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Caracterización epidemiológica de la infección por parásitos intestinales en poblaciones indígenas de la Provincia de Misiones y de la Provincia de Salta (Región del Norte Grande Argentino, Argentina)

TESIS DOCTORAL

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Las abajo firmantes, Dra. Maria Victoria Periago Roses, directora de la Tesis Doctoral, y Dra. Carla Muñoz Antoli-Candela, directora y tutora de la Tesis Doctoral del Doctorando **Ernesto Candela Sentí**, por la presente **informan favorablemente** de la Tesis Doctoral titulada *"Caracterización epidemiológica de la infección por parásitos intestinales en poblaciones indígenas de la provincia de Misiones y de la provincia de Salta (región del norte grande argentino, Argentina)"*, y autorizan el depósito de dicha Tesis Doctoral presentada por compendio de publicaciones con opción a mención internacional.

Y sin otro particular, se firma la presente en Burjassot-Valencia, a trece de junio de dos mil veinticuatro.

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"Perquè hi haurà un dia en que no podrem més llavors ho podrem tot"

Vicent Andrés Estellés

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ABSTRACT

Intestinal parasites (IPs) are widely distributed worldwide and are one of the major contributors to gastrointestinal disease. Their prevalence is associated with poor access to water, sanitation and hygiene (WASH). The objective of this study was to identify the prevalence of IPs, including soil-transmitted helminths (STH), and their relation to socioeconomic characteristics, as well as a first approach to molecularly characterize the types of *Giardia intestinalis*, *Blastocystis* sp. and *Entamoeba histolytica* present in an indigenous community from Puerto Iguazú, Misiones, Argentina and Salta province. A cross-sectional study was conducted in the rural settlement of Fortin Mbororé between January and March 2018. Socioeconomic variables, household characteristics, and stool and blood samples were collected. Standard coprological techniques were used to analyze stool samples, and a complete hemogram was performed on the blood samples. Moreover, blood samples were analyzed for the presence of specific antibodies to the STH *Strongyloides stercoralis*. *Giardia intestinalis* microscopy-positive samples were genetically typed by the β-giardin (bg) gene. Molecular identification of *Blastocystis* sp. subtypes and *E. histolytica* were carried out by amplification and sequencing of a partial fragment of the small subunit ribosomal RNA gene (SSU rDNA).

The overall prevalence of IPs was 92.7%, with 72.0% specifically for hookworm. IPs were significantly more prevalent in preschool- and school-age children ($P < 0.05$). No formal education ($P = 0.035$), the presence of unimproved floors ($P = 0.001$) and overcrowding ($P = 0.005$) were significantly associated with IP infection. Hookworm was associated with anemia ($P = 0.019$). Molecular characterization revealed the presence of *E. histolytica* sub-assemblages. AII (12.5%), AIII (87.5%) and BIV (100%); one case of sub-assemblage D for *G. intestinalis*; and the presence of subtypes ST1 (14.8%), ST2 (14.8%) and ST3 (70.4%) of *Blastocystis* sp. The prevalence of STHs varied across villages: 89.6% (43/48), in Mini-Marangatú, 80.8% (101/125) in Yriapú, and 68.5% (115/169) in Fortín Mbororé. Notably, there was a significant difference in hookworm infection among the villages (P=.02). The analysis highlighted the significant influence of specific environmental factors on STH presence and spatial distribution, particularly in relation to hookworm infection. Vegetation patterns represented by the Vegetation Heterogeneity Index, created ad hoc for this study, emerged as a critical factor, with 2

significant predictors related to it (P=.002 and P=.004) alongside impervious surface density with a significant predictor (P<.001). The multilinear regression model yielded a high F test score (F108=4.75, P<.001), indicating a strong fit (R2=0.5465). Furthermore, socioeconomic factors, including walking barefoot in houses with dirt floors and overcrowding, were significantly correlated with hookworm infection intensity (P<.001 and P=.001, respectively). We also used the multilinear regression model to calculate hookworm infection intensity (F110=21.15, P<.001; R2=0.4971).

Protozoans detected in this study are transmitted mainly through water contaminated with fecal matter, evidencing the need to improve the quality of water and sanitation for the inhabitants of Fortín Mbororé. Molecular characterization showed that domestic animals can be implicated in the zoonotic transmission of *G. intestinalis* and *Blastocystis sp*. to humans. A hyperendemic area for STH was found, with hookworm prevalence greater than 50%. Therefore, improvements in WASH as well as mass deworming programs need to be implemented in this area to control and decrease the prevalence of IPs in general and STH in particular. Villages with similar living conditions and environmental characteristics displayed varied STH prevalence and spatial distribution. Specific environmental factors, such as vegetation pattern and impervious surface density, played major roles in STH presence, demonstrating the crucial relationship between environmental factors and hookworm infection distribution. Moreover, our findings emphasize the significant influence of socioeconomic factors on hookworm infection intensity. By gaining insights into this complex interplay, our research contributes to a better understanding of STH transmission characteristics, thereby informing targeted public health interventions for effective control. Molecular characterization allowed us to evidence possible zoonotic or human-to-human transmission pathways for *G. intestinalis* or *Blastocystis sp*., while serology for *S. stercoralis* proved to be a useful screening tool for monitoring this parasite after treatment. In general, a decrease in the prevalence of STHs was observed in the area, from 60% to 2.9–20% for hookworms and from 51% to 1–9.3% for *S. stercoralis* four years after treatment, demonstrating the effectiveness and duration of anthelmintic treatment with these two drugs.

Keywords: instestinal parasites, soil-transmitted helminths, prevalence, environmental factors, NIE-ELISA, molecular study, Iguazú, Tartagal, Argentina.

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CAPÍTULO I:

INTRODUCCIÓN

Las infecciones por parásitos intestinales, que incluyen protozoos y helmintos, son uno de los mayores problemas de salud pública a nivel mundial, especialmente en los países en vías de desarrollo de las regiones tropicales y subtropicales y que suponen una pesada carga humana, social y económica, afectando a más de 3.500 millones de personas, sobre todo en las regiones tropicales y subtropicales (Hajare et al., 2021). La mayoría de estas infecciones parasitarias son cosmopolitas. El aumento de migraciones del campo a la ciudad ha supuesto un incremento de los puestos de distribución de comida. Algunos de estos establecimientos tienen unas condiciones sanitarias e higiénicas deficientes, lo que facilita la transmisión de estas enfermedades, especialmente a través de la manipulación de alimentos (Kamau et al., 2012). A pesar de que pueden afectar a cualquier grupo de edad o sexo, la población infantil sigue siendo la más afectada (Wördemann et al., 2006). Estos enteroparásitos se encuentran asociados a múltiples factores socioeconómicos, sociosanitarios o ambientales, que favorecen los continuos procesos de infección (Rivero et al., 2017). Además, la coexistencia de unas condiciones sanitarias deficientes, tales como la falta de acceso a agua potable y segura, la falta de un sistema adecuado de eliminación de excretas, deficientes prácticas higiénicas, etc., en poblaciones de escasos recursos económicos residentes en zonas rurales o periurbanas, como las poblaciones indígenas, con favorables condiciones medioambientales de temperatura y humedad, constituyen las condiciones ideales para el mantenimiento del ciclo biológico de los parásitos intestinales y, por tanto, el aumento de las posibilidades de infección. Estas infecciones, que muchas veces permanecen durante largos periodos de forma asintomática, a pesar de que su mortalidad es relativamente baja, su presencia supone un grave perjuicio para el desarrollo y crecimiento de estas poblaciones ya que su presencia puede causar múltiples desórdenes de salud como anemia, alteraciones nutricionales, malnutrición, deterioro cognitivo, incluso negativo impacto psicomotor y del lenguaje o disminución de la capacidad de trabajo o complicaciones en el crecimiento y desarrollo de la población infantil (Fauziah et al., 2022). Además, y dado que la morbilidad se encuentra asociada a la carga parasitaria, a consecuencia de estas infecciones, tanto en la forma de costes directos como indirectos, pueden reducir el tiempo disponible para actividades productivas, por lo que estas enfermedades parasitarias contribuyen a las desigualdades que existen tanto dentro de las sociedades como entre ellas (Evans & Jamison, 1994).

Entre los protozoos intestinales más prevalentes en el mundo, y los agentes etiológicos de la giardiasis, amebiasis o la criptosporidiosis, se encuentra la *Giardia intestinalis*, *E. histolytica*, *Cryptosporidium* spp, respectivamente, los cuales constituyen en su conjunto los mayores causantes de cuadros diarreicos en humanos (Squire & Ryan, 2017). En el caso de los helmintos, *Ascaris lumbricoides*, los Ancylostomátidos (*Ancylostoma duodenale* y *Necator americanus*) o *Trichuris trichiura* (Ahmed, 2023), junto con *Strongyloides stercoralis*, constituyen el grupo de los geohelmintos, un grupo de parásitos intestinales que causan infecciones humanas por el contacto con huevos o larvas que prosperan en el suelo, que afectan a más de 1.500 millones de personas, y se consideran infecciones frecuentes en el mundo (World Health Organization, 2022). En este listado, cabría añadir el microorganismo potencialmente patógeno *Blatocystis* sp. por su alta prevalencia.

De entre los protozoos, cabe hablar de *G. intestinalis*, una de las infecciones parasitarias más comunes y una de las causas más frecuentes de diarrea inducida por parásitos (AKC et al., 2019). Este enteroparásito, especialmente habitual en zonas con saneamiento deficiente y escaso tratamiento del agua, se transmite por vía directa fecal-oral, generalmente a través de agua o alimentos contaminados. Este parásito tiene dos fases en su ciclo vital: trofozoítos y quistes infecciosos. Los trofozoítos se desplazan mediante flagelos y se adhieren al epitelio intestinal, e inducen daños estructurales en la célula apical, comprometiendo las uniones celulares y aumentando así la permeabilidad de la barrera intestinal. Estos cambios estructurales pueden llegar a ocasionar dolor abdominal, malabsorción, anorexia o pérdida de peso. Si bien esta sintomatología suele remitir en algunas semanas, su presencia se ha asociado a secuelas postinfecciosas a largo plazo, como trastornos gastrointestinales funcionales, artrosis o retraso del crecimiento (Halliez & Buret, 2013). Hasta la fecha, se ha descrito *G. intestinalis* como un complejo zoonótico formado por 8 genotipos (assemblages) diferentes de especies causantes de giardiasis (A-H). Entre ellos, A y B se han asociado a enfermedades en humanos y mamíferos; mientras, los genotipos C y D se observan en perros; E en rumiantes; F en gatos; G en roedores; y, H en animales mamíferos. Aunque se piensa que los genotipos C-H son específicos del hospedador (Zajaczkowski et al., 2021), varios estudios han demostrado que los perros pueden desempeñar un papel importante en la transmisión de parásitos zoonóticos, ya que son hospedadores definitivos de varios protozoos con potencial zoonótico (S. V. Soriano et al., 2010). El contacto entre perros y humanos que

habitan zonas rurales es especialmente estrecho y, más concretamente, en regiones donde la ganadería es de importancia económica (Pierangeli et al., 2007) por lo que la contaminación del área con heces de perros representa un alto riesgo de contraer enfermedades zoonóticas. Se ha reportado casos humanos de *G. intestinalis* con infecciones de genotipos específicos de otros animales (Traub et al., 2009), por lo que el abordaje molecular se presenta como una importante herramienta para determinar posibles patrones zoonóticos. A su vez, el assemblage A y B se subtipifican en AI (principalmente zoonótico), AII (principalmente antroponótico), AIII (ungulados), BIII y BIV. Aunque se ha observado que el assemblage B es más virulento y prevalente que el assemblage A, todavía no existe una correlación clara entre las infecciones y los síntomas con los assemblages de *G. intestinalis* (Mahdavi et al., 2022).

Blastocystis sp., otro de los microorganismos más frecuentes que se localiza en el intestino de los humanos, se transmite a través de la ruta fecal-oral y su transmisión puede darse también de humano a humano y de animal a humano. Estudios basados en la subunidad pequeña del ARN ribosómico (ARNr) han descrito una amplia diversidad molecular, con hasta 28 subtipos (ST) (Jiménez et al., 2022). Los subtipos ST 1-9 y ST12 se han observado en humanos y el resto, a excepción del ST9, han sido detectados en animales, como los primates (ST1 y ST8), monos (ST2) o cerdos (ST5) (Maloney et al., 2019). El potencial patógeno sigue siendo objeto de debate; si bien la presencia de *Blastocystis* sp. se asocia a síntomas gastrointestinales, como diarrea, dolor abdominal, vómitos, o distensión abdominal entre otros, o manifestaciones cutáneas como urticaria (Wawrzyniak et al., 2013), la relación entre enfermedad y colonización sigue siendo incierta, y, a día de hoy, no se conoce exactamente el mecanismo de colonización y cómo afecta a su patogenicidad. Todo esto lleva a distintos autores a considerar a *Blastocystis* sp. como un verdadero patobionte (Muadica et al., 2020). La gran prevalencia de *Blastocystis* sp. en poliparasitismo acompañada de síntomas inespecíficos, no permite establecer asociaciones entre estas manifestaciones clínicas con su presencia, ni siquiera vincularlo a alguno de sus subtipos ribosómicos, aunque se intenta relacionar la sintomatología intestinal con la elevada carga presente en la población detectada.

Por último, de entre los parásitos entéricos con mayores prevalencias comentados anteriormente, se encuentra el género *Entamoeba*, un grupo de amebas endobióticas que colonizan especies humanas y animales. La persistencia de al menos siete especies de *Entamoeba* depende de su capacidad de colonizar el tracto intestinal (Guillén, 2023).

Aunque la mayoría de las infecciones en humanos están causadas por especies no patógenas, como *E. coli* o *E. hartmanni*, la única especie que puede ser virulenta y causar daños intestinales y extraintestinales es la especie *E. histolytica*, el agente causal de la amebiasis. Si bien la mayoría de sus infecciones cursan de manera asintomática, cerca del 10% de las personas infectadas desarrollan la amebiasis invasiva, que puede cursar con diarreas sanguinolentas y mucosidad, abscesos hepáticos o colitis amebiana entre otras complicaciones, ocasionando hasta 10.000 muertes anualmente (Chou & Austin, 2023). De hecho, se considera la tercera causa más común de muerte después de la malaria y la esquistosomiasis (Ouattara et al., 2010). Esta enfermedad infecciosa, aunque con una amplia distribución mundial y con una elevada prevalencia en países de bajos niveles socioeconómicos, también se encuentra en países industrializados, debido principalmente al retorno de viajeros o emigrantes de países endémicos (Shirley et al., 2018). Habitualmente, las infecciones amebianas, se han diagnosticado mediante la microscopia óptica, a través de la identificación de quistes o trofozoítos. Sin embargo, este tipo de metodología no permite diferenciar entre las distintas especies, como *E. histolytica*, *E dispar* o *E. moshkovskii*, la cuales son morfológicamente idénticas. Además, la presencia de otros protozoos puede dificultar su identificación (Hamzah et al., 2006). Los métodos moleculares existentes, así como la serología, gracias a su elevada especificidad, permiten diferenciar *E. histolytica* de *E. dispar* (Hamzah et al., 2006).

Además de estas especies patógenas, que pueden causar evidentes enfermedades en la población infectada, existen otros protozoos comensales que se encuentran en el intestino humano, como *Endolimax nana*, *Iodamoeba bütschlii*, o *Chilomastix mesnili*, entre otras, que, si bien no son causantes de enfermedad y/o sintomatología, siguen siendo importantes ya que su presencia puede servir como marcador biológico o índice de las condiciones sanitarias y de salud de una zona determinada, dado que su transmisión de forma directa (ano-mano-boca) o indirecta (agua o alimentos contaminados) sigue la misma vía de transmisión que las otras especies patógenas.

Como se ha comentado anteriormente, los helmintos transmitidos por el suelo, los llamados geohelmintos o soil-transmitted helminths (STH), son un grupo de gusanos nematodos parásitos que causan infección humana a través del contacto con huevos o larvas de parásitos que prosperan en los suelos cálidos y húmedos de los países tropicales y subtropicales del mundo. Estos helmintos adultos, pueden vivir durante años en el tracto intestinal que, aunque no sean causantes de mortalidad, sí que desarrollan infecciones

crónicas que conllevan diferentes patologías. En el caso de *A. lumbricoides*, se ha referido abscesos hepáticos, malabsorción, obstrucción intestinal u obstrucción de las vías aéreas (en caso de hiperinfección). Por su parte, *T. trichiura* puede ocasionar diarrea mucosa crónica con prolapso rectal, o anemia. Y, en el caso los Ancylostomátidos, se desarrolla una anemia grave e hipoproteinemia, con la repercusión en el estado general de salud, así como hemorragias digestivas (Prieto-Pérez et al., 2016) que suponen una merma en el desarrollo económico del núcleo familiar, así como deficiencias en el crecimiento infantil (Bethony et al., 2006).

La especie *A. lumbricoides*, es la que ostenta las mayores prevalencias mundiales, afectando entre 807-1.221 millones de personas, seguido de *T. trichiura*, con 604-795 millones de personas, y de Ancylostomátidos, con 576-740 millones de personas. (Bethony et al., 2006). Dentro de este característico grupo, hay que señalar que, para la Organización Mundial de la Salud (OMS) y/o la Organización Panamericana de la Salud (OPS), las geohelmintiasis se reducían a estos tres agentes dejando, por lo tanto, fuera del estudio y del control a la especie *S. stercoralis*, que ha sido hasta ahora posiblemente la enfermedad tropical más desatendida. Recientemente, la OMS ha considerado incluirla, junto con el resto de los geohelmintos, dentro de las Enfermedades Tropicales Desatendidas (ETD) (World Health Organization, 2020a). Aunque se desconocen datos precisos sobre su prevalencia en los países endémicos debido a: la baja sensibilidad de los métodos de rutina estándares aplicados para su diagnóstico; al complejo ciclo biológico con alternancia de generaciones; la posibilidad de autoinfecciones; y, la emisión intermitente de larvas en la materia fecal, se estima que afecta entre 30 y 100 millones de personas (Olsen et al., 2009).

Estos geohelmintos, que se consideran en conjunto, porque es común que un solo individuo, que generalmente vive en un país menos desarrollado quede crónicamente infectado con varias de estas especies, podrían incrementar la susceptibilidad a otras importantes enfermedades como malaria, tuberculosis o VIH (Fincham et al., 2003; Le Hesran et al., 2004), y, a pesar de su elevada importancia en salud pública y que los grandes flujos migratorios han facilitado su extensión por todo el mundo, siguen perteneciendo al grupo de las enfermedades destendidas (Bethony et al., 2006), debido a múltiples factores, como: se ha subestimado su importancia ya que muchas son asintomáticas y tienen largos periodos de incubación (Fenwick, 2012); las zonas de alta endemicidad suelen estar aisladas, lo que dificulta su tratamiento y prevención; además,

afectan principalmente a los países más pobres en vías de desarrollo (Hotez et al., 2006); no son una prioridad para la industria farmacéutica ya que no los consideran rentables porque sus pacientes son demasiado pobres como para costearse su tratamiento (Yamey, 2002).

En los últimos años, la comunidad mundial ha comenzado a reconocer la importancia de la estrongiloidiasis. Sin embargo, la falta de un método de diagnóstico estándar ha supuesto un impedimento para la comparación de resultados entre investigaciones y la evaluación de proyectos piloto. Por ello, se acordó que las técnicas serológicas, cuya mejor opción es el kit NIE-ELISA por su disponibilidad comercial, son las recomendadas para su control y diagnóstico, acompañada siempre que sea posible del método Baermann o el cultivo en placa de agar (World Health Organization, 2020b). En este mismo aspecto de intervención, la OMS fomenta el desarrollo de biomarcadores y pruebas de diagnóstico rápido más sensibles y específicas para esta geohelmintiasis (World Health Organization, 2020b).

En este sentido, la OMS ha introducido tres estrategias de quimioterapia preventiva para el control de las STH. La primera, la administración masiva de medicamentos a toda la población de un área, recibiendo tratamiento antihelmíntico en intervalos regulares, independientemente del estado de infección. La segunda, la quimioterapia dirigida, sobre grupos de riesgo específicos de la población, definidos en función de la edad, el sexo u otras características sociales. Y, la quimioterapia selectiva, tras un cribado de un grupo poblacional que vive en una zona endémica y todos los individuos infectados (o sospechosos) reciben tratamiento antihelmíntico (World Health Organization, 2011)

Así pues, se recomienda aplicar quimioterapia preventiva a base de Albendazol (400mg) y Mebendazol (500mg) a los niños en edad preescolar (12-23 meses) y en edad escolar, como primera línea de prevención, una vez al año si la prevalencia inicial en la comunidad por infecciones con helmintos transmitidos por el suelo es superior al 20% y, dos veces al año, si esta prevalencia supera el 50% (World Health Organization, 2011). Esto hace que los monitoreos y los estudios de prevalencia epidemiológica sean de inestimable ayuda a la hora de aplicar y diseñar correctamente los programas de dichos tratamientos. Además, estas medidas tienen un efecto máximo disminuyendo la morbilidad por STH, ya que los niños en edad escolar constituyen un grupo de alto riesgo. Conjuntamente, se acompaña dichas campañas antihelmínticas con educación en salud y

en buenas prácticas higiénicas, para tratar de reducir la transmisión y la reinfección al promover comportamientos saludables.

Aunque se ha demostrado que el albendazol reduce de manera eficaz la prevalencia de geohelmintiasis, se han informado de reinfecciones después del tratamiento antihelmíntico (Jia et al., 2012). Por lo tanto, es poco probable que la terapia antihelmíntica por sí sola, sea capaz de erradicar estas enfermedades, lo que significa que se requieren estrategias de control más sostenibles a largo plazo. Además, la inviabilidad económica y operativa de cribar toda la población susceptible de padecer estas infecciones, limita la actuación y efectividad de estas desparasitaciones masivas. Como se ha comentado, la capacidad de las geohelmintiasis de sobrevivir fuera del hospedador está determinada por la variabilidad de las condiciones climáticas, como la lluvia o la temperatura, y ambientales, como la vegetación o la composición del suelo (Aw et al., 2021; Campbell et al., 2017). Con la información proporcionada por los sensores remotos y la identificación de los factores ambientales específicos que favorecen la distribución de las geohelmintiasis, a través del análisis geoestadístico basado en modelos bayesianos, se puede mapear y predecir la distribución espacial de estas geohelmintiasis, para guiar los programas de prevención y control (Tsheten et al., 2024). Sin embargo, hay que tener en cuenta las limitaciones que este tipo de predicciones, debido a la dificultad de interpretar estos mapas, su extrapolación temporal o la implementación de medidas de control epidemiológicas, basadas en datos históricos, que pueden cambiar rápidamente debido a las mejoras y esfuerzos previos de desparasitación (Pullan et al., 2011).

