the three tests performed (one prior to the start of the intervention training, the second 6 months later, and the third 1 year after the first).	- Provide didactic and interactive talks and workshops adapted to each population group according to their age and educational level. - Include classes on healthy habits in at least one subject of the primary education curriculum. - Ensure that, in physical education, all students interact and that different activities and sports are included to ensure that they are motivated and that their needs adapted to. - Place advertising posters in strategic places within the schools. - Hand out diptychs and triptychs with relevant information. - Supervise the food served at school cafeterias. - Form school cooking groups, "Master chef", where students are taught how to prepare healthy meals with seasonal and local foods. - Mark walking trails within the schools so that students can walk them during recesses. - Invite a well-known athlete to the schools to share their experience. - Create ecological vegetable gardens in schools. - "Sports week": allocate a few curricular hours during the week to promote sports competitions among students, teachers, authorities and parents. -"Fair: Healthy Family Day": allocate one annual day where students, teachers, authorities and legal representatives prepare and share together, exhibitions and workshops are organized, and healthy activities are learned.

Reduce school malnutrition figures.	Malnutrition will decrease by at least 10% in the first year of the intervention.	Difference in prevalence percentages determined with Nutrimetry between the first intake (pre-intervention) and second intake of anthropometric values (post-intervention).	- Provide deworming/supplementation services for schoolchildren in need at the nearby health center. - Refer cases to specialized health personnel (in nearby medical center) that need specific interventions, and continue to follow them up. - Provide deworming services for teachers, authorities and parents who are in direct contact with the children if necessary. - Take at least two anthropometric measurements to verify the improvement of nutritional status per year at the population level and at the clinical level so that the improvement in the follow-up will be greater.

ANEXO IV. Documentos de aprobación de Comités de Ética en Investigación en Humanos, España (UV) y Ecuador (Universidad Central y Dirección Nacional de Investigación en Salud).

D. José María Montiel Company, Profesor Contratado Doctor Interino del departamento de Estomatología, y Secretario del Comité Ético de Investigación en Humanos de la Comisión de Ética en Investigación Experimental de la Universitat de València,

CERTIFICA:

Que el Comité Ético de Investigación en Humanos, en la reunión celebrada el día 1 de marzo de 2018, una vez estudiado el proyecto de investigación titulado:
"Análisis de factores de riesgo y manejo de la malnutrición en edad escolar. Protocolo de actuación "aquí" para extrapolar "allí"", número de procedimiento H1518738039128,
cuya responsable es Dña. Mónica María Gozalbo Monfort, ha acordado informar favorablemente el mismo dado que se respetan los principios fundamentales establecidos en la Declaración de Helsinki, en el Convenio del Consejo de Europa relativo a los derechos humanos y cumple los requisitos establecidos en la legislación española en el ámbito de la investigación biomédica, la protección de datos de carácter personal y la bioética.

Y para que conste, se firma el presente certificado en Valencia, a dos de marzo de dos mil dieciocho.

UNIVERSITAT DE VALÈNCIA

Av. Blasco Ibáñez, 13 tel: 9638 64109 vicerec.investigacio@uv.es
València 46010 fax: 9639 83221 www.uv.es/serinves

UNIVERSIDAD CENTRAL DEL ECUADOR

SUBCOMITÉ DE ÉTICA DE INVESTIGACIÓN EN SERES HUMANOS
Aprobado por MSP: Of. No.MSP-VGVS-2017-0955-O/21-11-2017

EL SUBCOMITÉ DE ÉTICA DE INVESTIGACIÓN EN SERES HUMANOS DE LA UNIVERSIDAD CENTRAL DEL ECUADOR SEISH - UCE

CERTIFICA:

Que, conoció el Protocolo de Investigación presentado por la **Dra. Gozalbo Monfort Mónica María,** de la Universidad de Valencia, código 001-UV-2019, con el tema:

Análisis de factores de riesgo y tratamiento de la malnutrición en edad escolar. Protocolo de actuación "Aquí" para extrapolar "Allí".

Una vez analizados los fundamentos metodológicos, bioéticos y jurídicos del mencionado estudio, el Subcomité certifica la **VIABILIDAD ÉTICA**.