La provisión de saneamiento adecuado también es importante, aunque no es siempre posible en entornos con pocos recursos. La mayoría de los parásitos intestinales se transmiten por contaminación del ambiente y, en este aspecto, el agua y los alimentos juegan un papel importante. Si las heces no se eliminan de manera apropiada, los quistes, ooquistes y huevos de los parásitos pueden quedar en el ambiente de las casas o contaminar fuentes de agua o cultivos regados con aguas residuales.

En Argentina, estudios descriptivos han informado de prevalencias de parasitosis por encima del 80 % en algunas localidades del norte y sur del país (S. V. Soriano et al., 2005; Taranto et al., 2003), mientras que en la zona central se registran menores porcentajes cercanos tan solo al 45 % (Basualdo et al., 2007).

La población más afectada son los niños entre 0 y 14 años, y para este grupo, el

1,5 % de las enfermedades se deben a infecciones intestinales por nematodos, principalmente debido a la mala calidad del agua, el escaso o insuficiente saneamiento y la falta de higiene (World Health Organization, 2008). Además, las diarreas causadas por protozoos intestinales son motivo de una morbilidad y mortalidad significativas, principalmente en aquellas poblaciones desfavorecidas (Pierce & Kirkpatrick, 2009).

En los trabajos previos llevados a cabo en Argentina, la gran mayoría de los nematodos encontrados en las muestras fecales fueron geohelmintos. La endemicidad de estos parásitos en Argentina y sus diferentes prevalencias, ha siso observada especialmente en regiones del norte del país (Echazú et al., 2017; Vargas et al., 2017). Si nos centramos en estas regiones del norte del país, cabe destacar que se ha referido la presencia de *S. stercoralis* y *T. trichiura* tanto en Misiones (Gamboa et al., 2009; Navone et al., 2006) como en Salta (Menghi et al., 2007). Sin embargo, la especie *A. lumbricoides* tan solo ha sido detectada anteriormente en Misiones (Gamboa et al., 2009; Navone et al., 2006). Por último, comentar, que los Ancilostomátidos se encontraron también en Salta (Menghi et al., 2007) y en Misiones (Gamboa et al., 2009; Navone et al., 2006). Las diferencias observadas en cuanto a la distribución de las especies de geohelmintos, puede deberse a las condiciones de humedad y temperatura ambiental, así como al tipo de sustrato en el cual se desarrollan y transmiten los huevos y las larvas. Las larvas de Ancylostomátidos y *S. stercoralis*, por ejemplo, tienen una vida corta en el suelo, ya que generalmente no sobreviven más de un mes (Sorensen et al., 1994). Está demostrado que la humedad y la temperatura elevadas, unidas al hábito de defecar a cielo abierto y andar descalzo, favorecen la transmisión y dispersión de estos geohelmintos.

En Argentina, viene desarrollando su actividad la Fundación Mundo Sano. Se trata de una fundación que ha ido creciendo siempre con el lema de reducir el impacto de la salud desatendida. Sus principales y actuales ejes de actuación son 5: Chagas, control de enfermedades transmitidas por mosquitos; leishmaniasis; geohelmintiasis; e hidatidosis. Además, su misión es desarrollar modelos de gestión eficaces que sean replicables, escalables y transferibles; a través de alianzas público-privadas, sobre la base de la investigación científica multidisciplinaria aplicada en las comunidades afectadas. A través de la combinación de conocimientos en geomática espacial, se desarrollan mapas de riesgo y modelos predictivos sobre la dinámica de estas enfermedades, incluyendo las geohelmintiasis. Este enfoque se adecúa con las consideraciones prioritarias de la OMS de garantizar el acceso a los medicamentos y al tratamiento. En la actualidad, se

recomienda el empleo de indicadores de impacto en el diseño de programas, para que éstos queden bien establecidos. Además, lo aconsejable es considerar conjuntar enfoques intersectoriales, para obtener datos diversos de alta calidad, que permitan una toma de decisiones eficaz a todos los niveles.

Una de las Directoras de la presente Tesis Doctoral, desde su posición como Coordinadora de Proyectos Científicos de Mundo Sano en Argentina, ha puesto todo su empeño es desarrollar sistemas de vigilancia y sistemas de mapeo para controlar la distribución y adherencia a tratamientos y monitorear posibles resistencias a fármacos. Para ello, se ocupó de diseñar un programa de control de la situación de la infección con geohelmintos en las Provincias del Norte de Argentina (Noreste en Misiones y, Noroeste en Salta). Por un lado, en Misiones se quería proceder de acuerdo con la OMS para conocer las prevalencias de geohelmintos, sobre todo de Ancylostomátidos, existentes y poder diseñar los oportunos programas de desparasitación. Por otro lado, en Salta, una vez detenido el programa antihelmíntico, y transcurridos 4 años de la última administración, se quería conocer la situación actual y comprobar si el programa y la educación sanitaria ofrecida a la población había alcanzado las expectativas deseadas. Además, y también de acuerdo con la OMS, una de las prioridades era aplicar métodos de diagnóstico más sensibles y menos invasivos. Por eso, poder contribuir a reforzar las capacidades de los test diagnósticos disponibles en la actualidad se ha considerado también una prioridad, como es el caso del empleo del kit NIE-ELISA para el diagnóstico de *S. Stercoralis*.

Con este fondo, arranca el diseño de la presente Tesis Doctoral, titulada "Caracterización epidemiológica de la infección por parásitos intestinales en poblaciones indígenas de la Provincia de Misiones y de la Provincia de Salta (Región del Norte Grande Argentino, Argentina)". Para llevarla a cabo se ha tenido que diseñar una completa marcha analítica de forma que se ha incluido distintos métodos de diagnóstico coprológico, métodos serológicos, métodos de diagnóstico molecular y, también, distintos test estadísticos referentes en la realización de mapeos epidemiológicos.

De entre los métodos de diagnóstico coprológico utilizados, comentar que además de una visión directa del material fecal fijado con Formalina 10% con ayuda del colorante lugol, también se ha aplicado: la técnica de concentración de Ritchie modificada, para la detección de protozoos y helmintos; la técnica de Baermann para la detección de larvas; y, la técnica de Kato-Katz, que permitió la medida de la intensidad de la infección

helmíntica, obteniendo el número de huevos (eggs) por gramo de heces (EPG), que permitió, a su vez, hacer la clasificación de clases de intensidad (leve, moderada o alta) de acuerdo a las directrices de la OMS (World Health Organization, 2012).

Los análisis hematológicos, que permitieron obtener el índice anémico de la población estudiada, se realizaron gracias a la punción venosa de los participantes. Posteriormente, un laboratorio privado procedió a facilitar los datos de hemoglobina (Hgb), recuento de glóbulos blancos, conteo relativo de eosinófilos y hematocrito. El nivel de Hgb se utilizó para asociar anemia con infección helmíntica. Además, una alícuota de suero mantenida a -20ºC permitió la aplicación de un test de ELISA doméstico (in house enzyme-linked immunosorbent assay (NIE-ELISA) para el diagnóstico de *S. stercoralis*, detectando anticuerpos IgG frente al antígeno recombinante NIE de las larvas L3 de *S. stercoralis*. (Cimino et al., 2020).

Para poder aplicar el diagnóstico molecular, a las alícuotas mantenidas y fijadas en etanol 70º, se les extrajo el DNA genómico previo a la aplicación de las reacciones de PCR convencional y/o PCR cuantitativa (qPCR), utilizando los cebadores específicos para los protozoos patógenos y/o potencialmente patógenos detectados. En el caso de *G. intestinalis* primero se determinó el gen de la subunidad pequeña del ARN ribosomal (SSU rRNA) (fragmento de 62 bp), seguido de una amplificación del gen B-giardina (fragmento de 753 bp). En el caso de *Blastocystis* sp se amplificó el gen SSU rRNA (fragmento de 600 bp) y, en el caso del complejo *E. hystolitica* y *E. dispar* se amplificaron fragmentos de 166 bp y 752 bp del gen SSU rRNA, respectivamente. A pesar de la existencia de varios estudios epidemiológicos llevados a cabo en el norte de Argentina, hasta la presente Tesis Doctoral en la Provincia de Misiones, en ninguno de ellos se abarcaba los aspectos de identificación molecular de *G. intestinalis*, *Blastocystis* sp. y *E. histolytica*.

El estudio del riesgo de infección con diferentes condiciones medioambientales se llevó a cabo aplicando test estadísticos a los datos recolectados gracias al empleo de cuestionarios estandarizados y empleados por la fundación Mundo Sano. Gracias a los datos recogidos usando sensores remotos (RS) y modelos de elevación digital (DEM), se calcularon distintos índices de vegetación, así como índices topográficos y, finalmente, se seleccionaron los mejores índices predictores de infección.

Como se ha comentado anteriormente, el desarrollo de la presente Tesis Doctoral

ha abarcado varias poblaciones indígenas del norte de Argentina. Por un lado, se estudió a los habitantes indígenas localizados en la periferia de la ciudad de Iguazú, en la Provincia de Misiones, que limita con Brasil y Paraguay. En este primer estudio los participantes estaban comprendidos entre 1 y 87 años. Se recogieron variables socioeconómicas, características de la vivienda, hábitos higiénicos, así como muestras sanguíneas y coprológicas. Para el análisis parasitológico se empleó el examen microscópico convencional de concentración de Ritchie modificado, como método de cribado, así como ensayos moleculares de PCR convencional, seguida de secuenciación Sanger, para identificar los protozoos intestinales presentes en la zona. Como análisis complementario, se realizó un hemograma para observar el estado general de salud de la población participante.

Los resultados en Iguazú mostraron una alta tasa de parasitación en las poblaciones analizadas, que oscilaba entre el 91.1% y el 95.8%, con una elevada prevalencia tanto de protozoos como de geohelmintos. El protozoo patógeno más prevalente fue *G. intestinalis* (29.7%-30.4%), y los geohelmintos más prevalentes fueron los Ancylostomátidos (67.5%-87.5%), seguidos por *S. stercoralis* (11.5%-35.5%). Se detectó que la elevada prevalencia de parásitos intestinales aparecía asociada a las deficientes características de las viviendas y, a bajos niveles educativos. En concreto, respecto a los geohelmintos, se encontró asociación con el tipo de suelo de la vivienda y con la anemia. Las condiciones de vida como el hacinamiento o caminar descalzo estuvieron implicadas en una mayor intensidad de infección por Ancylostomátidos y los diversos factores ambientales como la vegetación, la humedad del terreno y el índice de impermeabilidad de superficie, influenciado por el desarrollo urbano, se relacionaron con la distribución de las geohelmintiasis. Por último, los análisis moleculares confirmaron la presencia del patógeno *E. histolytica* y mostraron como los animales estaban implicados en la transmisión de genotipos y subtipos zoonóticos de *G. intestinalis* y *Blastocstis* sp., respectivamente.

Siguiendo con poblaciones del norte de Argentina, en segundo lugar, se abarcó las distintas aldeas de la comunidad de Tartagal, en la Provincia de Salta, que limita al norte con Bolivia. En este segundo estudio los estudios abarcaron únicamente población infantil de 1-15 años de las distintas aldeas. Sin embargo, junto a los análisis parasitológicos microscópicos y moleculares, se les realizó también un ensayo serológico (NIE-ELISA) como método de control de la reinfección por *S. stercoralis*, para

comprobar el estado de infección tras la interrupción del programa de desparasitación masiva aplicado anteriormente en dicha comunidad.

Los resultados en la comunidad de Tartagal mostraron una parasitación total en la población que oscilaba entre el 78.6% y el 92.8%, con elevadas prevalencias de protozoos, siendo el patógeno más prevalente *G. intestinalis* (19.6%-49.2%). En esta comunidad, objeto de un programa de desparasitación helmíntica anterior, se obtiene una menor prevalencia general de geohelmintos, apareciendo los Ancylostomátidos como los mayoritarios (2.9%-51.6%). Así, en este estudio se ha comprobado una disminución cercana al 60% en la prevalencia de los Ancylostomátidos. Igualmente, la prevalencia detectada para *S. stercoralis* se reduce entre el 1% y el 10.3%. Los análisis serológicos obtenidos demostraron la efectividad y la duración del efecto del tratamiento antihelmíntico y la importancia de la cobertura y su adhesión por parte de la población para controlar las geohelmintiasis. Esto aparece evidenciado debido a que, en una de las aldeas, donde su población reconocía la baja adhesión al programa de desparasitación, la prevalencia de geohlemintos detectada se mantenía elevada (58.8%) comparada con la detectada en el resto de las aldeas de la comunidad estudiada (3.9%-26.7%). Finalmente, todas las aldeas que participaron en los estudios recibieron charlas educativas y sanitarias, así como tratamiento para toda la comunidad

Como conclusiones, en general, se puede observar una elevada prevalencia de parasitación en las aldeas indígenas del norte de Argentina. Además, se detecta incluso zonas hiperendémicas de geohelmintos, en las cuales el medio ambiente juega un papel importante en el establecimiento y desarrollo de dichas infecciones. En dichas poblaciones se hace necesario implementar programas de desparasitación masiva para el control de las geohelmintiasis, en especial las debidas a Ancylostomátidos. Además, se comprueba que garantizar una correcta adherencia al tratamiento logra disminuir las prevalencias. Se debe considerar el uso de técnicas serológicas como herramienta útil para el seguimiento de la población tras el tratamiento, incluso para la detección precoz de *S. stercoralis*. Llama la atención las altas prevalencias de protozoos en zonas donde se mantiene la reducción de la transmisión de geohelmintos, lo que debe orientar a que su transmisión debe estar relacionada, o producirse, a través del agua contaminada con material fecal. Debido a que las variables ambientales no pueden ser modificadas, es importante trabajar sobre aquellos aspectos que sí pueden modificarse como mejorar la

calidad del agua, mejorar el acceso a un saneamiento adecuado, mejorar las características de la vivienda o la educación sanitaria. Por último, señalar que el uso de técnicas moleculares contribuye a diagnosticar correctamente la presencia de *E. histolytica*, y favorece el análisis de los patrones antropozoonóticos de *G. intestinalis* y *Blastocystis* sp. de forma que se puede aplicar las correctas y oportunas medidas de prevención.

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CAPÍTULO II:

OBJETIVOS

Objetivo General y Específico

Objetivo General

Estudiar la situación epidemiológica de las parasitosis intestinales de las diferentes poblaciones indígenas de Argentina, de la Región del Norte Grande Argentino, en concreto del Departamento de Puerto Iguazú en la Provincia de Misiones y del Departamento General San Martín en la Provincia de Salta. Este análisis permitirá identificar el espectro parasitológico de la población, previo a la propuesta de intervención, con la finalidad de mejorar su calidad de vida, ya que conocer las características específicas de la zona permitirá adaptar y adecuar su situación particular a las intervenciones, de tipo multidisciplinar.

Objetivos Específicos

- Determinar las especies parásitas intestinales, tanto de protozoos como de helmintos, presentes en poblaciones indígenas, mediante el uso de técnicas coprológicas y moleculares.
- Determinar las respectivas prevalencias de parasitación para cada especia parásita y su relación con los grupos de edad, sexo así como variables epidemiológicas relevantes, como distintos factores sociales, económicos y culturales que pueden influenciar en la transmisión de los parásitos intestinales.
- Determinar la intensidad de parasitación, tanto de las especies de protozoos como de las especies de helmintos, así como el nivel de multiparasitismo y las asociaciones entre distintas especies.
- Establecer y definir las características epidemiológicas de cada zona, con la finalidad de detectar similitudes o particularidades, y poder contribuir a desarrollar un patrón de intervención (tratamiento y educación sanitaria).

CAPITULO III:

ARTÍCULOS DE INVESTIGACIÓN

3.1 Prevalence of intestinal parasites and molecular characterization of *Giardia intestinalis*, *Blastocystis* spp. and *Entamoeba histolytica* in the village of Fortín Mbororé (Puerto Iguazú, Misiones, Argentina)

Ernesto Candela, Carolina Goizueta, María Victoria Periago, Carla Muñoz-Antoli

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Resumen

Los parásitos intestinales (PI) están ampliamente distribuidos por todo el mundo y son uno de los principales causantes de enfermedades gastrointestinales. Su prevalencia está asociada a un acceso deficiente al agua, el saneamiento y la higiene (WASH). El objetivo de este estudio fue identificar la prevalencia de PI, incluidos los helmintos transmitidos por el suelo (STH), y su relación con las características socioeconómicas, así como una primera aproximación para caracterizar molecularmente los tipos de *Giardia intestinalis*, *Blastocystis* spp. y *Entamoeba histolytica* presentes en una comunidad indígena de Puerto Iguazú, Misiones, Argentina. Se realizó un estudio transversal en el asentamiento rural de Fortín Mbororé entre enero y marzo de 2018. Se recolectaron variables socioeconómicas, características del hogar y muestras de materia fecal y sangre. Se utilizaron técnicas coprológicas estándar para analizar las muestras de heces, y se realizó un hemograma completo en las muestras de sangre. Las muestras positivas por microscopía para *G. intestinalis* se tipificaron genéticamente mediante el gen de la β-giardina (bg). La identificación molecular de los subtipos de *Blastocystis* spp. y *E. histolytica* se llevó a cabo mediante la amplificación y secuenciación de un fragmento parcial del gen del ARN ribosómico de subunidad pequeña (ADNr SSU).La prevalencia global de PI fue del 92,7%, con un 72,0% específico para anquilostoma. Los PI fueron significativamente más prevalentes en niños en edad preescolar y escolar ($P < 0,05$). La ausencia de educación formal ($P = 0,035$), la presencia de suelos no mejorados ($P = 0,001$) y el hacinamiento ($P = 0,005$) se asociaron significativamente con la infección por IP. La anquilostomiasis se asoció con la anemia ($P = 0,019$). La caracterización molecular reveló la presencia *E.* histolytica y de los subensamblajes AII (12,5%), AIII (87,5%), BIV (100%) y un caso del subensamblaje D de *G. intestinalis*; y la presencia de los subtipos ST1 (14,8%), ST2 (14,8%) y ST3 (70.4%) de *Blastocystis* spp. Los protozoos detectados en este estudio se transmiten principalmente a través del agua contaminada con materia fecal, lo que evidencia la necesidad de mejorar la calidad del agua y el saneamiento de los habitantes de Fortín Mbororé. La caracterización molecular demostró que los animales domésticos pueden estar implicados en la transmisión zoonótica de *G. intestinalis* y *Blastocystis* spp. al ser humano. Se halló una zona hiperendémica de STH, con una prevalencia de anquilostomiasis superior al 50%. Por lo tanto, deberían aplicarse mejoras en el agua, el saneamiento y la higiene, así como programas de desparasitación masiva en esta zona, para controlar y disminuir la prevalencia de las IP en general y de las STH en particular.

Palabras clave: helmintiasis transmitidas por el suelo, parásitos intestinales, caracterización molecular, *Giardia intestinalis*, *Blastocystisk* spp., *Entamoeba histolytica,* Puerto Iguazú, Misiones, Argentina.

Parasites & Vectors

RESEARCH Open Access

Prevalence of intestinal parasites and molecular characterization of *Giardia intestinalis*, *Blastocystis* spp. and *Entamoeba histolytica* in the village of Fortín Mbororé (Puerto Iguazú, Misiones, Argentina)

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Abstract

Background: Intestinal parasites (IPs) are widely distributed worldwide and are one of the major contributors to gastrointestinal disease. Their prevalence is associated with poor access to water, sanitation and hygiene (WASH). The objective of this study was to identify the prevalence of IPs, including soil-transmitted helminths (STH), and their relation to socioeconomic characteristics, as well as a first approach to molecularly characterize the types of *Giardia intestinalis*, *Blastocystis* spp. and *Entamoeba histolytica* present in an indigenous community from Puerto Iguazú, Misiones, Argentina.

Methods: A cross-sectional study was conducted in the rural settlement of Fortin Mbororé between January and March 2018. Socioeconomic variables, household characteristics, and stool and blood samples were collected. Standard coprological techniques were used to analyze stool samples, and a complete hemogram was performed on the blood samples. *Giardia intestinalis* microscopy-positive samples were genetically typed by the β-giardin (*bg*) gene. Molecular identification of *Blastocystis* spp. subtypes and *E. histolytica* were carried out by amplification and sequencing of a partial fragment of the small subunit ribosomal RNA gene (SSU rDNA).

Results: The overall prevalence of IPs was 92.7%, with 72.0% specifically for hookworm. IPs were significantly more prevalent in preschool- and school-age children ($P < 0.05$). No formal education ($P = 0.035$), the presence of unimproved floors ($P = 0.001$) and overcrowding ($P = 0.005$) were significantly associated with IP infection. Hookworm was associated with anemia ($P = 0.019$). Molecular characterization revealed the presence of *E. histolytica* sub-assemblages AII (12.5%), AIII (87.5%) and BIV (100%); one case of sub-assemblage D for *G. intestinalis*; and the presence of subtypes ST1 (14.8%), ST2 (14.8%) and ST3 (70.4%) of *Blastocystis* spp.

Conclusions: Protozoans detected in this study are transmitted mainly through water contaminated with fecal matter, evidencing the need to improve the quality of water and sanitation for the inhabitants of Fortín Mbororé. Molecular characterization showed that domestic animals can be implicated in the zoonotic transmission of *G. intestinalis* and *Blastocystis* spp. to humans. A hyperendemic area for STH was found, with hookworm prevalence greater than

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50%. Therefore, improvements in WASH as well as mass deworming programs need to be implemented in this area to control and decrease the prevalence of IPs in general and STH in particular.

Keywords: Soil-transmitted helminths, Intestinal parasites, Molecular characterization, *Giardia intestinalis*, *Blastocystis* spp., *Entamoeba histolytica*, Puerto Iguazú, Misiones, Argentina

Background

Intestinal parasite infections (IPIs) are a global public health problem due to their high prevalence and worldwide distribution, especially in populations from tropical and subtropical areas of the developing world [1]. Although they can affect any age group, children are the most affected by the consequences of infection [1, 2]. Some of the most important causal agents are protozoans (e.g. *Entamoeba histolytica*, *Giardia intestinalis*) and helminths, with soil-transmitted helminths (STH, referring to *Strongyloides stercoralis, Ascaris lumbricoides, Trichuris trichiura* and hookworm) the most prevalent [2], and listed as part of the neglected tropical diseases (NTDs) by the World Health Organization (WHO) [3]. Several studies reported varying prevalence of IPIs in the population, which depends on socioeconomic status, sanitary and environmental conditions and access to water, as well as changes in lifestyle as a result of acculturation and environmental degradation processes [4–7]. Even though most of these infections are asymptomatic, intestinal parasites (IPs) cause malabsorption, nutritional syndromes, malnutrition, morbidity and deficiencies in children's growth [8, 9]. Moreover, STH infections are implicated in the etiology of iron-deficiency anemia (IDA) in developing countries [10]. Specifically, moderate and heavy hookworm infections have been strongly associated with the development of anemia, due to chronic intestinal blood loss [11], as well as with cognitive impairment in school-age children and negative impact on psychomotor and language development of preschool-age children [12, 13]. All these factors contrib- ute to the economic impact of the disease and perpetua- tion of poverty, causing more than 100,000 annual deaths [2] and susceptibility to develop other diseases as adults [14].

Several studies have described the presence of IPs in northern Argentina, showing a high prevalence of protozoans with a predominant presence of *G. intestinalis* and *Blastocystis* spp., as well as STH [5, 15]. Additionally, Argentina has a heterogeneous prevalence of STH infections, with high prevalence in the north [16]. Anemia is considered a public health problem in the country, particularly in the northwest, where 38% of preschool-age children and 19% of women are anemic [17]. However, the prevalence found could be higher than the one reported due to difficulty in diagnosis if the parasite load is low and given that larval/egg output tends to be irregular in helminths [18]. Moreover, the diagnostic methods commonly used for STH detection have a low sensitivity for *S. stercoralis* or fail to detect it altogether [19, 20].

Resolution WHA62.12 from the WHO World Health Assembly (WHA) urges authorities to "implement interventions against neglected tropical diseases" [21] for STH in endemic areas based on mass drug administration (MDA), normally using a single dose of either albendazole or mebendazole for *A. lumbricoides*, *T. trichiura* and hookworm, and ivermectin for *S. stercoralis*. Currently, Argentina does not have a deworming program at either the national or provincial level.

Although several epidemiological studies have been carried out in northern Argentina, none of them have delved into the molecular aspect of *G. intestinalis*, *Blas- tocystis* spp. and *E. histolytica*. These enteric protozo- ans, including *Cryptosporidium* spp., are regarded as the most common and important causes of protozoan-diar- rheal disease in humans globally. Even though the effects caused by *Blastocystis* spp. are still debatable, several pieces of evidence have emerged pointing to its patho- genic role in intestinal disorders [22, 23]. Determining the molecular frequency and subtype of these protozoans is important to ascertain the sources of infection, trans- mission dynamics and zoonotic potential [23].

Giardia intestinalis, one of the major worldwide contributors to diarrheal disease, is a complex formed of eight genotypes identified to date (A–H), with several sub-assemblages; but only genotype A and B have been associated with human infections [24]. With respect to *Blastocystis* spp., based on its high level of genetic diversity, it is classified into different global ribosomal subtypes (STs). Currently, 17 subtypes have been described in different areas, with ST1 to ST9 and ST12 colonizing humans. In humans from Europe, ST1, 2, 3 and 4 reportedly occur most commonly, whereas ST1, 2 and 3 commonly occur in South America [22, 25].

The aim of this study was to identify the prevalence of intestinal parasites, including STH, due to the presence of living conditions appropriate for their transmission; identify any association between prevalence and socioeconomic characteristics; and perform a first approach to molecularly type *G. intestinalis*, *Blastocystis* spp. and *E. histolytica* present in an indigenous community from Puerto Iguazú, Misiones, Argentina. The results from this

study constitute the first published report on *G. intestinalis* and *Blastocystis* genotypes circulating in the province of Misiones.

Methods

Study area and study population

This study was conducted in the city of Puerto Iguazú, located in the province of Misiones (25° 35′ 52″ S, 54° 34′ 55″ W), a subtropical province of northeastern Argen- tina (Fig. 1) and part of the most biodiverse region of the country [26], which houses the Iguazú National Park and Iguazú Falls. Puerto Iguazú is in the tri-border area of Argentina, Brazil and Paraguay; the three countries are naturally divided by the Paraná and Iguazú Rivers. The region is characterized by a subtropical climate with no dry season. The median annual temperature is 21 °C, with annual rainfall of 1883.2 mm [27]. The predominant soil type is lateritic of deep red color [26].

Around the city's periphery, Mbyá Guaraní aboriginal communities have settled into villages, including the village of Fortín Mbororé, which is composed of around 200 families. The primary source of income is from guided tours organized to visit the village, handcrafts and social plans. These communities also all share simi- lar water and sanitation conditions and are homogene- ous in their economic status, with low monthly incomes [16]. The living conditions in Fortín Mbororé are charac- terized by a lack of water and sanitation [16] and houses made of adobe bricks with unimproved roofs and dirt floors, and practically the entire population, both chil- dren and adults, walk barefoot (Fig. 2). Most houses have a single room for sleeping, and therefore overcrowding is common.

Study design

A cross-sectional study was conducted in Fortín Mbororé between January and March 2018, as a community-wide intervention. The study included individuals older than 1 year. A total of 61 households were visited, georeferenced and characterized using a house-by-house questionnaire,

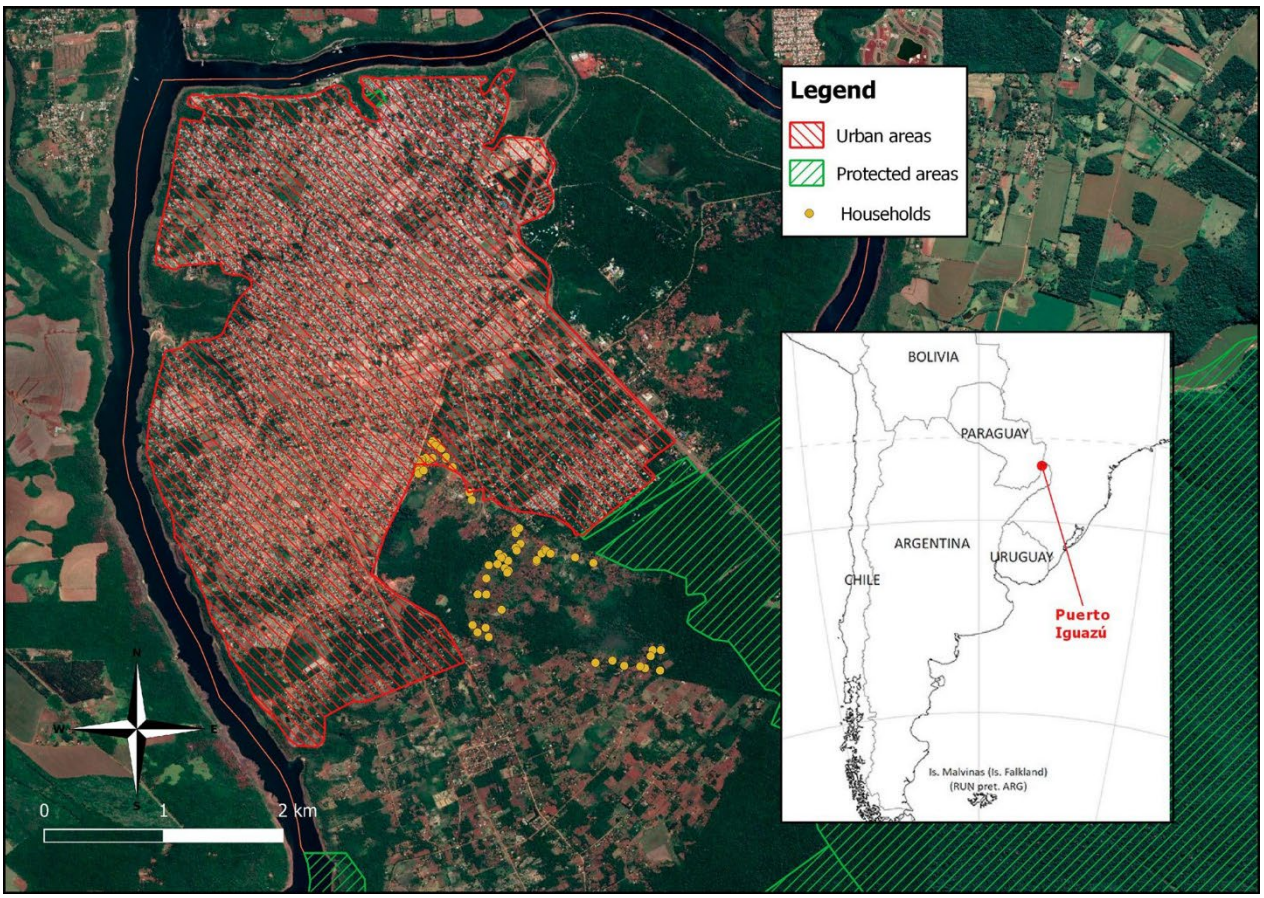

Fig. 1 Map of the study area. The households from the village of Fortín Mbororé are shown as yellow dots, which are located just outside the urban area of the city of Puerto Iguazú, Misiones, Argentina. South America image was obtained from www.mapasparacolorear.com. This map was created with QGIS 3.14 (www.qgis.org)

Fig. 2 An example of a household (**a**), a water well (**b**) and a latrine (**c**) in the Fortin Mbororé Village, Puerto Iguazú, Misiones (Argentina)

collecting data on all members of the household, includ- ing socioeconomic variables; these are detailed in the results (Table 4).

Oral bilingual (Spanish/Mbyá-Guaraní) explanations on sample collection were provided along with sample collection containers without any fixative [one per person], and were retrieved on the following day. The fresh samples were transported without fixative in a refrigerated icebox and kept at 4 °C in the lab until analysis within 24 h of collection.

Together with stool samples, blood samples were drawn though venipuncture into H2PP tubes (VITIS®, Prunus SLR, Argentina) containing EDTA-K3 anticoagulant and transported to a private clinical laboratory (Clínica SAM) located in Puerto Iguazú for hematological analysis. A complete hemogram was performed, including hemoglobin values (Hgb), white blood cell counts, eosinophil relative count and hematocrit. Subjects were classified as anemic or not anemic using the thresholds to define anemia according to sex and age as defined by WHO [28]. All participants were offered anthelminthic treatment according to the WHO preventive chemotherapy for human helminthiasis [29] and national guidelines [30], with the inclusion of ivermectin (200 µg/kg), together with WASH [water, sanitation, and hygiene] education workshops to improve hygiene habits.

Stool examination

To determine the presence of intestinal parasites, samples were processed with four different diagnostic techniques [31–33] to optimize the detection of a diverse parasite spectrum. The techniques used included the Ritchie concentration technique for detection of both proto- zoan and helminth parasites, Baermann concentration

for the detection of larvae, Kato–Katz to measure infection intensity of helminth parasites, and modified Ziehl–Neelsen stain for detection of coccidia. The Kato–Katz technique was used only if eggs or larval stages were previously detected by the Ritchie or Baermann method; results were recorded as eggs per gram (EPG) and classified as light, moderate or heavy infection following WHO guidelines [34]. Aliquots of the fresh samples were stored either in 10% formalin for confirmatory techniques or at −20 °C with no fixative for molecular biology techniques. If the sample volume was insufficient to perform all diagnostic methods, the sedimentation technique was prioritized due to its overall higher sensitivity [35]. The findings from each of the different methods were recorded in a database.

DNA extraction

Genomic DNA was extracted from 200 mg of concentrated fecal material using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions, with slight modification; fecal samples were mixed with stool lysis buffer and incubated for 10 min at 95 °C. The DNA was eluted in 100 µl of elution buffer and stored at −20 °C.

Molecular identification of *Giardia* spp., *Blastocystis* spp. and *Entamoeba* spp.

PCR reactions were performed using an MJ Mini Thermal Cycler PTC-1148 (Bio-Rad Laboratories, Inc.). Sterile water was used as a negative PCR control, and previously tested fecal samples containing *G. intestinalis*, *Blastocys- tis* spp., *E. histolytica* and *E. dispar* were used as positive controls. The oligonucleotides used for molecular iden- tification and characterization of *G. intestinalis*, *Blasto- cystis* spp. and *Entamoeba histolytica/dispar* appear in Additional file 1: Table S1. PCR products from all of the reactions were run on a 1% agarose gel, except for the PCR products for *Blastocystis* spp., which were run on a 2% agarose gel.

Molecular detection of *Giardia intestinalis*

Samples positive for *Giardia* spp. through microscopy were screened by a quantitative PCR (qPCR) method targeting a specific 62-bp region of the small subunit rRNA (SSU rRNA) gene of the parasite [36]. Amplification reac- tions were conducted in total volumes of 25 µl with 3 µl template DNA, 12.5 pmol of primers and 1× TaqMan Gene Expression Master Mix (Applied Biosystems, CA, USA). Reactions were run using the following protocol: an initial hold step of 2 min at 60 °C, 10 min at 95 °C and 45 cycles of 15 s at 95 °C and 1 min at 60 °C.

Molecular typing of *G. intestinalis*

Giardia intestinalis isolates that tested positive by qPCR with cycle threshold values less than 37 (Ct < 37) were genotyped to assemblage level using a nested PCR encoding a 753-bp fragment of the β-giardin (*bg*) gene of the parasite [37, 38]. In general, PCR mixtures (25 µl final volume) consisted of 8.5 µl of MyTaq Reaction Buffer, containing 5 mM dNTPs and 15 mM $MgCl_2$, 2.5 units (U) of MyTaq DNA polymerase (Bioline GmbH, Luckenwalde, Germany), 1 µl of each 10 µM primer pair and 5 µl of extracted DNA for the first PCR reaction. The amplification condition for the first PCR reaction was as follows: initial denaturation at 95 °C for 7 min, followed by 35 cycles (95 °C for 30 s, 65 °C for 30 s and 72 °C for 60 s), and the final extension was at 72 °C for 7 min. For the second PCR reaction, 3 µl of the product from the first PCR reaction was added, and the reaction was performed under the same conditions as above except for the cycling time, which was instead 95 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s.

Molecular typing of *Blastocystis* spp.

Characterization of the *Blastocystis* subtypes from the microscopic-positive samples was achieved by PCR, targeting the SSU rRNA gene of the parasite, amplifying a PCR product of ~ 600 bp [39]. The reaction mixture (25 µl) contained 2.5 U of MyTaq DNA polymerase (Bioline GmbH, Luckenwalde, Germany), 5× MyTaq Reaction Buffer, 5 µl of template DNA and 0.5 µM of each primer. Amplification conditions consisted of one step of 95 °C for 3 min, followed by 30 cycles of 1 min each at 94 °C, 59 °C and 72 °C, with an additional 2 min final extension at 72 °C.

Molecular detection of *Entamoeba histolytica/dispar*

Entamoeba histolytica/dispar (*Entamoeba* complex) are morphologically identical species. In this study, spe- cific primers were used to identify either *E. histolytica* or *E. dispar*. To identify a 166-bp product for *E. histol- ytica* and a 752-bp product *E. dispar* DNA, samples were screened using a specific PCR based on SSU rRNA [40]. The reaction mixture contained 5 µl of DNA, 1.25 µl of each primer, 2.5 U of Taq polymerase (MyTaq DNA polymerase, Bioline GmbH) and 5× MyTaq Reaction Buffer containing 5 mM dNTPs. PCR amplification started with an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C 1 min, 58 °C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 7 min.

Sequencing and phylogenetical analysis

All PCR amplicons obtained from *G. intestinalis*-positive samples were purified using an mi-PCR Purification Kit

(Metabion International AG, Martinsried, Germany) and were sent for sequencing in both direction using the corresponding internal primers to the Central Service for sequencing for Experimental Research (SCSIE, University of Valencia, Spain). β-giardin DNA sequences, both forward and reverse directions, obtained from *G. intestinalis*-positive samples, were viewed using the Chromas 2.6.6v (Technelysium Pty Ltd.) sequence analysis program. The BLASTn tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare β-giardin nucleotide sequences with sequences retrieved from the NCBI GenBank database. Generated DNA consen- sus sequences from the β-giardin fragment were aligned using the ClustalW algorithm, and a phylogenetical tree was constructed using the neighbor-joining method in MEGA X version 10.1.7 (Molecular Evolutionary Genet- ics Analysis) software. The reliability of the phylogenetic tree at each branch node was estimated by the boot- strap method using 500 replications. Reference β-giardin sequences chosen for comparison were from isolates of human and animal origin collected throughout the world and from Italian clinical samples, which are described in Additional file 2: Table S2. *Giardia muris* was used as the outgroup.

Blastocystis sequences were submitted to the *Blastocystis* 18S database (http://pubmlst.org/blastocystis/) for subtype confirmation and allele identification.

Statistical analysis

Data were analyzed using Stata 14.2 software (STATA Corp., TX, USA). Measures were evaluated using proportions with 95% confidence intervals (95% CI) and means with standard deviations (SD). The Chi-square test was used to compare significant associations between different variables. Associations were obtained compar- ing presence or absence of protozoa and helminth (by age group and by parasitic species). A probability (P) value < 0.05 was considered as evidence of statistical sig- nificance. Odds ratios (OR) with 95% confidence inter- vals (CI) were used to measure the strength between dependent and independent variables.

Results

Study population

In total, 218 individuals from 61 households provided stool samples. Population distribution was 47.7% male (n = 104) and 52.3% female (n = 114), with a mean age of 19.9 years and a range from 1 to 87 years. Individuals younger than 15 years represented half of the participants (50.9%). Over half of the underage population were in school (57%), and 76% of adults had gone to school, although not all of them had completed their education; 72.6% had attended primary school and only 24.7% high school. Nonetheless, more than half of the population (64.0%) knew how to read and write.

Prevalence of intestinal parasites

The stool samples from all 218 individuals were analyzed through the Ritchie sedimentation technique. Two hundred and three samples had enough material for the Baermann concentration technique, and only those positive for either one of the previous techniques were processed by the Kato–Katz technique (n = 134) in order to quantify helminth eggs. Additionally, 209 of the samples were processed using the modified Ziehl–Neelsen stain. Due to the small amount of fecal material received from nine of the 218 samples, only the Ritchie technique was performed.

The overall prevalence of intestinal parasites was 92.7% (202/218), including 82.6% protozoans and 78.4% helminths (Table 1). The most prevalent protozoans were *Blastocystis* spp. (57.3%), followed by *Entamoeba coli* (41.7%) and *G. intestinalis* (24.8%), while the most

Table 1 Prevalence of intestinal parasites and polyparasitism in individuals (n = 218) from Fortín Mbororé Village (Puerto Iguazú, Misiones, Argentina)

Parasite	*N*	% Prevalence [95% CI]
Protozoa	180	82.6 [76.9-87.1]
Entamoeba coli	91	41.7 [35.1-48.3]
Entamoeba complex	22	10.1 [6.1-14.1]
Entamoeba hartmanni	31	14.2 [9.5-18.9]
Endolimax nana	45	20.6 [15.2-26.1]
Iodamoeba bütschlii	11	5.0 [2.1-8.0]
Chilomastix mesnili	12	5.5 [2.3-8.6]
Giardia intestinalis	54	24.7 [19-30.5]
Protist	125	57.3 [50.7-64.0]
Blastocystis spp.	125	57.3 [50.7-64.0]
Helminths	171	78.4 [72.9-83.9]
Enterobius vermicularis	3	1.4 [0.2-2.9]
Hymenolepis nana	34	15.6 [10.7-20.5]
Trichuris trichiura	1	0.5 [0.4-1.4]
Ascaris lumbricoides	3	1.4 [0.2-2.9]
Hookworms	157	72.0 [66.0-78.0]
Strongyloides stercoralis	25	11.5 [7.2-15.7]
Total positives	202	92.7 [88.3-95.5]
Polyparasitism	178	88.1 [82.8-91.9]
Double	53	29.8 [23.5-37.0]
Triple	53	29.8 [23.5-37.0]
Quadruple	45	25.3 [19.4-32.2]
Quintuple	19	10.7 [6.9-16.2]
Sextuple	7	3.9 [1.9-8.1]
Octuple	1	0.5 [0.1-3.9]

CI confidence interval

prevalent helminth was hookworm (72%). There was also an 11.5% prevalence of *S. stercoralis*. *Cryptosporidium* spp. infection was not detected in any of the analyzed samples, and *Enterobius vermicularis* was practically undetected. Polyparasitism was frequent (88.1%), mainly with two or three parasite species (29.8%), with one sample harboring up to eight parasites (Table 1). The most common co-infections found were *E. coli* + *Blastocystis* + hookworm ($n = 47$) and *Blastocystis* + *G. intestinalis* + hookworm ($n = 24$) (Table 1).

Giardia intestinalis and *C. mesnili* were the most frequent IPIs among preschool-age children ($\chi^2 = 18.83$, $df = 1$, $P = 0.001$), while *Hymenolepis nana* and *Blastocystis* spp. were the most frequent among school-age children ($\chi^2 = 20.92$, $df = 1$, $P = 0.007$). Hookworm was the most frequent IP found in adults ($\chi^2 = 9.29$, $df = 1$, $P = 0.007$), while *S. stercoralis* was the most frequent among the female gender ($\chi^2 = 4.39$, $df = 1$, $P = 0.003$). There was an association ($\chi^2 = 4.69$, $df = 1$, $P = 0.035$) between no formal education and the presence of intestinal parasites (Table 2).

The intensity of hookworm, measured as EPG of feces using the Kato–Katz technique, was mostly of light (66.1%) and heavy (26.2%) infection, and no statistically significant differences were found either between age groups or by sex. The distribution of hookworm infection intensity by age group is reflected in Fig. 3, which shows the frequency of light, medium or heavy infection within each age group. The intensity of the only *T. trichiura* infection found was light (48 EPG), while the *A. lumbricoides* infections detected were of light and heavy intensity (168, 408 and 14,352 EPG, respectively).