Quito, 22 de enero de 2019

Dr. Fernando Salazar Manosalvas
PRESIDENTE

Dr. Patricio Pazán León
SECRETARIO

SUBCOMITÉ DE ÉTICA DE INVESTIGACIÓN EN SERES HUMANOS
UNIVERSIDAD CENTRAL DEL ECUADOR
SEISH

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Oficio Nro. MSP-DIS-2020-0219-O

Quito, D.M., 18 de mayo de 2020

Asunto: Respuesta a la solicitud de evaluación del protocolo MSPCURI000336-3: "Análisis de factores de riesgo y tratamiento de la malnutrición en edad escolar. Protocolo de actuación "Aquí" para extrapolar "Allí"

Doctora
Monica Maria Gozalbo Monfort
En su Despacho

De mi consideración:

En respuesta al oficio Nro. MSP-DNGA-SG-10-2020-2696-E, ingresado al Ministerio de Salud Pública, el 20 de febrero de 2020, en el que la Dra. Mónica María Gozalbo Monfort, en calidad de Investigadora principal, remite el protocolo del estudio observacional denominado: *«Análisis de factores de riesgo y tratamiento de la malnutrición en edad escolar. Protocolo de actuación "Aquí" para extrapolar "Allí"»,* codificado por la Dirección Nacional de Inteligencia de la Salud (DIS) como: MSPCURI000336-3; contando con la Carta de aprobación del protocolo por parte del Subcomité de Ética de Investigación en Seres Humanos reconocido por el MSP (Subcomité de Ética de Investigación en Seres Humanos de la Universidad Central del Ecuador-SEISH-UCE), cumpliendo los requisitos mínimos para la evaluación de la investigación y contando con los criterios favorables de la Dirección Nacional de Estrategias de Prevención y Control y de la Dirección Nacional de Promoción de la Salud del Ministerio de Salud Pública-MSP, se **APRUEBA** la versión adjunta del protocolo y se recomienda cumplir con las recomendaciones finales realizadas en el informe adjunto.

El presente estudio observacional de prevalencia transversal, de acuerdo a lo indicado, se desarrollará en las siguientes Unidades Educativas: Unidad Educativa San Juan de Bucay (Guayas), mismas que se encuentran bajo la dependencia del Ministerio de Educación, a quien ponemos en conocimiento para los fines pertinentes.

De acuerdo a lo indicado en el presente estudio el financiamiento será cubierto en su totalidad por fondos del grupo de investigación de la Universidad de Valencia y su patrocinadora es la Dra. Mónica María Gozalbo Monfort.

El presente proyecto no contempla el almacenamiento de las muestras biológicas humanas obtenidas en el mismo para futuras investigaciones. Por lo tanto, las muestras biológicas deberán ser destruidas/eliminadas una vez que culminen con los análisis referentes a la presente investigación.

Adicionalmente, se indica que el proyecto contempla la exportación de muestras biológicas humanas a España (Departamento de Parasitología de la Universidad de

Oficio Nro. MSP-DIS-2020-0219-O

Quito, D.M., 18 de mayo de 2020

Valencia), por lo tanto, previo a realizar este procedimiento, las investigadoras deberán obtener la aprobación de la Agencia Nacional de Regulación, Control y Vigilancia Sanitaria-ARCSA.

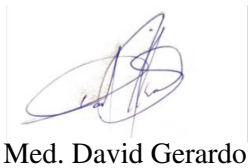
Le recordamos que una vez finalizada la investigación, es responsabilidad de la investigadora principal, enviar a esta Dirección, a la Dirección Nacional de Estrategias de Prevención y Control y a la Dirección Nacional de Promoción de la Salud del Ministerio de Salud Pública, los resultados de la misma; así como, las publicaciones que se realicen como producto de este estudio.

La Dirección Nacional de Inteligencia de la Salud, aprueba los protocolos de los estudios observacionales en el ámbito de sus competencias, en base a una revisión de calidad metodológica y ética de los estudios. Sin embargo, el contenido, la autoría y la responsabilidad sobre los resultados del estudio corresponden a la Patrocinadora - Investigadora Principal, exonerando al Ministerio de Salud Pública de cualquier acción legal que se derive por esta causa.

Cabe mencionar que si bien los resultados podrían contribuir a la salud pública, éstos no son de carácter vinculante para esta Cartera de Estado.