Intestinal parasites and anemia

Approximately 72.4% of the population presented with anemia, although females were significantly more affected ($\chi^2 = 6.57$, $df = 1$, $P = 0.010$). Only hookworm infections were significantly associated with anemia (OR = 1.97; 95% CI 0.9–4.3), with statistical association for male sex ($P = 0.02$; OR = 3.80; 95% CI 1.20–12.04). No association between the presence of anemia and educa- tion level was found (Table 3).

Household characteristics

The main characteristics from the houses and their relationship with the presence of intestinal parasites are detailed in Table 4. Most of the inhabitants live in overcrowded houses (68.4%), generally with only one room for the whole family. An association between the presence of intestinal parasites and overcrowding ($\chi^2 = 7.62$,

Table 2 Descriptive characteristics of participants from Fortín Mbororé, Puerto Iguazú (Misiones, Argentina) and prevalence of intestinal parasites (IPs)

Characteristics	Total participants ($n = 218$)	Presence of IP ($n = 202$)	Absence of IP ($n = 16$)	Percentage of infected per characteristic [95% CI]	*P*-value
Age group (years) 1-					
5	44	36	8	81.8 [66.9-90.9]	*0.002*[a]
6-12	54	53	1	98.1 [87.3-99.8]	0.075[b]
13-19	26	25	1	96.2 [74.6-99.5]	0.467
19-27	33	30	3	90.0 [74.1-97.2]	0.675[c]
> 27	61	58	3	95.1 [85.4-98.5]	0.393[c]
Gender					
Female	114	107	7	93.6 [87.6-97.1]	0.477[d]
Male	104	95	9	91.3 [84.0-95.5]	0.477
Education					
In preschool	3	3	0	100	0.718
In elementary school	82	78	4	95.1 [87.5-98.2]	0.280
In high school	19	17	2	89.5 [62.9-97.7]	0.577
Finished elementary school	27	26	1	96.3 [75.5-99.5]	0.439
Incomplete elementary school	3	3	0	100	0.718
Finished high school	9	9	0	100	0.724
No formal education	69	60	9	86.9 [76.5-93.2]	*0.035*

Values in italic indicate the relationship was statistically significant $P < 0.05$

CI confidence interval

[a] *G. intestinalis* and *C. mesnili* $P = 0.001$ at this age group

[b] *H. nana* and *Blastocystis* spp. $P = 0.007$ at this age group

[c] Hookworm $P = 0.007$ at this group

[d] *S. stercoralis* $P = 0.03$ at female group

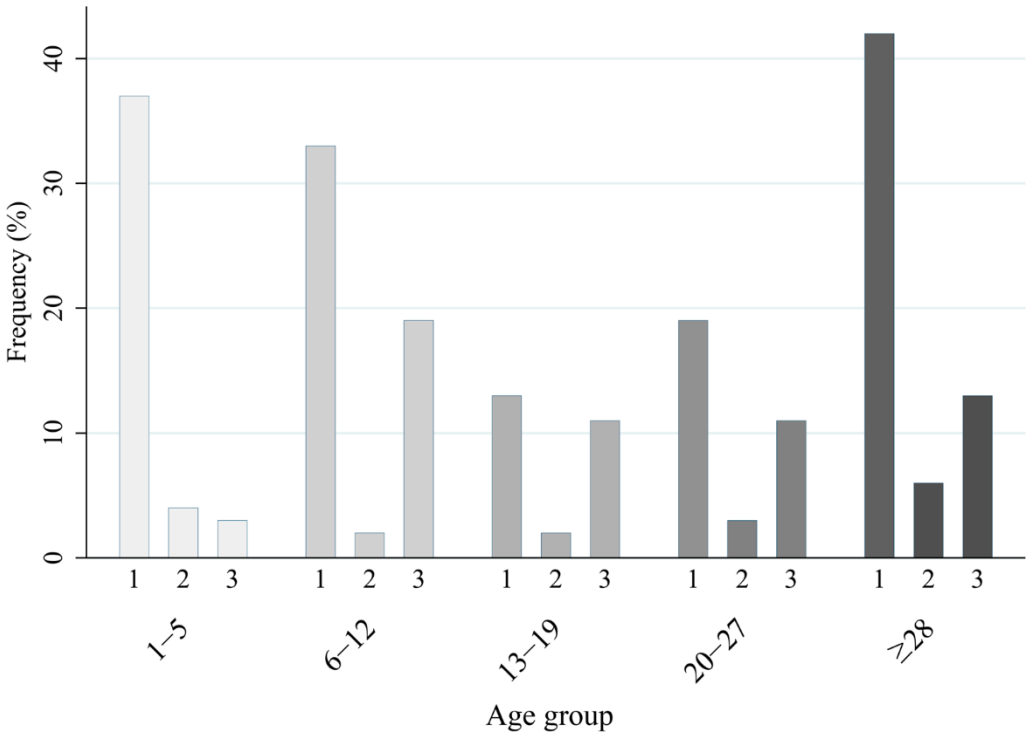

Fig. 3 Intensity of hookworm infection by age group. Number of participants from Fortin Mbororé, Puerto Iguazú (Misiones, Argentina) with light (1), moderate (2) or heavy (3) hookworm infection, by age group (1-5, 6-12, 13-19, 20-27, and 28 years of age or older)

$df = 1, P = 0.005$) was found, with the odds of having intestinal parasites increased up to four times. The employment situation of the families was precarious, and the main livelihoods were animal farming (53.9%) and crafts (71.6%), while some families benefited from social plans (27.4%). Almost all the families (90.4%) had at least one dog, so practically the entire population lived with animals in their environment (97.2%).

Most of the houses were made of wooden walls (83.3%) or thin wooden panels (55.8%) and dirt floors (50.7%). This type of floor was associated with increased transmis- sion of STH ($\chi^2 = 15.02$, $df = 1$, $P = 0.001$), and decreased by 72.2% (0.278, 95% CI 0.1–0.6, $P = 0.001$) for those living in houses with cement floors. Additionally, 87.6% of the participants walked barefoot and 16.2% practiced open defecation, although most had a latrine with simple ground excavation (79.1%). Nonetheless, no significant associations were observed between these factors and infection with IPs.

The most prevalent protozoan parasites observed were those transmitted by the fecal–oral route and through water. Most of the community (63.7%) obtained water from boreholes, and the rest of the families used tap water. None of the families stated that they treated the water for drinking or cooking by either boiling or use of disinfectants (i.e. bleach). There was no association between infection with protozoans as a group and water source, but families drinking from tap water had a higher prevalence of waterborne parasites. When analyzed by protozoan species, infection with *E. histolytica/dispar* was significantly associated with the use of tap water ($\chi^2 = 4.39$, $df = 1$, $P = 0.020$).

Molecular characterization of *G. intestinalis* isolates

Through the standard coprological techniques used, 54 samples were found to be positive for *Giardia* spp. Of these, only those with a high number of cysts/slide were selected for molecular analysis ($n = 32$), and 29 of the 32 DNA isolates tested positive for *G. intestinalis* by qPCR. Generated Ct values ranged from 28.3 to 39.1 (median: 32.2; 25th centile: 30.5; 75th centile: 34.0). Only DNA isolates with Ct values ≤ 37 ($n = 28$) were used for genotyping.

Of the 28 DNA isolates selected, 92.9% (26/28) were successfully amplified at the β-giardin locus and are described in Additional file 3: Table S3. Sequence analyses revealed the presence of assemblages A (30.8%; 8/26) and B (65.4%; 17/26); one canine assemblage (D) was also detected. Type A assemblage sequences were assigned to either sub-assemblage AII (12.5%; 1/8) or AIII (87.5%;

Table 3 Association between sex, age and soil-transmitted helminths (STH) and presence of anemia in participants from Fortín Mbororé Village (Puerto Iguazú, Misiones, Argentina)

	Presence of anemia (*N*)		Percentage (%) [95% CI]	*P*-value
	Yes	No		
Population	118	45	72.4 [64.9–78.8]	
Sex				
Male	47	28	62.7 [51.0–73.0]	
Female	71	17	80.7 [70.9–87.8]	*0.010*
Age group				
1–5	11	10	52.4 [30.2–73.7]	
6–12	30	9	76.9 [60.5–87.9]	
13–19	17	5	77.3 [53.5–90.9]	0.116
19–27	26	5	83.9 [65.4–93.5]	
> 27	34	16	68 [53.5–79.7]	
Hookworm				
Male				
Yes	41	18	69.5 [56.3–80.1]	*0.019*
No	6	10		
Female				
Yes	55	13	80.9 [69.5–88.7]	0.930
No	16	4		
S. stercoralis				
Male				
Yes	2	2	50 [24.7–97.5]	0.590
No	45	26		
Female				
Yes	11	3	78.6 [46–94]	0.830
No	60	14		

Values in italic indicate the relationship was statistically significant $P < 0.05$

CI confidence interval

7/8), while all type B sequences were assigned to the BIV sub-assemblage. The phylogenetic analysis performed with the sequences obtained revealed that the sample sequences found herein clustered with the corresponding assemblage and sub-assemblage A–D sequences used as references, although some samples tended to group into independent subgroups, reflecting noticeable changes at the nucleotide level (Fig. 4).

Molecular characterization of *Blastocystis* isolates

Through the standard coprological techniques used, 125 samples were found to be positive for *Blastocystis* spp.; only those with high numbers of cyst/slide were selected for molecular analysis ($n = 66$). After rejecting unreadable or poor-quality sequences typically associated with faint bands on agarose gels, 27 isolates were successfully subtyped (40.9%) and are detailed in Fig. 5. Sequence analysis at the SSU rDNA (barcode region) gene of the parasite revealed the presence of three subtypes (ST): ST1 (14.8%; 4/27), ST2 (14.8%, 4/27) and ST3 (70.4%; 19/27). Three different alleles were observed for ST1 (2, 4 and 88), three for ST2 (11, 12, 15), and a single allele (34) for ST3.

Molecular characterization of *E. histolytica/dispar*

Through the standard coprological techniques used, 22 samples were found to be positive for the "*Entamoeba* complex" and processed by PCR. Molecular characterization of the isolated samples showed that 45.5% of them were positive for *E. histolytica*, and *E. dispar* was not identified in any of the samples.

Discussion

The overall prevalence of IPs found in individuals from Fortín Mbororé Village, located in Puerto Iguazú, Misiones, Argentina was 92.7%, with a high prevalence of both protozoa and helminths. The most prevalent patho- genic protozoans were *G. intestinalis* (24.7%) and *Blas- tocystis* spp. (57.3%), whose pathogenic capacity is still a debatable issue. The most prevalent STH was hookworm (72%), followed by *S. stercoralis* with a prevalence of 11.5%. The percentage of individuals with polyparasitism was higher than 88%, evidencing the need for an urgent improvement of the health and living conditions of the population. These results are similar to previous studies carried out in other areas from northern Argentina, in populations who presented higher socio-environmental vulnerability [5, 15].

In the multivariate analysis of the data, a higher presence of IPs was observed in preschool- and school-age children. Also, a significant relation between no formal education and a higher presence of IPs was observed, as previously reported [41]. In contrast, parasitism was not associated with gender. The high prevalence of parasites transmitted by the oral–fecal route suggests that water quality is not adequate. Furthermore, the population that obtained water from the public water system was positively associated with infection by *E. histolytica/dispar* and had a higher probability of harboring a parasitic infection (OR = 1.77). Some of the household characteristics of the study area, such as dirt floor or overcrowding, were associated with a higher prevalence of STH or IPs, as observed in other studies [7, 42]. Unimproved sanitation, walking barefoot or practicing open defeca- tion are risk factors that may contribute to an increase in skin-penetrating parasite infections like hookworm. In the present study, households with cement floors showed lower incidence of STH, so an initial improvement of the material conditions of the houses might have an impact on the prevalence of this type of parasite, which

Table 4 Household characteristics and their association with the presence or absence of intestinal parasites in Fortín Mbororé, Puerto Iguazú, Misiones, Argentina

Characteristics	Presence of IP ($n = 199$)	Absence of IP ($n = 16$)	OR (95% CI)	*P*-value
Animal farming				
Yes	175	12	2.43 (0.7-8.1)	0.139
No	24	4	0.41 (0.1-1.4)	
Water source				
Borehole	125	12	0.56 (0.2-1.8)	0.329[a]
Tap water	74	4	1.776 (0.6-5.7)	
Overcrowding				
Yes	141	6	4.05 (1.4-11.6)	*0.005*
No	58	10	0.25 (0.1-0.7)	
Wall				
Improved (wood)	165	14	0.7 (0.2-3.3)	0.479
Unimproved	34	2	1.4 (0.3-6.5)	
	Presence of STH ($n = 137$)	Absence of STH ($n = 45$)		
Floor				
Cement	33	24	0.28 (0.1-0.6)	*0.001*
Wood	24	8	0.9 (0.4-2.4)	0.982
Dirt floor	80	13	3.5 (1.7-7.2)	*0.001*
Latrine				
Yes	110	40	0.5 (0.2-1.4)	0.189
No	27	5	1.96 (0.7-5.4)	
Barefoot				
Yes	119	40	1.16 (0.5-2.9)	0.762
No	18	7	0.9 (0.3-2.2)	

Values in italic indicate the relationship was statistically significant $P < 0.05$

[a] *E. histolytica/dispar* associated with tap water source ($P = 0.020$)

would theoretically decrease even more with the use of footwear.

The high prevalence of hookworm and *S. stercoralis* detected in Iguazú coincides with rates reported previously [4, 15], although a large difference in prevalence was observed for *S. stercoralis* (41.9% vs. 11.5%). These differences may be due to the variability in parasite expulsion in the stool and the low sensitivity and detection limitations of the nonmolecular techniques used [19, 43]. There were practically no cases of *A. lumbri- coides* or *T. trichiura*; these parasites are more influ- enced by fecal–oral hygiene habits, and tap water and hand washing could contribute to a lower prevalence detected in our study [6]. Although studies investigat- ing risk factors for *S. stercoralis* infection have mostly reported a higher risk among men, generally attrib- uted to men's extensive exposure to soil during farming activities [44], in our study this parasite was associ- ated more with females. We can only hypothesize on the reason for this, although it is important to note that there was no bias in sampling, since the participa- tion between males and females was not statistically different. One plausible explanation is that men in this area do not perform farming activities; they usually travel outside the village to tourist areas to sell their crafts, while women mostly stay at home to care for the children.

Additionally, 72.4% of the population studied was anemic. The relationship between STH infection, malnutrition and anemia has been extensively shown [15]. In this study, a significant association was observed between the presence of hookworm and anemia in men. Furthermore, the intensity of hookworm infection is related to anemia and morbidity and is a key indicator for measuring the success of large-scale deworming programs [45, 46]. Also, deleterious nutritional conditions and protein malabsorption have been associated with elevated parasite loads [15]. However, no association was found between heavy intensity (26.2%) and a greater presence of anemia. This is probably because most of the population studied had light-intensity hookworm infections (66.1%). The low nutritional status of the population previously reported [15] could be influenced, among other factors, by para- site incidence, since, as the study points out, these two

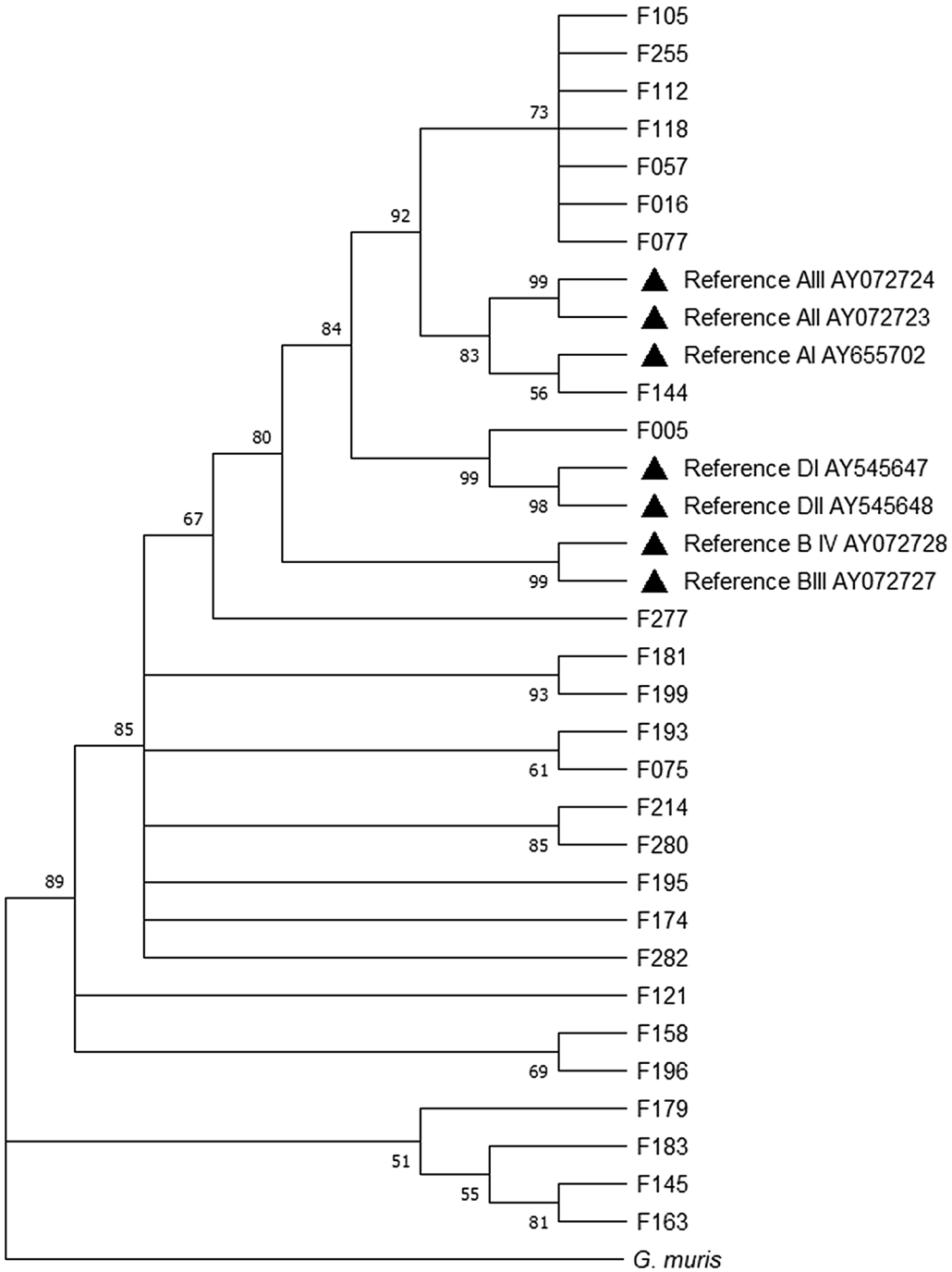

Fig. 4 Phylogenetic relationships of *Giardia intestinalis* inferred by neighbor-joining analysis of the β-giardin nucleotide sequences. Filled triangles represent reference sequences obtained from GenBank, described in Additional file 2: Table S2. A sequence from *Giardia muris* was used as the outgroup. Bootstrap values are based on 500 replicates, and only bootstraps > 50% are indicated

factors are associated. Guidelines for anemia and STH recommend implementing deworming programs to improve the public health situation of communities with high prevalence of anemia [10, 47].

Infection with *E. histolytica* can result in invasion of the colon wall and damage to other host tissues (amebiasis), and remains a cause of morbidity and mortality in developing countries [48]. The clinical diagnosis of amebiasis

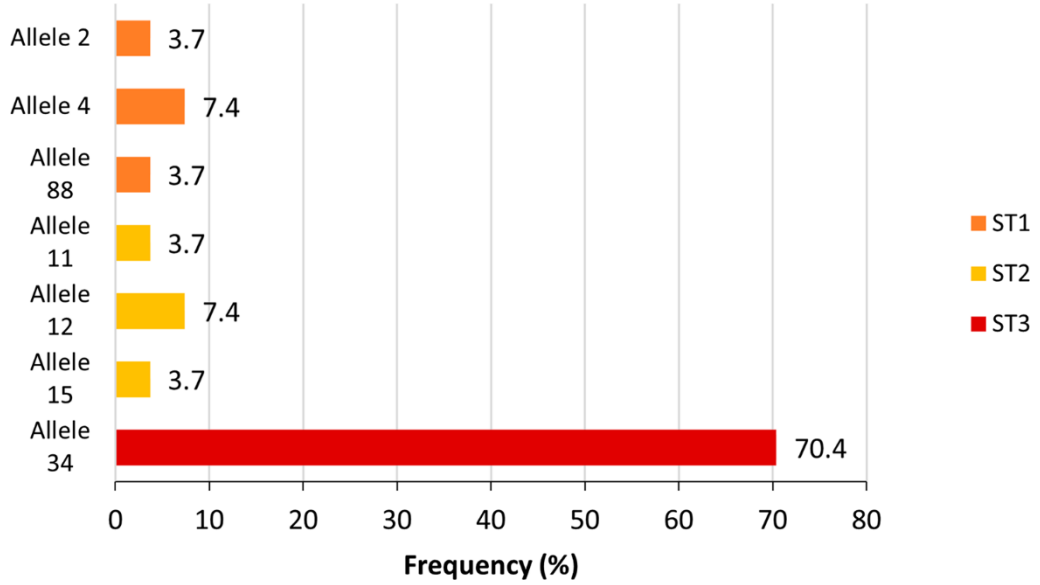

Fig. 5 Diversity and frequency of *Blastocystis* spp. subtypes and 18S alleles identified from positive samples, identified through microscopy, of participants from Fortín Mbororé (Puerto Iguazú, Misiones, Argentina)

is usually confirmed by visualization of the parasite by light microscopy, but this has the limitation of being una- ble to distinguish *E. histolytica* from *E. dispar* and *E. moshkovskii* cysts. Furthermore, the presence of other *Entamoeba* spp., *Iodamoeba* spp. or *Endolimax* spp. can make the diagnosis even more difficult [40]. With the aid of molecular techniques, it was possible to identify 10 *E. histolytica*-infected individuals in 22 microscopy-posi- tive samples. The remaining 12 negative samples by PCR suggest they are cysts that may belong to another *Enta- moeba* spp. The low prevalence of this protozoan in the current study agrees with previous studies from northern Argentina [49, 50].

On the other hand, *Blastocystis* spp. was the most prevalent species detected in this study, which is a zoonotic parasite that colonizes humans and multiple domestic and wild animals. The high prevalence contrasts greatly with data obtained in another study on the same population (57.3% vs. 5.9%) [15], although another study conducted in the Mbyá-Guaraní communities (Misiones, Argentina) has reported a higher prevalence [4]. The genetic diversity of *Blastocystis* spp. can be classified based on their polymorphic regions of its small subu- nit of the ribosomal RNA gene [51]. Some studies have reported that the most frequent subtypes in humans in Latin America are ST1 and ST3 [22, 25]. Although its immunopathogenesis is a matter of study and controversy, the ST3 subtype has been associated with a higher frequency in symptomatic patients [52]. This first molecular approximation of *Blastocystis* spp. in Iguazú indicates that the majority of the population was infected with the ST3 (87%) subtype, although gastrointestinal symptoms were not the subject of an associative analysis in the current study. Considering the zoonotic nature of the parasite, found in almost 60% of the population, and the fact that practically all families live with domestic or farm animals, these may represent a significant zoonotic source of this parasite for the community of Fortín Mbororé.

With respect to *G. intestinalis*, assemblage B showed a higher prevalence (65.4%) compared to assemblage A (30.8%); sub-assemblages AIII and BIV were the most frequent. These results coincide with the prevalence reported in other studies [24, 53], and it is commonly believed that humans are infected only with assemblages A and B. Surprisingly, a case of infection by assemblage D (identified from dogs) was found in this study, coinciding with the recent identification of unusual *G. intestinalis* genotypes, such as assemblages C, D, E and F in humans [54–56]. These results indicate the need to carry out greater genotyping of *G. intestinalis* infections in order to increase our knowledge on the transmission routes of this parasite.

Conclusion

This study represents the first molecular approach in Puerto Iguazú, describing the genotype of *G. intesti- nalis, Blastocystis* spp. and molecular detection of *E. histolytica*. There was a significant inverse association between age and parasitism, and a higher prevalence

of IPs was associated with no formal education and with poor household characteristics. Domestic animals were found to be implicated in the zoonotic transmis- sion of *Giardia* spp. and *Blastocystis* spp. The protozo- ans detected in the population are transmitted through water contaminated with fecal matter, evidencing the need to improve the quality of water and to improve access to appropriate sanitation. A hyperendemic area for STH was found, with hookworm infections associ- ated with anemia. Mass deworming programs, together with WASH and health education, need to be imple- mented in this area to control and decrease the preva- lence of IPs in general and STH in particular.

Abbreviations

bg: β-Giardin; CI: Confidence interval; Ct: Cycle threshold; EPG: Eggs per gram; Hgb: Hemoglobin; IDA: Iron-deficiency anemia; IP: Intestinal parasites; IPI: Intestinal parasite infection; NCBI: National Center for Biotechnology Information; OR: Odds ratio; qPCR: Quantitative real-time polymerase chain reaction; SD: Standard deviation; SSU rRNA: Small subunit ribosomal RNA; ST: Subtypes; STH: Soil-transmitted helminths; WASH: Water, sanitation and hygiene; WHA: World Health Assembly; WHO: World Health Organization.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-021-04968-z.

Additional file 1: Table S1. Oligonucleotides used for the molecular identification and characterization of *Giardia intestinalis*, *Blastocystis* spp. and *Entamoeba histolytica*/*dispar* in this study.

Additional file 2: Table S2. Reference sequences of *Giardia intestinalis* used in the comparative analysis to build the phylogenetic tree.

Additional file 3: Table S3. List of *Giardia intestinalis*-positive samples from participants of Fortín Mbororé, Puerto Iguazú, Misiones (Argentina) identified by microscopy and qPCR that were also identified at the assemblage level using the β-giardin gene.

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Authors' contributions

EC carried out laboratory analyses, analyzed and interpreted the data, and wrote the draft of the manuscript. CG was involved in the acquisition of data and community relations with the cacique. MVP was involved in the conception and design of the study, acquisition of data, analysis and interpretation, and revising the manuscript. CMA was involved in the conception and design of the study, analysis and interpretation, and revising the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data supporting the conclusions of this article are included within the article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All participants provided written informed consent prior to study participation, and parents provided informed consent on behalf of minors (younger than 16 years of age). An assent form was also provided from children between the ages of 6 and 13 years. The research protocol and the informed consent/assent forms were approved by the Bioethics Committee of the Ministry of Public Health of the Government of the Province of Misiones, Argentina. All infected individuals were treated according to national guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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3.2 The Relationship Between Soil-Transmitted Helminth Infections and Environmental Factors in Puerto Iguazú, Argentina: Cross-Sectional Study

Ernesto Candela, Carolina Goizueta, Leonardo Sandon, Carla Muñoz-Antoli, María Victoria Periago

JMIR Public Health Surveill. 2023; 9:e41568

Resumen

Los helmintos transmitidos por el suelo (STH) están ampliamente distribuidos por todo el mundo. Diversos factores, como el medio ambiente, las características socioeconómicas y el acceso al agua y el saneamiento, desempeñan un papel importante en la propagación y persistencia de estos parásitos en las comunidades. Éstos, a su vez, afectan al crecimiento y desarrollo de los miembros de la comunidad, especialmente de los niños. Estudios realizados en las provincias del norte de Argentina han mostrado una prevalencia variable de STHs, pero los factores asociados a su presencia no han sido completamente dilucidados. Este estudio transversal tuvo como objetivo identificar los factores socioeconómicos y ambientales relacionados con la infección por STH en aldeas indígenas ubicadas en Puerto Iguazú (Misiones), Argentina. Entre 2018 y 2019, se recogieron muestras de heces de individuos ≥1 año residentes en 3 aldeas: Mini-Marangatú, Yriapú y Fortín Mbororé. Se utilizaron métodos parasitológicos estándar para determinar la prevalencia de STH. Se utilizaron cuestionarios normalizados para evaluar los hábitos, costumbres y características de las viviendas, y se obtuvieron datos ambientales mediante imágenes de satélite. Se utilizó la regresión multilineal con variables por pasos del criterio de información de Akaike para explorar asociaciones relevantes. Un total de 342 personas de las tres aldeas participaron en el estudio. La prevalencia de las STH varió entre aldeas: 89.6% (43/48) en Mini-Marangatú, 80.8% (101/125) en Yriapú y 68.5% (115/169) en Fortín Mbororé. En particular, hubo una diferencia significativa en la infección por anquilostomas entre las aldeas (P=0.02). El análisis puso de relieve la influencia significativa de factores ambientales específicos sobre la presencia y distribución espacial de las STH, particularmente en relación con la infección por anquilostoma. Los patrones de vegetación representados por el Índice de Heterogeneidad de la Vegetación, creado ad hoc para este estudio, surgieron como un factor crítico, con 2 predictores significativos relacionados con él (P=.002 y P=.004), junto con la densidad de superficie impermeable con un predictor significativo (P<.001). El modelo de regresión multilineal arrojó una alta puntuación en la prueba F (F_{108}=4.75, P<.001), lo que indica un fuerte ajuste (R^2=0.5465). Además, factores socioeconómicos, como andar descalzo en casas con suelo de tierra y el hacinamiento, se correlacionaron significativamente con la intensidad de la infección por anquilostomas (P<.001 y P=.001, respectivamente). También se utilizó el modelo de regresión multilineal para calcular la intensidad de la infección por anquilostoma (F_{110}=21.15; P<0,001; R^2=0.4971). Nuestro estudio subraya la complejidad de la transmisión de las STH, ya que aldeas con condiciones de vida y características ambientales similares mostraron una prevalencia y una distribución espacial de las STH variables. Factores ambientales específicos, como el patrón de vegetación y la densidad de la superficie impermeable, desempeñaron papeles importantes en la presencia de STH, lo que demuestra la relación crucial entre los factores ambientales y la distribución de la infección por anquilostomas. Además, nuestros hallazgos subrayan la

significativa influencia de los factores socioeconómicos en la intensidad de la infección por anquilostoma. Al comprender mejor esta compleja interacción, nuestra investigación contribuye a una mejor comprensión de las características de la transmisión de la anquilostomiasis, lo que permite orientar las intervenciones de salud pública para un control eficaz.

Palabras clave: helmintiasis transmitidas por el suelo, anquilostomas, prevalencia, intensidad, distribución, Iguazú, Argentina.

Original Paper

The Relationship Between Soil-Transmitted Helminth Infections and Environmental Factors in Puerto Iguazú, Argentina: Cross-Sectional Study

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Abstract

Background: Soil-transmitted helminths (STHs) are widely distributed throughout the world. Various factors, including the environment, socioeconomic characteristics, and access to water and sanitation, play an important role in the spread and persistence of these parasites within communities. They, in turn, affect the growth and development of members of the community, especially children. Studies in the northern provinces of Argentina have shown variable prevalence of STHs, but the factors associated with their presence have not been completely elucidated.

Objective: This cross-sectional study aimed to identify the socioeconomic and environmental factors related to STH infection in indigenous villages located in Puerto Iguazú (Misiones), Argentina.

Methods: Between 2018 and 2019, stool samples were collected from individuals ≥1 year residing in 3 villages: Mini-Marangatú, Yriapú, and Fortín Mbororé. Standard parasitological methods were used to determine STH prevalence. Standardized questionnaires were used to assess participants' habits, customs, and household characteristics, and environmental data were obtained through satellite imagery. Multilinear regression with Akaike information criterion stepwise variables was used to explore relevant associations.

Results: A total of 342 individuals from the 3 villages participated in this study. The prevalence of STHs varied across villages: 89.6% (43/48), in Mini-Marangatú, 80.8% (101/125) in Yriapú, and 68.5% (115/169) in Fortín Mbororé. Notably, there was a significant difference in hookworm infection among the villages (P=.02). The analysis highlighted the significant influence of specific environmental factors on STH presence and spatial distribution, particularly in relation to hookworm infection. Vegetation patterns represented by the Vegetation Heterogeneity Index, created ad hoc for this study, emerged as a critical factor, with 2 significant predictors related to it (P=.002 and P=.004) alongside impervious surface density with a significant predictor (P<.001). The multilinear regression model yielded a high F test score (F_{108}=4.75, P<.001), indicating a strong fit (R^2=0.5465). Furthermore, socioeconomic factors, including walking barefoot in houses with dirt floors and overcrowding, were significantly correlated with hookworm infection intensity (P<.001 and P=.001, respectively). We also used the multilinear regression model to calculate hookworm infection intensity (F_{110}=21.15, P<.001; R^2=0.4971).

Conclusions: Our study underscores the complexity of STH transmission, as villages with similar living conditions and environmental characteristics displayed varied STH prevalence and spatial distribution. Specific environmental factors, such as vegetation pattern and impervious surface density, played major roles in STH presence, demonstrating the crucial relationship between environmental factors and hookworm infection distribution. Moreover, our findings emphasize the significant influence of socioeconomic factors on hookworm infection intensity. By gaining insights into this complex interplay, our research contributes

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to a better understanding of STH transmission characteristics, thereby informing targeted public health interventions for effective control.

(JMIR Public Health Surveill 2023;9:e41568) doi: 10.2196/41568

KEYWORDS

soil-transmitted helminths; hookworm; prevalence; intensity: distribution: Iguazú; Argentina

Introduction

Soil-transmitted helminth (STH) infections are the most prevalent among the neglected tropical diseases (NTDs) worldwide, affecting over 1.5 billion people as of 2023 [1]. These diverse diseases have an enormous impact on individuals, families, and communities in low- and middle-income countries, and they constitute a serious obstacle to socioeconomic development and quality of life, leading to loss of productivity and exacerbating poverty [2]. NTDs are mainly prevalent in tropical and subtropical areas, where they mostly affect impoverished communities and disproportionately affect women and children [2,3]. STHs are intestinal parasites (IPs), with the most common species affecting humans being *Ascaris lumbricoides, Trichuris trichiura,* and the hookworms *Necator americanus* and *Ancylostoma duodenale* [4]. Although *Strongyloides stercoralis* is a common STH in Latin America and the Caribbean, it is not included in this group due to its complex life cycle and specific characteristics for diagnosis, quantification, and treatment [5,6].

Previous studies have shown the involvement of different factors in the transmission of STHs, including socioeconomic factors, such as the Human Development Index; nutritional and immunological factors; and environmental factors, such as the Normalized Difference Vegetation Index (NDVI) and the Enhanced Vegetation Index (EVI) [7-9]. All these factors exert an economic impact on the population and thus also play a role in the perpetuation of poverty [10,11].

Argentina has a heterogeneous prevalence of STHs (between 0% and 88.9%) throughout the country, and the northeast and northwest provinces of Misiones, Chaco, or Salta are identified as endemic [12-16], with varying rates of infection depending on socioeconomic status, sanitary and environmental conditions, and access to water [16-19]. Despite its high prevalence of STHs, Argentina does not currently have a deworming program as approved by the World Health Assembly (WHA) through resolution WHA54.19 [20].

The Misiones province is composed of 10,218 indigenous people from the Mbyá-Guaraní ethnic group distributed among 116 communities that live mostly in rural areas [21,22] under precarious and poor sanitary conditions, with high rates of malnutrition among children [17]. IPs are highly endemic in this area, especially among indigenous communities [23], with varying prevalence rates depending on age, hygiene habits, access to water and basic sanitation, and nutritional conditions, among others [16,17,24]. These factors can cause or aggravate malnutrition, leading to anemia and growth delays in children [18,25].

Geographic information system (GIS), remote sensing (RS), and digital elevation model (DEM) technologies provide information to identify and analyze determinants of disease distribution, thereby facilitating the development of risk prediction models in relation to environmental variables. This, in turn, aids the design of strategies to identify and prevent these infections. In this study, we collected parasitological and socioeconomic data from different Mbyá-Guaraní villages of Puerto Iguazú, Misiones, Argentina, as well as environmental variables, to determine the main factors associated with STH infection in this region.

Methods

Study Area

Puerto Iguazu is a city located in the province of Misiones in northeastern Argentina. It is naturally divided by the Paraná and Iguazú Rivers, which act as the physical borders between Argentina, Brazil, and Paraguay. According to the most recent national census data, the city has over 42,800 inhabitants [26].

This study was carried out in the rural area of Puerto Iguazú, around the city´s periphery, where Mbyá Guaraní indigenous communities have settled into 3 villages: Fortín Mbororé, Mini-Marangatú, and Yriapú. Previous studies in this area have shown a high prevalence of IPs, specifically STHs [12,16,27]. Moreover, these villages are adequately sized to enable the enrollment of enough participants to explore the relationship between STH infection and its determinants, with 200 families in Fortín Mbororé, 35 in Mini-Marangatú, and 100 in Yriapú (Figure 1).

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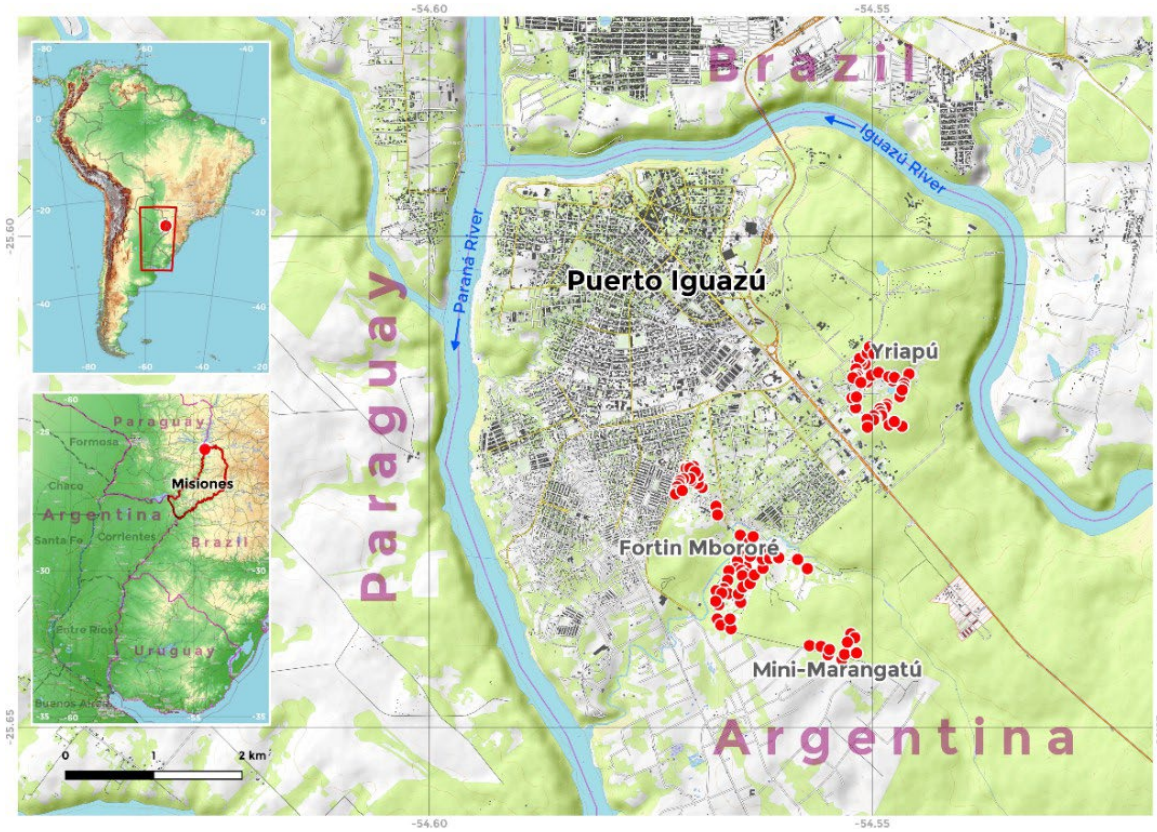
Figure 1. Map of the study area. Points represent each of the georeferenced households from the 3 villages included in the study, Fortín Mbororé, Mini-Marangatú, and Yriapú, which are adjacent to Puerto Iguazú, Misiones, Argentina. Map created using QGIS with overlay imagery from OpenTopoMap (CC-BY-SA) via the QuickMapServices plugin (version 0.19.29). Copyright belongs to OpenStreetMap contributors [28]. Map data are from August 27, 2021.



The family subsistence economy of the communities that participated in this study is from guided tours organized to visit the village, handcrafts, and social plans [12]. These communities share similar water and sanitation conditions and are homogeneous in their economic status, with low monthly incomes. Living conditions are characterized by a lack of water and sanitation [19]. Houses are scattered, surrounded by vegetation, and made of adobe bricks with unimproved roofs and dirt floors.

Study Design

Stool samples were collected from participants from all 3 villages. The households were visited, georeferenced, and characterized using a questionnaire. Stool containers were provided, along with verbal instructions on how to collect the samples, and retrieved on the following day. The fresh samples were transported without a fixative in a refrigerated icebox and kept at 4 °C in the lab until analysis within 24 hours of collection. The inclusion criteria were based on age (participants had to be at least 1 year of age) and willingness to participate, evidenced through the informed consent process. Individuals who lived or worked for long periods of time outside the study area and those with conditions that impaired an understanding of the consent process were excluded from the study. Considering that the area is in the tropical forest biome, which involves deforestation of tropical and subtropical forests for agriculture, the extension of grasslands may affect the viability of STH eggs and larvae. Nevertheless, several publications have suggested that the forest mass is in the process of forest transition, where the recovery of natural systems such as forests is taking place [29].

Ethics Approval

The institutional review board of the Ministry of Health of the Province of Misiones approved this study protocol and consent forms (171403/2018). Written consent was obtained from all the parents/guardians for children under 16 years of age. All individuals aged 16 years and older are considered adults in Argentina; therefore, written consent was obtained from them directly.

Parasitological Analysis

The collected stool samples were analyzed using the Ritchie concentration technique, Baermann concentration, and Kato-Katz technique to measure the infection intensity of STH, as previously described [12]. The parasitological parameters used were the prevalence of IPs and the intensity of STH infections in eggs per gram of feces.

Living Conditions and Environmental Characterization

Habits, customs, and household characteristics were analyzed at the household level using GIS. The variables, which were collected using a standardized questionnaire, are summarized in Table 1.

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Table 1. Variables analyzed at individual, household, and village levels.

Variables	Description	Results
Education	Education level of household inhabitants	From none to university level
Income	Source of income	Formal employment, public sector, tourism, animal farming, crafts, and social plans, among others
Animal farming	Presence of pets or animal farming	Dogs, cats, chickens, pigs, ducks, and other
Health coverage	Type of health insurance	Public system, private health insurance, and prepaid health insurance, among others
Household characteristics		
Roof	Type of material	Wood, branches, adobe, and metal sheets
Wall	Type of material	Wood, cement, dirt, bricks, and adobe
Floor	Type of material	Wood, dirt floor, cement, and smoothed floor
Electricity	Source of electricity	None, public network, generator, and other
Water source	For human consumption, cooking, or washing	Borehole, tap water, well, and other
Water treatment	Any type of treatment	Boiling, chemical treatment, and other
Excreta disposal	Excretal disposal	Open defecation or latrine
Cooking	Source of heat for cooking	Gas stove or oven, electric stove, wood stove, and other
Garbage disposal	Type of disposal	Municipal system, burning, burying, and other
Behavioral aspects		
Barefoot	Use of footwear	Yes or no
Hand washing	Practice of handwashing before eating and after defecation	Yes or no

Environmental and geographical data were collected using RS together with DEM. From the RS, the Soil-Adjusted Vegetation Index (SAVI), Vegetation Heterogeneity Index (VHI), Enhanced Normalized Difference Impervious Surfaces Index (ENDISI), and Bare Soil Index (BSI) were obtained. From the DEM, the Topographic Position Index (TPI) and Topographic Wetness Index (TWI) were calculated. The characteristics and sources of these indices are detailed in Table 2 and Multimedia Appendix 1.

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Table 2. Environmental and geographical indices used to determine their association with the presence of soil-transmitted helminths (STH) in villages from Puerto Iguazú, Misiones, Argentina.