Me despido con sentimientos de distinguida consideración y alta estima.

Atentamente,



Med. David Gerardo Armas Ruiz
DIRECTOR NACIONAL DE INTELIGENCIA DE LA SALUD

Referencias:
- MSP-DNGA-SG-10-2020-2696-E

Anexos:
- 2696_0126036001582210295.pdf
- protocolo_336_1ra.pdf
- protocolo_336_2da.pdf
- protocolo_336_3ra.pdf
- protocolo_336_4ta.pdf
- protocolo_336_5ta.pdf
- protocolo_336_6ta.pdf
- protocolo_336_7ma.pdf
- protocolo_336_8va.pdf
- protocolo_336_9na.pdf
- informe_tècnico_2_mspcuri000336-3-1.pdf

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Quito, D.M., 18 de mayo de 2020

Copia:

Señor Magíster
Esteban Andres Moreno Flores
Director Nacional de Investigación Educativa
MINISTERIO DE EDUCACIÓN

Doctor
Fernando Marcelo Salazar Manosalvas

Señor Doctor
Fausto Guillermo Patiño Mosquera
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Señor Doctor
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Director Nacional de Estrategias de Prevención y Control

Señora Ingeniera
Gianina Lizeth Suárez Rodríguez
Especialista de Investigación y Análisis 1

Señora Magíster
Miriam del Rocío Obando Rodríguez
Analista

nh

VNIVERSITAT DE VALÈNCIA Facultat de Medicina i Odontologia

ANEXO V. INFORME DIRECTORAS PRODUCCIÓN CIENTÍFICA DE LA DOCTORANDA

Artículos científicos publicados en revistas indexadas de alta impacto:

1. Assessment of the Health Status of Spanish Schoolchildren Based on Nutrimetry, Lifestyle and Intestinal Parasites

- Autores: Estephany Tapia-Veloz, Marisa Guillén, María Trelis, Tannia Valeria Carpio-Arias, Mónica Gozalbo
- Revista de publicación: *Nutrients*
- Fecha de publicación: 19 Junio 2023
- Doi: https://doi.org/10.3390/nu15122801
- Factor de impacto de la revista: 5,9 NUTRITION & DIETETICS 17/88 **Q1, D2** (2022)

2. Prevalence and associated risk factors of intestinal parasites among schoolchildren in Ecuador, with emphasis on the molecular diversity of Giardia duodenalis, Blastocystis sp. and Enterocytozoon bieneusi

- Autores: Estephany Tapia-Veloz, Mónica Gozalbo, Marisa Guillén, Alejandro Dashti, Begoña Bailo, Pamela C Köster, Mónica Santín, David Carmena y María Trelis
- Revista de publicación: *PLoS Neglected Tropical Diseases*
- Fecha de publicación: 24 Mayo 2023
- Doi: https://doi.org/10.1371/journal.pntd.0011339
- Factor de impacto de la revista: 3,8 TROPICAL MEDICINE 2/24 **Q1, D1** (2022)

3. Evaluation of School Children Nutritional Status in Ecuador Using Nutrimetry: A Proposal of an Education Protocol to Address the Determinants of Malnutrition
   - Autores: Estephany Tapia-Veloz, Mónica Gozalbo, Gabriela Tapia-Veloz, Tannia Valeria Carpio-Arias, María Trelis y Marisa Guillén
   - Revista de publicación: *Nutrients*
   - Fecha de publicación: 6 Septiembre 2022
   - Doi: https://doi.org/10.3390/nu14183686
   - Factor de impacto de la revista: 5,9 NUTRITION & DIETETICS 17/88 Q1, D2 (2022)

e D/Dña. **ESTEPHANY TAPIA VELOZ,** estudiante del Programa de Doctorado **3139 Medicina** (RD99/2011) en Medicina de la Universitat de València, ha publicado sus tres artículos de la **TESIS POR COMPENDIO** en revistas de alto impacto e indexadas en JCR.

Fecha: Valencia, a 27 de julio de 2023

MONICA MARIA\| GOZALBO\| MONFORT
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Fdo. Mónica Gozalbo

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Fdo. Marisa Guillén

MARIA TRELIS VILLANUEVA
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Fdo. María Trelis

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