Index	Source	Characteristics
Topographic Position Index [30]	30-m resolution DEM[a] from the IGN[b] [31]	• This index measures the altitude of a point with respect to its surrounding area. • Positive values indicate that the central point is located higher than its average surroundings (such as ridges and hilltops), negative values indicate a position lower than the average (valley and sinkholes), and a near-zero value indicates a flat or continuous slope. • The index used is a multiscale mean of 100-, 200- and 500-meter radii.
Topographic Wetness Index [32]	30-m resolution DEM from IGN [31]	• It identifies potential points of water accumulation (humidity) based on topographic elements. • This index is used as a proxy for the measurement of soil humidity.
Soil-Adjusted Vegetation Index [33]	2019 mean annual surface reflectance from Sentinel 2 imagery [34], retrieved and processed with GEE[c] [35]	• This is a modification of the Normal Density Vegetation Index, which corrects for the brightness of the soil when vegetation is scarce. • It is used to estimate the quantity, quality, and development of vegetation through RSd.
Vegetation Heterogeneity Index	2019 mean annual surface reflectance from Sentinel 2 imagery [34], retrieved and processed with GEE [35]	• The index is used to identify areas of more or less vegetation with respect to their surroundings (80 m radius). • The SD of these values was also mapped to show the heterogeneity of the vegetation in the study area. Low values indicate a subtle variation, while high levels show abrupt changes in the vegetation around each household.
Enhanced Normalized Difference Impervious Surfaces Index [36]	2019 mean annual surface reflectance from Sentinel 2 imagery [34], retrieved and processed with GEE [35]	• It detects impervious surfaces (buildings, asphalted roads, etc). • A threshold of 0.15 was applied to set a value of 1 for impervious areas and 0 for other surfaces. • This binary map was used to get a map with the imperviousness index within a radius of 500 m for each household, with continuous values from 0 to 1.
Bare Soil Index [37]	2019 mean annual surface reflectance from Sentinel 2 imagery [34], retrieved and processed with GEE [35]	• This index combines blue, red, and infrared bands to capture variations in the soil. • Short infrared and red bands are used to quantify the mineral composition of the soil, while blue bands and infrared bands are used for vegetation cover. • Values range between −1 and 1, with higher values indicating bare soil.

[a]DEM: digital elevation model.

[b]IGN: Instituto Geográfico Nacional (National Geographical Institute).

[c]GEE: Google Earth Engine.

[d]RS: remote sensing.

Statistical Analyses

Data were analyzed using Stata 12 software (StataCorp) and RStudio (R Foundation for Statistical Computing). Measures were evaluated using proportion with 95% CIs and means with SDs. The chi-square test was used to compare significant associations between different variables. The age variable was categorized into 5 groups: group 1 (0-5 years), group 2 (6-10 years), group 3 (11-20 years), group 4 (21-40 years), and group 5 (>40 years).

To determine the spatial distribution of STH infection in the study area, an algorithm based on the kernel density estimation (KDE) [38-40] technique was used to identify areas where infection was more prevalent than expected, assuming a homogenous distribution (null hypothesis), by calculating the difference from it using the SD as the unit of measurement. Therefore, positive or negative values indicated higher or lower values of infection than expected under the null hypothesis. With the main results and this same method (a quartic kernel shape and bandwidth of 200 m), the distribution of other variables was calculated: intensity of hookworm infection, households with dirt floors where inhabitants walked barefoot, and households with overcrowding. Variables that showed a significant correlation with the presence and intensity of hookworm infection were then used in a multiple linear regression analysis [41]. Predictors of infection were selected using a stepwise method that selected the best predictors using the Akaike information criterion. Values were considered significant at $P<.05$, with a 95% CI. The full statistical report is available in Multimedia Appendix 2.

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Results

Study Population

A total of 342 individuals from the 3 villages participated in this study and provided stool samples: 169 (49.4%) individuals from Fortin Mbororé, 125 (44.5%) from Yriapú, and 48 (14%) from Mini-Marangatú. The population distribution in the 3 communities was 53.8% (91/168) men and 46.2% (78/169) women in Fortin Mbororé, 56.8% (71/125) men and 43.2% (54/125) women in Yriapú, and 50% (24/48) for both sexes in Mini-Marangatú. The mean age of participants was 21 (SD 17.9) years in Fortin Mbororé, 10.4 (SD 11.43) years in Yriapú, and 15 (SD 12.21) years in Mini-Marangatú.

Prevalence of IPs

The overall prevalence of I Ps in the 3 villages was 95.8% (46/48) in Mini-Marangatú, 95.2% (119/125) in Yriapú, and 91.1% (154/169) in Fortín Mbororé. Protozoan infection ranged from 87.5% (42/48) in Mini-Marangatú to 81.6% (102/125) in Yriapú, while helminth infections were highest in Mini-Marangatú (44/448, 91.7%), followed by Yriapú (104/125, 83.2%) and lower in Fortin Mbororé (126/169, 74.6%) (Table 3). The STH prevalence was 68.1% (115/169) in Fortín Mbororé, 80.8% (101/125) in Yriapú, and 89.6% (43/48) in Mini-Marangatú. Infection caused by *T. trichiura* was only detected in Mini-Marangatú village, with only 1 (2.1%) case, and no *A. lumbricoides* infections were detected in this village. The most prevalent STH was hookworm, reaching statistically different infection rates (χ^2_2=7.6, *P*=.02) between the 3 villages: Mini-Marangatú (42/48, 87.5%), Yriapú (92/125, 73.6%), and Fortín Mbororé (114/169, 67.5%). The descriptive characteristics of STH infections in the 3 villages are provided in Table 3.

Table 3. Descriptive characteristics and prevalence of intestinal parasites in individuals from Fortin Mbororé, Yriapú, and Mini-Marangatú.

Characteristics	Fortin Mbororé	Yriapú	Mini-Marangatú
Age (years), mean (SD)	21 (17.9)	10.4 (11.43)	15 (12.21)
Age range	1-87	1-54	1-49
Gender, n (%)			
Female	78 (46.2)	54 (43.2)	24 (50)
Male	91 (53.8)	71 (56.8)	24 (50)
Prevalence of protozoans, n (%); range (95% CI)	138 (81.7); 75-86.8	102 (81.6); 73.7-87.5	42 (87.5); 74.2-94.4
Entamoeba coli	69 (40.8); 33.6-48.5	73 (58.4); 49.5-66.8	22 (45.8);32-60.4
Entamoeba complex	18 (10.7); 6.8-16.3	12 (9.6);5.5-16.3	3 (6.3);1.9-18.3
Entamoeba hartmanni	19 (11.2); 7.3-17	24 (19.2); 13.1-27.2	12 (25); 14.5-39.6
Endolimax nana	34 (20.1); 14.7-26.9	13 (10.4); 6.1-17.2	10 (20.8); 11.3-35.2
Iodamoeba butschlii	10 (5.9); 3.2-10.7	2 (1.6);0.4-6.3	1 (2.1); 0.3-14.2
Chilomastix mesnili	6 (3.6); 1.6-7.7	16 (12.8); 7.9-20	6 (12.5); 5.6-25.8
Giardia intestinalis	40 (23.7); 17.8-30.7	38 (30.4); 22.9-39.1	14 (29.2); 17.8-44
Blastocystis spp.	93 (55); 47.4-62.4	56 (44.8); 36.2-53.7	32 (66.7); 51.8-78.8
Prevalence of helminths, n (%); range (95% CI)	126 (74.6); 67.4-80.6	104 (83.2); 75.5-88.8	44 (91.7); 79.2-96.9
Enterobius vermicularis	3 (1.8); 0.6-5.4	5 (4); 1.7-9.4	—[a]
Hymenolepis nana	28 (16.6); 11.6-23	36 (28.8); 21.5-37.5	6 (12.5); 5.6-25.8
Trichuris trichiura	—	—	1 (2.1); 0.3-14.2
Ascaris lumbricoides	3 (1.8); 0.6-5.4	21 (17.2); 11.4-25.1	—
Hookworm	114 (67.5); 60-74.2	92 (73.6); 65.1-80.7	42 (87.5); 74.2-94.4
Strongyloides stercoralis	11 (6.5); 3.6-11.4	44 (35.48); 27.5-44.4	14 (29.2); 17.8-44

[a]—: not available.

Hookworm infection was higher in the age groups ranging from 0 to 5 years and from 6 to 10 years in Yriapú and lower in Fortin Mbororé, especially in the western area located near the urban area of Puerto Iguazú. Moreover, statistical differences between age groups were observed within Fortin Mbororé (χ^2_4=27.9, *P*<.001) and Mini-Marangatú (χ^2_4=17.5, *P*=.002). Mixed infections with different STH species were also observed (Tables 4 and 5), together with the intensity of infection. *Trichuris trichiura* and *A. lumbricoides* were present mainly as light-intensity infections. On the other hand, heavy-intensity hookworm infections were detected in all 3 villages. The highest rate of individuals with heavy infections (22/42, 52.4%) were found in Mini-Marangatú, but no statistical differences were observed between the types of intensity.

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Table 4. Intensity of soil-transmitted helminth (STH) infections in individuals from Fortin Mbororé, Yriapú, and Mini-Marangatú.

Infections	Fortin Mbororé, n (%)			Yriapú, n (%)			Mini-Marangatú, n (%)		
	Light	Moderate	Heavy	Light	Moderate	Heavy	Light	Moderate	Heavy
Hookworm	67 (58.8)	12 (10.5)	35 (30.7)	61 (66.3)	11 (12)	20 (21.7)	15 (35.7)	5 (11.9)	22 (52.4)
A. lumbricoides	2 (66.7)	—[a]	1 (33.3)	12 (57.1)	6 (28.6)	3 (14.3)	—	—	—
T. trichiura	—	—	—	—	—	—	1 (100)	—	—

[a]—: not available.

Table 5. Number of mixed soil-transmitted helminth (STH) infections in individuals from Fortin Mbororé, Yriapú, and Mini-Marangatú.

Mixed STH infections	Fortin Mbororé, n (%)	Yriapú, n (%)	Mini-Marangatú, n (%)
Hookworm/*S. stercoralis*	10 (76.9)	37 (56.9)	13 (92.9)
Hookworm/*A. lumbricoides*	3 (23.1)	19 (29.2)	—[a]
Hookworm/*S. stercoralis*/*A. lumbricoides*	—	9 (13.9)	—
Hookworm/*S. stercoralis*/*T. trichiura*	—	—	1 (7.1)

[a]—: not available.

Living Conditions

Living conditions between the villages were usually similar. The average number of inhabitants per household was 5.3 for Fortin Mbororé, 5.1 for Yriapú, and 5.8 for Mini-Marangatú. Most households had a single room for sleeping; therefore, overcrowding was common. Generally, houses were made of wooden walls and dirt floors—90% (43/48) in Mini-Marangatú and 50% (61/121) in Yriapú. In the case of Fortin Mbororé, this figure was reduced to 35% (58/166) since 40% (66/166) of the households had cement floors. Practically the entire population, both children and adults from all 3 villages, walked barefoot. Although 22.9% (38/166) of households practiced open defecation, most had a latrine that consisted of a simple ground excavation. With respect to the source of drinking water, all the families in Mini-Marangatú obtained their water from boreholes, along with 75% (92/121) in Yriapú and 49% (81/166) in Fortin Mbororé. Family incomes were low and precarious, mostly coming from animal farming, crafts, or social plans. In the newer village of Mini-Marangatú, which branched off from Fortin Mbororé, 60% (99/166) of the families obtained their income from crafts.

Only the type of floor was observed to be associated with hookworm transmission, with a significantly higher prevalence of hookworm found in individuals from Yriapú village living in dirt floor houses (χ^2_3=8.8, P=.03). Lack of sanitation and hygiene, water source, the use of a latrine with simple ground excavation, and source of income were not related to a higher prevalence of hookworms. Walking barefoot and living in overcrowding conditions were significantly related to the intensity of hookworm infection (P<.001 and P=.003, respectively; F_{110}=46.2, P<.001), indicating the significance of the model (R^2=0.46).

Environmental Characterization

Figure 2 shows the distribution of the different environmental indices in the study area. TPI helps discriminate between areas that are depressed and those that have some prominence and thus are less prone to accumulating water, and TWI detects hydrological flow paths and thus proximity to streaming water and other bodies of water. The values of these 2 indices indicate that the study area was irregular with depressed areas and small hills (Figure 2A), with many water courses running through it, including the Mbocay stream (Figure 2B). Yriapú is located at the edge of a slightly depressed area, while Fortin Mbororé is divided into 2 parts by the Mbocay stream. Both sides of the village are on flat ground. Mini-Marangatú is located close to the crest of a small hill.

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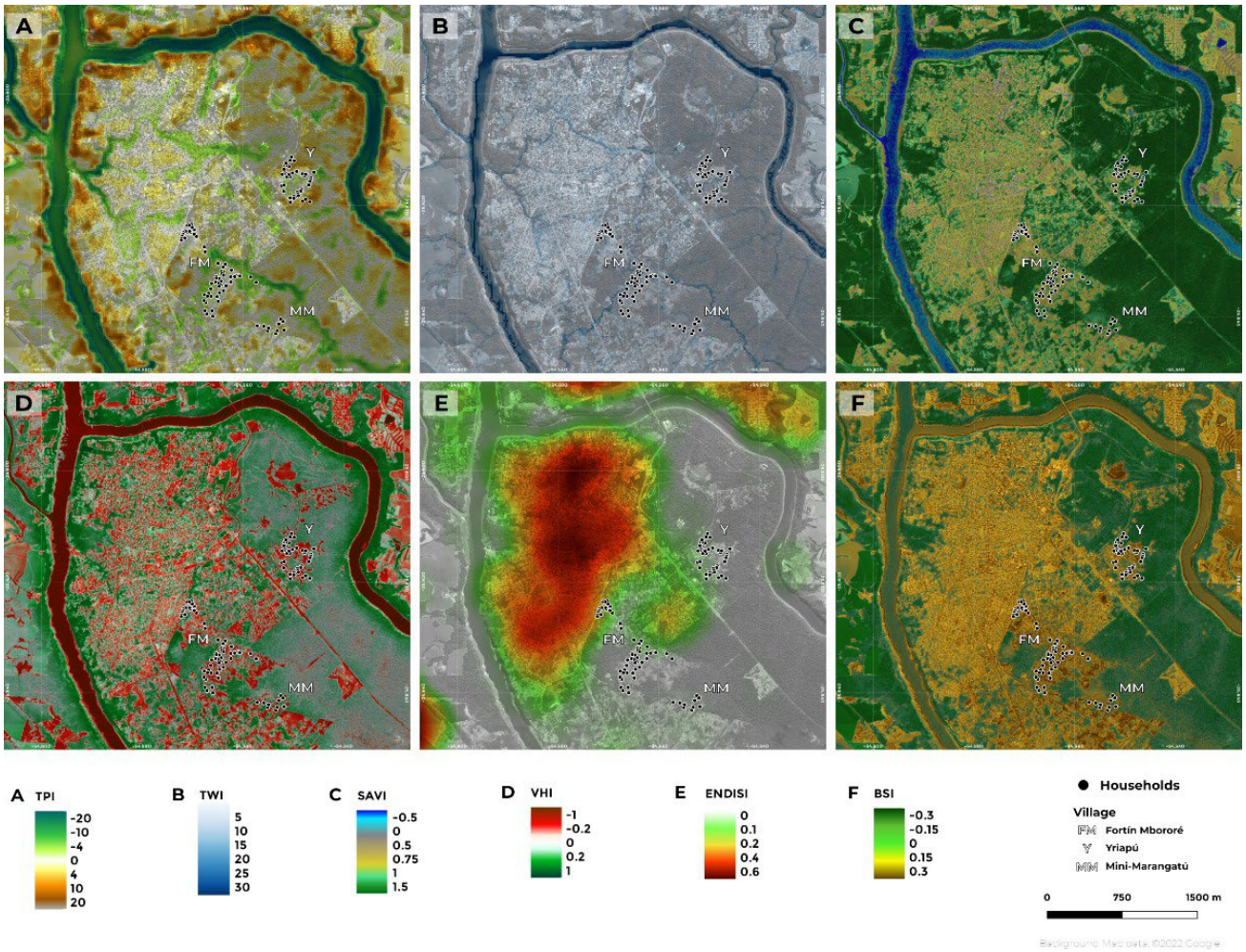
Figure 2. Distribution of the different indexes used in the study area from Puerto Iguazú, Misiones, Argentina. (A) Topographic Position Index (TPI). (B) Topographic Wetness Index (TWI). (C). Soil-Adjusted Vegetation Index (SAVI). (D) Vegetation Heterogeneity Index (VHI). (E) Enhanced Normalized Difference Impervious Surface Index (ENDISI). (F) Bare Soil Index (BSI). Map created using QGIS with background imagery from Google Maps via the QuickMapServices plugin (version 0.19.29). Copyright 2021 Google. Map data 2021 Google.



For the presence of vegetation, SAVI and VHI were used, with SAVI measuring vigor and VHI estimating the heterogeneity of the vegetation landscape. As depicted in Figures 2C and D, some differences in the distribution of the SAVI were observed between the villages. Western Fortin Mbororé had lower values than the central area of the village, while both Mini-Marangatú and Yriapú were surrounded by more vigorous vegetation (Figure 2C). Through the VHI, the difference between bare soil and the presence of vegetation was greatest in Mini-Marangatú and very small in Fortin Mbororé. The other 2 indices, ENDISI and BSI, were used to indicate the presence of bare soil (Figure 2E and 2F) but with different focuses: ENDISI on urbanized areas and BSI on natural soil. Again, Fortin Mbororé had a greater presence of bare soil around the houses, especially in the western area where households were located close to the urban city of Puerto Iguazú, whereas both Yriapú and Mini-Marangatú had patches of bare soil and vegetation.

Spatial Distribution of STH

The KDE technique was used to analyze the spatial distribution of STH in the study area and observe differences between the villages. As shown in Figure 3A, the distribution of hookworm-positive individuals was not entirely homogenous, and the differences in the SD were not pronounced and were present throughout the entire study area. There was a slight concentration of cases in Mini-Marangatú, followed by Yriapú. Some differences were observed within Fortin Mbororé, where households closer to the urbanized area of Puerto Iguazú (northwest) had lower values than those farther away (southwest).

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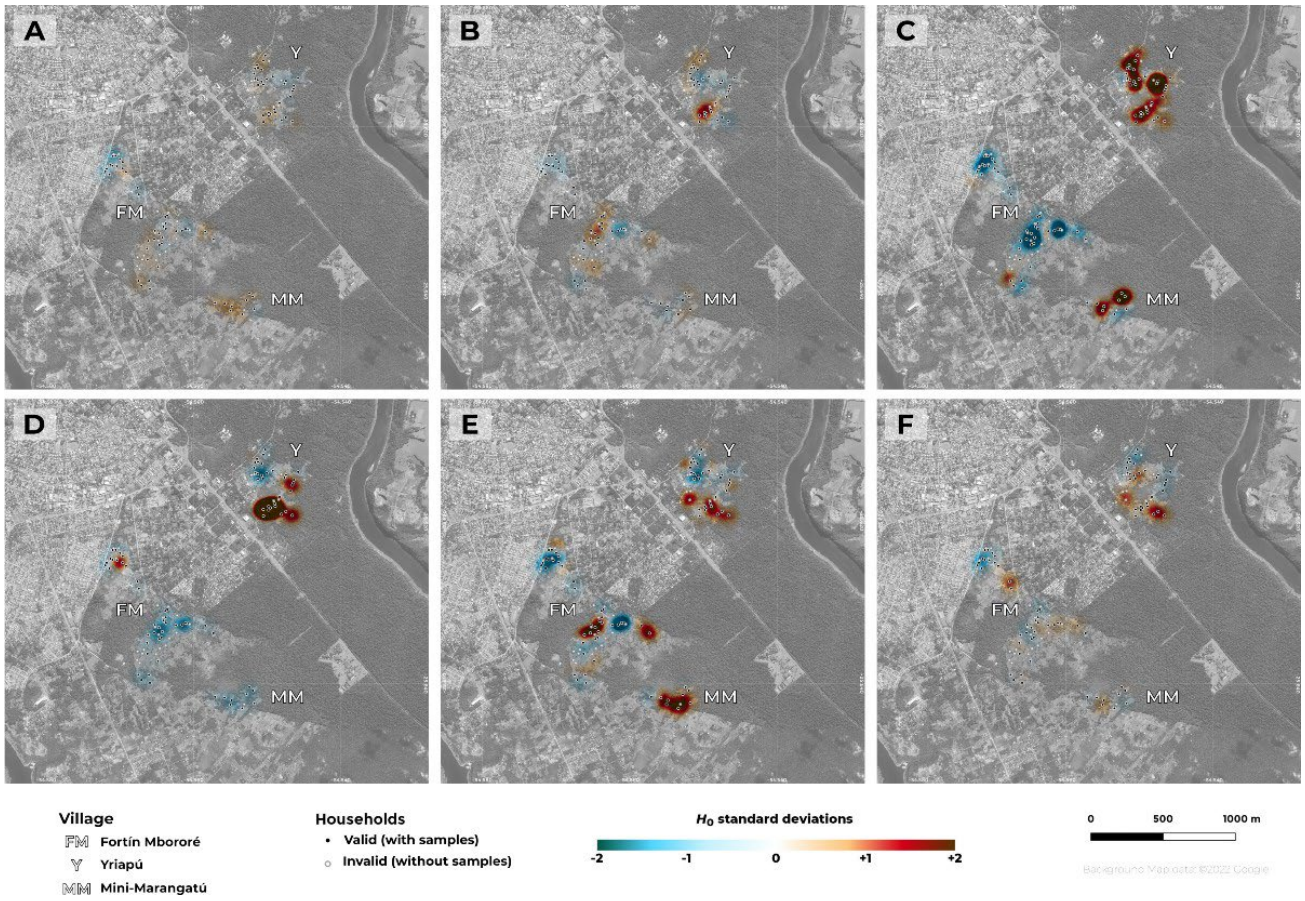
Figure 3. Spatial distribution obtained using the kernel density estimation (KDE) technique for (A) hookworm infection, (B) hookworm intensity, (C) Strongyloides stercoralis infection, (D) Ascaris lumbricoides infection, (E) households with dirt floors where inhabitants walk barefoot, and (F) households with overcrowding. Map created using QGIS with background imagery from Google Maps via the QuickMapServices plugin (version 0.19.29). Copyright 2021 Google. Map data 2021 Google.



With respect to the intensity of hookworm infection (Figure 3B), positive cases from Yriapú were mostly of light intensity, while higher-intensity cases were clustered only in the southern area of Yriapú. Although the prevalence of other STH was lower than that of hookworm, the KDE analysis also showed a heterogeneous distribution, with a marked difference in the SD of *S. stercoralis*, showing a high concentration of positive cases in Yriapú and Mini-Marangatú compared to Fortin Mbororé (Figure 3C). *Ascaris lumbricoides* infections were detected mostly in Yriapú village (Figure 3D).

Figure 3E shows the distribution of households with dirt floors and individuals who usually walk barefoot. Given that many houses in Fortin Mbororé had cement floors, the difference in the SD was more evident in Mini-Marangatú and southwestern Fortin Mbororé compared to Yriapú. The distribution of households with overcrowding (Figure 3F) was homogeneous in the study area, although there was a clustering in Yriapú.

The multivariate model used to identify the factors most related to the distribution of hookworm in the study area selected the following predictors: mean VHI, SD of VHI, and ENDISI and households with dirt floors and individuals who walk barefoot. In this model, only the environmental variables were significantly associated *(P*<.001) with the presence of hookworm infection, which explained 56% (248/342) of the variability observed in the distribution of cases in the study area (F_{108}=34.75, *P*<.001). The predictors for hookworm intensity selected by the multivariate model were the distribution of dirt floor households with individuals that walked barefoot and households with overcrowding. Both variables showed a significant association (*P*<.001) with the intensity of hookworm infection and predicted 45% (153/342) of the variability observed in the distribution of the cases.

Discussion

Principal Findings

This study uncovers factors associated with the presence and intensity of STH infection, especially hookworm, in rural areas of Puerto Iguazú. The high prevalence observed coincides with previous studies in other rural areas of the region [5,15,42,43], as well as a higher prevalence in the infant population [3,5]. Hookworm prevalence was above 70% in all villages (114/169 in Fortín Mbororé, 92/125 in Yriapú, and 42/48 in Mini-Marangatú), with the highest prevalence observed in Mini-Marangatú, located in the easternmost area.

In the multivariate analysis for hookworm infection, barefoot walking and overcrowding were implicated in an increase in the intensity of infection, while the type of household floor material was associated with an increase in transmission. These results coincide with those already observed in other studies [12,44,45]. Although other studies indicate that hygienic conditions, water, type of latrine, and level of education are related to a higher hookworm prevalence [16,19,46-48], in this

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study, these factors did not show statistical significance. These different results may be due to the uniformity of these variables throughout the different villages since most of the households shared the same characteristics. Moreover, although the villages were independent population centers managed by their own community leader or cacique, they were relatively close to each other, thus sharing similar environmental and economic conditions.

Since hygienic conditions have been shown to greatly influence transmission through the fecal-oral route [19], as in the case of *A. lumbricoides*, the proximity to the Mbocay stream in the villages of Fortin Mbororé (eastern zone) and Mini-Marangatú could have generated different *A. lumbricoides* infection rates between the villages. In addition, the high density of construction observed in the western area of Fortin Mbororé, through the ENDISI, may have affected the transmission of this parasite, given that low humidity can hinder egg embryonation [9].

With respect to environmental factors and their predictors, previous studies have highlighted the role of humidity, temperature, and soil type in the transmission of STH [16,45,48,49]. Higher humidity and temperature have been shown to be favorable conditions for the survival of heterogonic stages of *S. stercoralis* [50,51]. A joint analysis of the TPI and TWI allowed us to determine that the village of Yriapú is situated in a depressed area with higher humidity accumulation, which could be a factor behind the high prevalence of *S. stercoralis* observed in this village. Available studies also show the association of humidity and vegetation indices in the prevalence of *S. stercoralis* [52].

To analyze the effect of vegetation on the transmission of STH, the VHI, which was developed specifically for this study, helped identify areas with more or less vegetation with respect to each surrounding household. The algorithm is similar to the TPI; however, instead of elevation, the data source is a vegetational index (in this case, SAVI). High values of this index around households were associated with increased hookworm infection, which indicates the importance not only of the vigorousness of vegetation but also its distribution pattern in the landscape of the study area. High values of this index around the Mini-Marangatú households coincided with the highest prevalence of hookworm in this area. These vegetation and humidity indices, which demonstrate the influence of vegetation on hookworm distribution, coincided with the risk of hookworm infection in areas of VHI values reported in other studies [44,49,53].

Previous studies suggested that infection is more probable in households surrounded by bare soil, among other factors [52,54-56]. Fortin Mbororé has bare soil surrounding its houses, especially in the western area located close to the urbanized area of the city. Although bare soil is an environmental factor that has been shown to be associated with STH infections, this area had lower hookworm infection rates.

The ENDISI aids in detecting impervious surfaces, particularly the amount of water stored. The survival of hookworm larvae depends on the soil's water-retaining properties. When the soil dries out, the water is restricted to the thin film around individual soil particles, and the infective larval stage remains quiescent in the moisture film until it makes contact with its host [57]. The lower hookworm infection rates observed in Fortin Mbororé may be because this area is highly influenced by the ENDISI since urban development and infrastructure density modify soil permeability and moisture. This can have a negative effect on the persistence and survival of this type of parasite, which requires humidity to survive and develop its larval stages [51,58].

In general, villages sharing similar living conditions and environmental characteristics contributed to the high infection rates observed. This study demonstrated that living conditions play a role in the intensity of hookworm infection, and environmental variables are significantly associated with its presence. However, the specific differences observed in certain areas aid in elucidating how human development and social and sanitary conditions may influence lower infection rates among individuals and villages located in endemic areas. Evidence from previous studies and the results obtained herein show that environmental factors such as temperature, vegetation, and humidity play a role in the presence and maintenance of STHs in the soil. Therefore, environmental changes caused by climate change could modify the distribution of these parasites, although deforestation, bare soil, high temperature, and lack of humidity could restrict their presence.

The limitations of this study include the sensitivity of the techniques used to detect STHs since low burdens of infection could be missed. Additionally, in this area, hookworm was the most prevalent STH; therefore, studies conducted in areas with a greater presence of other species of the group would be beneficial. Further studies in more heterogeneous communities with similar environmental characteristics could help aid our understanding of the socioeconomic and building characteristics that determine the presence and intensity of STH infections.

Conclusion

This study, conducted in an endemic area for STHs, especially hookworms, reinforces the importance of the environment in the establishment of this group of parasites, which require passage through the soil for their development. Additionally, we observed that living conditions, like walking barefoot, having dirt floors, or overcrowding, are associated with the intensity of hookworm infection. Given that environmental variables cannot be changed, it is important to work on those aspects that can be modified, such as the characteristics of the house, the availability of water and sanitation, and periodic deworming as suggested by the World Health Organization deworming guidelines [59].

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Acknowledgments

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Data Availability

Data supporting the conclusions of this article are included within the article. The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

Authors' Contributions

EC carried out laboratory analyses, analyzed and interpreted the data, and wrote the draft of the manuscript. CG was involved in the acquisition of data and community relations with the cacique. LS developed, analyzed, and interpreted the mathematical models as well as the figures. CMA was involved in the conception and design of the study, analysis, and interpretation, and revising the final draft of the manuscript. MVP was involved in the conception and design of the study, acquisition of data, analysis and interpretation, and revision of the final draft of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Environmental index formulas.
[DOCX File , 16 KB-Multimedia Appendix 1]

Multimedia Appendix 2

Report of the statistical models used.
[DOCX File , 20 KB-Multimedia Appendix 2]

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Abbreviations

BSI: Bare Soil Index
DEM: digital elevation model
ENDISI: Enhanced Normalized Difference Impervious Surfaces Index
EVI: Enhanced Vegetation Index
GIS: geographic information system
IP: intestinal parasite
KDE: kernel density estimation
NDVI: Normalized Difference Vegetation Index
NTD: neglected tropical disease
RS: remote sensing
SAVI: Soil-Adjusted Vegetation Index
STH: soil-transmitted helminth **TPI:** Topographic Position Index **TWI:** Topographic Wetness Index
VHI: Vegetation Heterogeneity Index
WHA: World Health Assembly

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3.3 Prevalence of Intestinal Parasites, Protozoans and Soil-Transmitted Helminths, in Children from Communities of Northern Argentina after the Interruption of Deworming

Ernesto Candela, Rubén Oscar Cimino, Leonardo Sandon, Carla Muñoz-Antoli, María Victoria Periago

Parasitologia. 2024, 4.

Resumen

Los helmintos transmitidos por el suelo (STHs) son un grupo de parásitos que se encuentran distribuidos mundialmente y constituyen la enfermedad desatendida (ETD) de mayor prevalencia en América Latina y el Caribe (LAC); su presencia está asociada a problemas de salud y desarrollo. En Argentina, su distribución es heterogénea, existiendo zonas altamente endémicas en el norte del país. La Organización Mundial de la Salud (OMS) recomienda la desparasitación masiva en los niños como estrategia de primera línea para la prevención y el control de las STHs y, recientemente, también fomenta el desarrollo de pruebas de diagnóstico más sensibles. El objetivo de este estudio fue determinar la prevalencia de STHs en Tartagal (Salta, Argentina) tras cuatro años de interrupción en la desparasitación con albendazol e ivermectina. Se analizaron 437 muestras fecales mediante técnicas coprológicas estándar, de las cuales se seleccionaron un subconjunto para tipificar molecularmente los parásitos protozoarios; 257 muestras de sangre se analizaron para determinar la presencia de anticuerpos específicos contra el STH *Strongyloides stercoralis*. Las especies de protozoos más prevalentes fueron G. *intestinalis* (19.6-49.2%) y *B. hominis* (19.1-38.5%). La caracterización molecular permitió evidenciar posibles vías de transmisión zoonótica entre humanos para *Giardia intestinalis* o *Blastocystis* spp., mientras que la serología para *S. stercoralis* demostró ser una herramienta de cribado útil para el seguimiento de este parásito tras el tratamiento. En general, se observó una disminución de la prevalencia de STH en la zona, del 60% al 2.9-20% para los anquilostomas y del 51% al 1-9.3% para *S. stercoralis* cuatro años después del tratamiento, lo que demuestra la eficacia y duración del tratamiento antihelmíntico con estos dos fármacos.

Palabras clave: Helmintiasis transmitidas por el suelo, parásitos intestinales, NIE-ELISA, *Giardia intestinalis*, *Blastocystis* spp, Tartagal, Salta, Argentina.

parasitologia

MDPI

Article

Prevalence of Intestinal Parasites, Protozoans and Soil-Transmitted Helminths, in Children from Communities of Northern Argentina after the Interruption of Deworming

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Abstract: Soil-transmitted helminths (STHs) are a group of parasites that are globally distributed and are the most prevalent neglected disease (NTD) in Latin America and the Caribbean (LAC); their presence is associated with health and development problems. In Argentina, their distribution is heterogenous, and there are highly endemic areas in the north of the country. The World Health Organization (WHO) recommends the mass deworming of children as a first-line strategy for the prevention and control of STHs and recently also encourage the development of more sensitive diagnostic tests. The aim of this study was to determine the prevalence of STHs in Tartagal (Salta, Argentina) after four years of deworming interruption with albendazole and ivermectin. A total of 437 fecal samples were analyzed using standard coprological techniques, a subset of which were selected to molecularly typify protozoan parasites; 257 blood samples were analyzed for the presence of specific antibodies to the STH Strongyloides stercoralis. The most prevalent protozoan species were *G. intestinalis* (19.6–49.2%) and *B. hominis* (19.1–38.5%). Molecular characterization allowed us to evidence possible zoonotic or human-to-human transmission pathways for *Giardia intestinalis* or *Blastocystis* spp., while serology for *S. stercoralis* proved to be a useful screening tool for monitoring this parasite after treatment. In general, a decrease in the prevalence of STHs was observed in the area, from 60% to 2.9–20% for hookworms and from 51% to 1–9.3% for *S. stercoralis* four years after treatment, demonstrating the effectiveness and duration of anthelmintic treatment with these two drugs.

Keywords: soil-transmitted helminths; intestinal parasites; NIE-ELISA; *Giardia intestinalis*; *Blastocystis* spp.; Tartagal; Salta; Argentina

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1. Introduction

Soil-transmitted helminths (STHs) are a group of parasites that comprises *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, and hookworms. They are the most prevalent neglected tropical disease (NTD) in Latin America and the Caribbean (LAC), are considered the most common infection of humankind [1,2], and are transmitted by eggs present in human feces that contaminate the soil in areas with poor sanitation systems. Several studies have highlighted the importance of these infections and the health problems associated, mainly related to children's nutrition and growth, and how the environment and sanitary conditions influence their prevalence and distribution, as well as their impact on population development and the perpetuation of poverty [1,3,4].

The endemicity of these parasites in Argentina and their different prevalences, especially in the northern regions of the country, have been observed [2,5]. Preventive chemotherapy (PC) through the mass drug administration (MDA) of albendazole or mebendazole is recommended by the World Health Organization (WHO) as a first-line strategy for the prevention and control of STHs [6]. Although the National Ministry of Health of Argentina implemented a nationwide MDA program (2005–2007) with mebendazole, it was short lived, and currently, there aren't any active deworming plans at the provincial or national level in the country.

Among STHs, *S. stercoralis* infections may be underestimated due to the low sensitivity of standard diagnostic methods [7,8] and the particularity of the parasite's life cycle, with autoinfection cycles where L3 larvae reinfect the host through the intestinal mucosa and the perianal skin, leading to persistent and lifelong infections [5,9]. Recently, the WHO categorized *S. stercoralis* as an STH, particularly regarding preventive chemotherapy, and encourages the development of more sensitive and specific rapid diagnostic tests, as well as biomarkers and procedures for disease control [10]. In this regard, previous studies have suggested that the use of serological techniques may solve the problem of having to use additional and specific parasitological methods for *S. stercoralis* for diagnosis and follow-up [5,11].

One of the areas with a high prevalence of intestinal parasites (IPs), and specifically STHs, in Argentina is in the northern province of Salta, bordering Bolivia. Both parasitological and serological studies conducted in the past have demonstrated the presence of these IPs [1,2,12]. In addition, the Ministry of Public Health of Salta launched a triennial antiparasitic plan with albendazole for children aged 2–15 years old (2013–2015) in this area to mitigate the transmission of parasites through periodic deworming [13]; ivermectin was also used for deworming in Tartagal, given the presence of *S. stercoralis* in the area [1,2]. Nonetheless, although these studies reported the presence of protozoan parasites such as *Giardia intestinalis* and *Blastocystis* spp., molecular typification has not been carried out and only one study has detected the presence of *Entamoeba histolytica* through molecular techniques (in Orán, Salta province) [14].

All of these parasitic infections associated with health, control, and distribution related to human behavior should unite multidisciplinary efforts under the concept of One Health to try to improve their diagnosis, control, and prevention. Given the previous work conducted in this area, the main objective of this study was to determine the current prevalence of STHs, as well as the prevalence of other IPs, using standard coprology and *S. stercoralis* NIE-ELISA. We also evaluated the long-term effectiveness of the STH deworming program that was implemented previously and up to 2018 [2,5,13] and determine the need for MDA specifically for STHs after deworming program interruption. Finally, molecular methods were used to determine the species, types, or subtypes of the most important species of protozoans present in the study area.

2. Results

2.1. Study Population

A total of six communities were visited (Km 3, Km 4, Km 5, Km 6, Lapacho, and Las Moras) and 580 plastic containers for fecal collection were distributed. A total of 437 fecal samples were returned (75.3% participation). For the serological study, 257 samples were obtained from four communities (n = 405), with a participation rate of 63.5%. Population distribution ranged from 41.5% to 59.5% females and 40.5% to 58.5% males depending on the community. Participants had a mean age of 6.5 to 7.6 years and an age range of 1–15 years. Descriptive characteristics are detailed in Table 1.

Table 1. Descriptive characteristics and prevalence of intestinal parasites in individuals aged between 1 and 15 years from different communities in Tartagal located along National Route 86, in the Department of San José de San Martín, Province of Salta, Argentina.

	Km 3	**Km 4**	**Km 5**	**Km 6**	**Lapacho**	**Las Moras**
Age. mean ± SD	7.2 ± 4.2	6.5 ± 3.9	7.6 ± 4.6	6.9 ± 3.9	6.9 ± 4.2	6.8 ± 3.9
Gender. *n* (%)						
Female	43 (57.3)	29 (52.7)	25 (59.5)	50 (51.5)	51 (49.5)	27 (41.5)
Male	32 (42.7)	26 (47.3)	17 (40.5)	47 (48. 5)	52 (50.5)	38 (58.5)
N	75	55	42	97	103	65
Prevalence of protozoans. *n* (%) [CI 95%]	54 (72) [60.6-81.2]	47 (85.4) [73.0-92.7]	28 (66.7) [50.6-79.6]	61 (62.9) [52.7-72.0]	73 (70.9) [61.2-78.9]	52 (80) [68.2-88.2]
Entamoeba coli	22 (29.3) [20.0-40.8]	23 (41.8) [29.2-55.5]	15 (35.7) [22.3-51.8]	31 (32) [23.3-42.0]	39 (37.9) [28.9-47.7]	25 (38.5) [27.2-51.1]
Entamoeba complex	7 (9.3) [4.4-18.6]	3 (5.5) [1.7-16.1]	2 (4.8) [1.1-18.0]	7 (7.2) [3.4-14.5]	2 (1.9) [0.5-7.6]	5 (7.7) [3.2-17.5]
Entamoeba hartmanni	12 (16) [9.2-26.4]	5 (9.1) [3.7-20.5]	3 (7.1) [2.2-20.7]	33 (34) [25.1-44.2]	13 (12.6) [7.4-20.7]	15 (23.1) [14.2-35.2]
Endolimax nana	8 (10.7) [5.3-20.2]	5 (9.1) [3.7-20.5]	5 (11.9) [4.9-26-4]	13 (13.4) [7.9-21.9]	14 (13.6) [8.1-21.8]	14 (21.5) [13.0-33.5]
Iodamoeba butschlii	-	-	-	-	2 (1.9) [0.5-7.6]	-
Chilomastix mesnili	8 (10.7) [5.3-20.2]	9 (16.4) [8.6- 29.0]	-	7 (7.2) [3.4-14.5]	1 (1) [0.1-6.8]	2 (3.1) [0.7-11.9]
Giardia intestinalis	24 (32) [22.3-43.6]	23 (41.8) [29.2- 55.5]	15 (35.7) [22.3-51.8]	19 (19.6) [12.8-28.9]	26 (25.2) [17.7-34.7]	32 (49.2) [37.0-61.5]
Blastocystis spp.	22 (29.3) [20.0-40.8]	15 (27.3) [16.9-40.9]	8 (19.1) [9.5-34.4]	19 (19.6) [12.8- 28.9]	28 (27.2) [19.4-36.7]	25 (38.5) [27.2-51.1]
Helminths	41 (54.7) [43.1-65.6]	28 (50.91) [37.5-64.2]	11 (26.2) [14.8-42.1]	69 (71.1) [61.2-79.4]	38 (36.9) [28.1-46.8]	29 (44.6) [32.8-57.1]
Enterobius vermicularis	2 (2.7) [0.6-10.3]	2 (3.6) [0.9-13.9]	-	5 (5.2) [2.1-12.0]	3 (2.9) [0.9-8.8]	2 (3.1) [0.7-11.9]
Hymenolepis nana	24 (32) [22.3-43.6]	18 (32.7) [21.4-46.5]	6 (14.3) [6.3-29.1]	24 (24.7) [17.1-34.5]	32 (31.1) [22.8-40.8]	18 (27.7) [18.0-40.1]
Trichuris trichiura	-	-	-	-	-	-
Ascaris lumbricoides	-	1 (1.8) [0.2-12.5]	-	14 (14.4) [8.7-23.1]	-	-
Hookworms	15 (20) [12.3-30.8]	9 (16.4) [8.6-29.0]	6 (14.3) [6.3-29.1]	50 (51.6) [41.5-61.5]	3 (2.9) [0.9-8.8]	13 (20) [11.8-31.8]
Strongyloides stercoralis	7 (9.3) [4.4-18.6]	2 (3.6) [0.9-13.9]	-	10 (10.3) [5.6-18.3]	1 (1) [0.1-6.8]	3 (4.2) [1.5-13.7]
STH	20 (26.7) [17.7-38.0]	10 (18.2) [9.9-31.1]	6 (14.3) [6.3-29.1]	57 (58.8) [48.6-68.3]	4 (3.9) [1.4-10.0]	14 (21.5) [13.0-33.5]
Total positives	63 (84) [73.6-90.8]	51 (92.7) [81.7- 97.3]	34 (80.9) [65.6-90.5]	90 (92.8) [85.5-96.6]	81 (78.6) [69.5-85.6]	54 (83.1) [71.6-90.5]

2.2. Prevalence of Intestinal Parasites

The overall prevalence of IPs in the communities ranged from 78.6% to 92.8%, and many of them were polyparasitized with more than one species (35.7% to 70.8%). The prevalence of protozoan infection ranged from 62.9% to 85.4%, while helminth infections ranged from 26.2% to 71.1%. The most prevalent protozoan species were *G. intestinalis* (19.6–49.2%) and *B. hominis* (19.1–38.5%); *Hymenolepis nana* was the most common helminth, with a prevalence of between 14.3% and 32.7%. The cumulative STH prevalence throughout the study ranged from 3.9% to 58.8%; the species found were hookworm (2.9–51.6%), *S. stercoralis* (1–10.3%), and *A. lumbricoides* (1.8–14.4%). The latter was found in only two of the six groups, while hookworm was the most prevalent. More specifically, hookworm

was most prevalent in the community of Km 6 ($p < 0.001$). Concerning the results of the serological analysis, 96.1% (247/257) of the participants were negative, and only 72 of the participants had matching parasitological and serological results. Of these, 2 were found to be positive by both coprological and serological techniques, while eight were positive only according to serology, with negative coprological results. Additionally, 23 participants were found to be infected with *S. stercoralis* through stool examination, but these lacked a blood sample, so serological evaluation using NIE−ELISA was not possible.

2.3. Molecular Characterization of Giardia instestinalis Isolates

Through the standard coprological techniques used, 139 samples were found to be positive for *Giardia* spp., of which 41 could be preserved in ethanol (29.5%) for molecular analysis, and 37 of the 41 DNA samples (90%) were successfully amplified at the β-giardin locus (described in Additional file 1: Table S1). Sequence analyses revealed the presence of assemblages A (24.3%; 9/37) and B (75.7%; 28/37).

2.4. Molecular Characterization of Blastocystis Isolates

Through the standard coprological techniques used, 117 samples were found to be positive for *Blastocystis* spp., and 40 of these were preserved in ethanol (34.2%) for molecular characterization. Finally, 31 samples with high numbers of cysts/slides were selected. After rejecting unreadable or poor-quality sequences typically associated with faint bands on the agarose gels, 19 isolates were successfully subtyped (61.3%). Sequence analysis of the SSU rDNA (barcode region) gene of the parasite revealed the presence of three subtypes (ST): ST1 (10.5%; 2/19), ST2 (26.3%; 5/19), and ST3 (63.2%; 12/19). A single allele was observed for ST1 (4), while three were found for ST2 (9,12,34) and two for ST3 (34,57).

2.5. Molecular Characterization of Entamoeba histolytica/dispar

According to the standard coprological techniques used, 26 samples were found to be positive for the "*Entamoeba* complex", and 14 (53.8%; 14/26) could be processed by PCR. Molecular characterization of the isolated samples showed that 21.4% of them (n = 3) were positive for *E. histolytica*, and *E. dispar* was not identified in any of the samples.

3. Discussion

The current WHO strategy for the control of STHs as a public health problem is focused on PC and the development of new, more-sensitive diagnostic techniques [10]. Previous studies carried out in this area reveal that the morbidity of these diseases is associated with poverty and poor access to water and sanitation [1,15,16].

The overall prevalence of intestinal parasites in the current study, performed between 2021 and 2022, ranged from 78.6% to 92.8%, in accordance with results from other studies in the area [17–19]. The prevalence of the most common fecal–oral-transmitted protozoans, such as *G. intestinalis* and *Blastocystis*, has remained high throughout different studies dating from 2003 to recent years [17,19,20]. This may be due to the lack of appropriate drugs for the treatment of protozoan parasites (i.e., metronidazole), which are not included in MDA programs, or, as the studies indicate, due to the living conditions of the population remaining the same, causing a lack of basic sanitation and access to safe water [1].

Nonetheless, despite a lack of substantial changes in the living conditions of the population, a clear decrease in the prevalence of STHs can be observed. The prevalence of hookworm was reduced from values of approximately 60.0% [5,17,18] to those currently detected (between 2.9 and 20%). A decrease in prevalence was also observed for *S. stercoralis*, from an initial prevalence of 51% to a current range between 1.0 and 10.3%. In the case of *A. lumbricoides*, practically all the infections (n = 13/14) were observed in children from Km 6; this STH was not detected in any of the other areas. Furthermore, it should also be noted that the prevalence of hookworms was significantly higher ($p < 0.001$) in this community (51.6%; 50/97) than in the rest of the areas. This may be due to two reasons: first, the location of the area. Although all the communities are peri-urban neighborhoods

along NR 86, Km 6 is more distant and isolated from the urban area of Tartagal. The other reason, and perhaps the most important, is that in previous studies carried out in the area, the chemotherapy coverage offered only reached 40% of the population, in contrast to the rest of the communities where coverage values reached up to 98.2% [2]. In this context, in order to avoid well-known antimicrobial drug resistance, the WHO recommends improving awareness and understanding of antimicrobial resistance, strengthening knowledge through surveillance and research, reducing the incidence of infection, optimizing the use of antimicrobial agents, and ensuring sustainable investment in countering antimicrobial resistance [21].

The serologic results of the NIE−ELISA assay [5], applied to evaluate the serological situation in response to previous interventions, were negative in 96.1% (n = 247/257) of the cases. This was corroborated using the Baermann technique (n = 70/72), demonstrating the effectiveness and duration of the effect of anthelmintic treatment, as has been observed in other studies [22–25]. Although other authors have pointed out that intervals of 6 months between MDA rounds is not enough to keep STH levels below the 20% threshold [26,27], in this study, the prevalence has not yet bounced back to original values.

Studies indicate that antibody levels specific for *S. stercoralis* tend to decrease after effective treatment [28–30]; therefore, serological tests are currently the only available method to assess impact of treatment for patients with (false) negative fecal test results, given the low sensitivity of standard coprological techniques. Additionally, since it takes several months to demonstrate negativization through serology, patients should be monitored 6 and 12 months after treatment. One limitation of the use of serological techniques at baseline and after treatment might be the need for a properly equipped laboratory and trained technician.

Infections with protozoan parasites can cause different gastrointestinal problems and interfere with the absorption of nutrients [31,32]. Additionally, infection with *E. histolytica* can lead to amoebiasis and is an important cause of dysentery [33,34]. Clinical diagnosis of this amoeba is usually confirmed by visualization of the parasite through light microscopy, but this has the limitation of being unable to distinguish *E. histolytica* from *E. dispar* and *E. moshkovski* cysts, which are not considered pathogenic. Herein, with the aid of molecular techniques, it was possible to identify three *E. histolytica*-infected individuals, and the remaining PCR-negative samples suggest they are microscopically identified cysts that may belong to another *Entamoeba* spp. Despite the low prevalence found in the current study, in agreement with previous studies from Northern Argentina [14,34,35], it was possible to confirm the presence of this pathogenic species though molecular techniques, as other authors have previously confirmed in fecal samples from Puerto Iguazu (Misiones) [20] and Tartagal (Salta) [18]. All cases of *E. histolytica* were detected in Km 6.

On the other hand, the pathogenicity of the protist Blastocystis is still controversial, and several studies have shown that this might be related to the specific lineage [36–38]. The genus *Blastocystis* comprises a variety of lineages, called STs, and more than 90% of human isolates are grouped into STs 1–4 [39]. The distribution of STs in our samples demonstrated that ST3 was the most frequently detected ST, followed by ST2 and ST1, as other studies have reported [20,40,41]. Several authors have reported gastrointestinal symptoms associated with ST3 [42–44], and more specifically, the possible pathogenic association of allele 34, the most common in our study (57.9%; 11/19) with urticaria [39,42].

Finally, with respect to *G. intestinalis* with zoonotic potential, genetic characterization has revealed the existence of eight groups (assemblages A to H); assemblages A and B appear to be the main *G. intestinalis* assemblages that most often infect human subjects [45]. In this study, assemblage B showed a higher prevalence (75.7%; 28/37), compared to assemblage A (24.3%; 9/37). These results coincide with the prevalence reported in a few studies from Northern Argentina and the Chaco region [20,46], where, in addition to assemblages A and B, the presence of assemblage D, usually found in animals [18], was detected, highlighting its potential zoonotic nature. Although several studies have reported correlations between assemblages and symptoms, it has not been possible to determine

which one is directly related to symptomatology [14,46,47]. Even though other studies in Argentina have detected protozoans using molecular techniques [14,20,46], our results are the first to use a molecular approach to determine the genotypes of *G. intestinalis* and *Blastocystis* present in communities from the city of Tartagal and the province of Salta. Considering the zoonotic potential of *G. intestinalis* and *Blastocystis*, domestic and wild animals such as dogs, cats, rodents, and mammals could be involved in the transmission cycle as risk agents.

It is important to point out the integral approach to balance and enhance the health of people and ecosystems with a variety of complementary techniques used for the correct identification of parasitic species or a better diagnostic sensitivity that at the same time allows for better control of the diseases.

4. Materials and Methods

4.1. Study Area and Population

This study was conducted in schools in peri-urban neighborhoods in Tartagal, a city with 64,530 inhabitants [48], located in the Department of San Jose de San Martín in the Province of Salta (22°30′54″ S and 63°47′56″ O), a tropical province of Northwestern Argentina (Figure 1), in a transition area between the "Yunga" Rainforest and the "Gran Chaco" biome. The climate is tropical with an annual average temperature of 21 °C, an annual rainfall of 1232 mm [49], and alfisol soil, composed mostly of clay, which can retain water humidity [50].

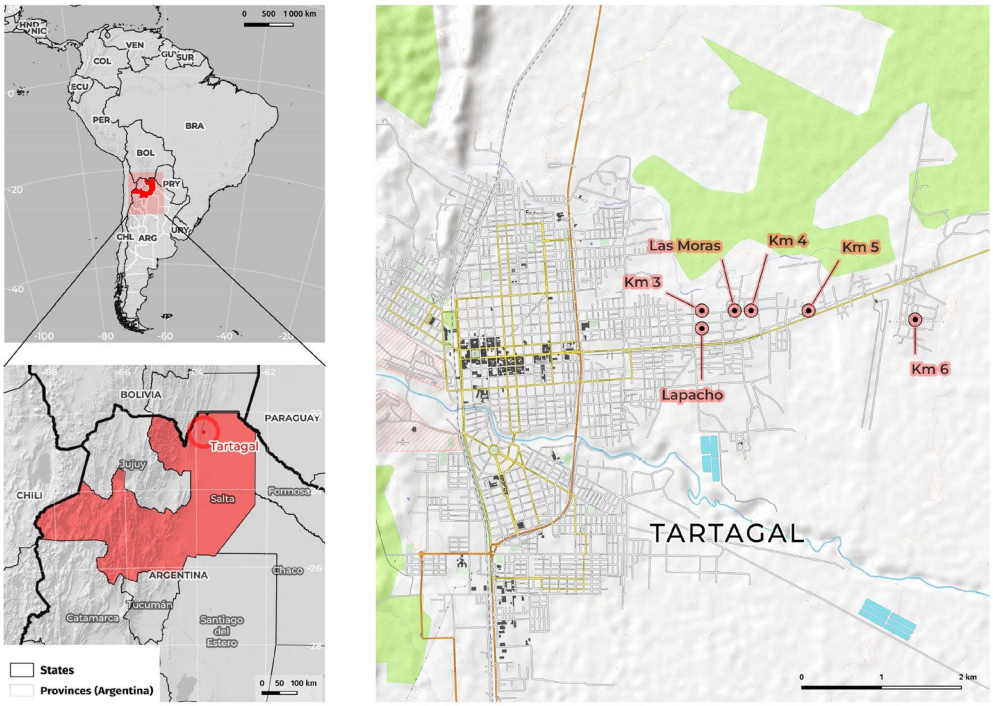

Figure 1. Map of the study area and the different communities in Tartagal.

The different indigenous communities in this area live in neighborhoods spread along National Route (NR) 86, which connects Tartagal to the rest of the province. Members of the communities Km 3, Km 4, Km 5, Km 6, Lapacho, and Las Moras, whose populations range from 91 (Km 3) to 1096 inhabitants (Km 6), were invited to participate. These communities share poor sanitation and water conditions. Most houses are made of adobe bricks, with unimproved roofs and dirt floors, and their main sources of income are government social plans and temporary jobs [2,20].

4.2. Study Design

The study was designed as a cross−sectional study, and field activities were conducted over one year (October 2021–September 2022). In each neighborhood, the study team performed house−by−house visits to enroll voluntary participants aged 1 to 15 years of age. Once signed informed consent and assent forms were obtained from parent/guardians and children, sterile containers were distributed for the collection of fecal samples. A single stool sample was collected from each individual, transported without fixative in a refrigerated icebox, and kept at 4 °C in the lab for parasitological and molecular analysis. Together with the stool samples, blood samples were drawn through venipuncture for analysis using the NIE enzyme-linked immunosorbent assay (NIE−ELISA), specifically for the detection of *S. stercoralis* antibodies [5].

4.3. Parasitological Analysis

The stool samples were processed within 24 h of collection using the modified Ritchie concentration technique [51] for the detection of both protozoan and helminth parasites. The Baermann concentration technique [52] was used for the detection of nematode larvae, and the Kato–Katz thick smear technique [53] was used to measure STH infection intensity. Aliquots of fresh fecal samples were stored partly in 10% formalin for confirmatory techniques and partly in ethanol 70% for molecular techniques. If the sample volume was insufficient to perform all the methods, the concentration technique was prioritized due to its overall higher sensitivity [54]. The present study did not consider the study of *Cryptosporidium* spp.

4.4. Serologic Test for Strongyloides Stercoralis

The individuals enrolled in the study, who had agreed and signed an informed consent form, had a 5 mL blood sample drawn through venipuncture during a second surveillance visit. All blood samples were centrifuged, and an aliquot of serum was preserved frozen at −20 °C and analyzed using the in-house enzyme−linked immunosorbent assay (NIE−ELISA) method for the diagnosis of *S. stercoralis*. NIE−ELISA detects IgG antibodies against the NIE recombinant antigen of *S. stercoralis* L3 larvae, as has been described previously [12]. A standard curve was used, and values (Unit/mL) were interpolated from that standard curve. The cut−off was defined using negative and positive control sera from stool-positive *S. stercoralis*-infected patients and healthy non−infected individuals. The cut−off was set at 120 Units/mL corresponding to a sensitivity and specificity of 75% and 95%, respectively, reported in a blinded study [12]. Patients' sera were tested in duplicate and compared to a standard positive IgG curve based on a standard curve run on each plate. The averages of duplicate results were calculated and corrected for background reactivity (no serum added). All subjects whose IgG titers against the NIE antigen were above the selected cutoff value as determined by ELISA were defined as seropositive for *S. stercoralis* and added to a database as cases [12].

4.5. DNA Extraction

Genomic DNA was extracted from 200 mg of concentrated fecal material previously washed three times with PBS using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions with brief modification. Fecal samples were mixed with stool lysis buffer and incubated for 10 min at 95 °C. DNA was eluted in 100 µL of elution buffer and stored at −20 °C for posterior use.

4.6. Molecular Identification of Giardia spp., Blastocystis spp., and Entamoeba spp.

PCR reactions were performed using an MJ Mini Thermal Cycler PTC-1148 (Bio- Rad Laboratories Inc., Hercules, CA, USA). Sterile water was used as a negative PCR control, and previously tested fecal samples containing only *G. intestinalis*, *Blastocystis* spp., *E. histolytica*, or *E. dispar* were used as positive controls. The oligonucleotides used for the molecular identification and characterization of *G. intestinalis*, *Blastocystis* spp., and

Entamoeba histolytica/dispar appear in Additional file 1: Table S1. PCR products from all of the reactions were run on a 1% agarose gel, except for the PCR products for *Blastocystis* spp., which were run on a 2% agarose gel.

4.7. Molecular Detection of Giardia intestinalis

Samples positive for *Giardia* spp. through microscopy were screened by a quantitative PCR (qPCR) method targeting a specific 62 bp region of the small subunit rRNA (SSU rRNA) gene of the parasite [55]. Amplification reactions were conducted in total volumes of 25 µL: contents included 3 µL of template DNA, 12.5 pmol of primers, and 1X TaqMan-GeneExpression Master Mix (Applied Biosystems, Foster City, CA, USA). Reactions were run using initial hold steps of 2 min at 60 ˚C, 10 min at 95 ˚C, 45 cycles of 15 s at 95 ˚C, and 1 min at 60 ˚C.

4.8. Molecular Typing of Giardia intestinalis

Giardia intestinalis isolates that tested positive by qPCR with cycle threshold values less than 37 (Ct < 37) were genotyped to an assemblage level using a nested PCR encoding a 753 bp fragment of the β-giardin (bg) gene of the parasite [56,57]. In general, the PCR mixtures (25 µL final volume) consisted of 8.5 µL of MyTaq Reaction Buffer containing 5 mM dNTPs and 15 mM $MgCl_2$, 2.5 units (U) of MyTaq DNA polymerase (Bioline GmbH, Luckenwalde, Germany), 1 µL each of a 10 µM primer pair, and 5 µL of extracted DNA for the first PCR reaction. The amplification condition for the first PCR reaction was as follows: initial denaturation at 95 ˚C for 7 min followed by 35 cycles (95 ˚C for 30 s. 65 ˚C for 30 s and 72 ˚C for 60 s), and the final extension was at 72 ˚C for 7 min. For the second PCR reaction, 3 µL of the product from the first PCR reaction was added, and the reaction was performed under the same conditions as used previously except for the cycling time, which was 95 ˚C for 30 s, 55 ˚C for 30 s, and 72 ˚C for 60 s.

4.9. Molecular Typing of Blastocystis spp.

Characterization of the *Blastocystis* subtypes from the microscopically positive samples was achieved by PCR by targeting the SSU rRNA gene of the parasite and amplifying a PCR product of ~600 bp. [58]. The reaction mixture (25 µL) contained 2.5 U of MyTaq DNA polymerase (Bioline GmbH, Luckenwalde, Germany), 5× MyTaq Reaction Buffer, 5 µL of template DNA, and 0.5 µM of each primer. The amplification conditions consisted of one step of 95 ˚C for 3 min, followed by 30 cycles of 1 min each at 94 ˚C, 59 ˚C, and 72 ˚C, with an additional 2 min final extension at 72 ˚C.

4.10. Molecular Detection of Entamoeba histolytica/dispar

Entamoeba histolytica/dispar (*Entamoeba* complex) are morphologically identical species that need to be distinguished through molecular techniques. In this study, specific primers from a PCR based on SSR rRNA [59] were used to identify either *E. histolytica* (through a specific 166 pb product) or *E. dispar* (through a specific 752 bp product). The reaction mixture contained 5 µL of DNA, 1.25 µL of each primer, 2.5 U of Taq polymerase (MyTaq DNA polymerase. Bioline GmbH, Luckenwalde, Germany), and 5× MyTaq reaction buffer containing 5 mM dNTPs. PCR amplification started with an initial denaturation at 94 ˚C for 3 min, followed by 30 cycles at 94 ˚C for 1 min, 58 ˚C for 1 min, and 72 ˚C for 1 min, with a final extension at 72 ˚C for 7 min.

4.11. Data Analysis

Data were analyzed using Stata 12 software (STATA Copr., College Station, TX, USA) and RStudio (PBC, Boston, MA, USA). Measures were evaluated using proportions with 95% confidence intervals (95% CI) and means with standard deviations (SD). The Chi-square test was used to compare significant associations between different variables.

4.12. Ethical Approval

The research protocol and the informed consent/assent forms were approved by the Ministry of Public Health of Salta province (Approval N° 0100321-111265/2018-0). All infected individuals were treated according to National Guidelines [60]. All participants provided written informed consent (children 14 and 15 years old) or informed assent (children 6 to 13 years old) prior to study participation, and parents provided informed consent/assent on behalf of minors (1 to 15 years).

5. Conclusions

This study highlights the efficacy and importance of adherence to treatment in reducing the prevalence of STHs, and the sustained effect over time. However, the high protozoan infection prevalence implies the need to improve access to drinking water, sanitation and jointly adapt treatments to the different parasitic species. The prevalence of *G. intestinalis* assemblage B found in the study undeniably shows that infection is mainly the ST3 *Blastocystis* subtype among human subjects, with the most common allele being 34, which could be associated with gastrointestinal symptoms. The use of serological techniques may be useful for post-treatment population monitoring as well as screening for the early detection of *S. stercoralis* infections, due to the sensitivity, rapidity, and diagnostic accuracy of NIE-ELISA. One Health integration in these communities could contribute to a better understanding of the environmental and transmission pathways of these diseases, allowing for greater action on the population and health system.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/parasitologia4020015/s1, Table S1: Oligonucleotides used for the molecular identification and characterization of *Giardia intestinalis*, *Blastocystis* spp., and *Entamoeba histolytica/dispar* in this study.

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CAPÍTULO IV:
CONCLUSIONES

The main conclusions that can be deduced from this Doctoral Thesis are:

1. A hyperendemic area of STH has been detected in the Province of Misiones, with a high prevalence of hookworms, related to the presence of anemia, so it is recommended to establish mass deworming programs. These programs, whose anthelmintic effectiveness is maintained in the medium term, are an effective measure for the reduction and control of the prevalence of STH, as long as the correct administration and adherence to treatment are guaranteed. A small population group can constitute a source of infection that allows the maintenance of the parasitic cycle;

2. It has been shown that in these populations, deficient socioeconomic factors, such as housing characteristics, overcrowding, walking barefoot, etc., are related to the infection and intensity of hookworms. It should also be noted that certain environmental factors such as vegetation or construction index (density of the impermeable surface) play a fundamental role in the distribution of STH;

3. The serological techniques applied in the Province of Salta are a useful tool for screening for STH reinfections, as well as a post-treatment evaluation method;

4. The high prevalence of protozoa detected, whose main route of transmission is human-human fecal-oral, suggests the need to improve the quality of drinking water and the sanitation system of these populations. It should also be noted that it has been shown that domestic animals are involved in the zoonotic transmission cycles of *Giardia intestinalis* and *Blastocystis* sp.

ANEXOS

ANEXO I. Tablas suplementarias de los artículos de investigación:

3.1 Prevalence of intestinal parasites and molecular characterization of *Giardia intestinalis*, *Blastocystis* spp. and *Entamoeba histolytica* in the village of Fortín Mbororé (Puerto Iguazú, Misiones, Argentina).

3.3. Prevalence of Intestinal Parasites, Protozoans and Soil-Transmitted Helminths, in Children from Communities of Northern Argentina after the Interruption of Deworming.

Additional file 1: Table S1. Oligonucleotides used for the molecular identification and characterization of *Giardia intestinalis*, *Blastocystis* spp. and *Entamoeba histolytica/dispar* from human fecal samples collected during the study conducted in Fortín Mbororé Village (Puerto Iguazú, Misiones, Argentina) and the study conducted in Tartagal (Salta, Argentina).

Target organism	Gene fragment	Oligonucleotide	Sequence (5′–3′)	Reference
Giardia intestinalis	SSU rRNA	Primers	CCCGCGGCGGTCCCTGCTAG	[36]
		Gd-80F	GACGGCTCAGGACAACGGTT	[36]
		Gd-127R	TTGCCAGCGGTGTCCG	[36]
	β-giardin	G7-F	AAGCCCGACGACCTCACCCGCAGTGC	[37]
	β-giardin	G759-R	GAGGCCGCCCTGGATCTTCGAGACGAC	[37]
	β-giardin	G99-F	GAACGAACGAGATCGAGGTCCG	[38]
	β-giardin	G609-R	CTCGACGAGCTTCGTGTT	[38]
Blastocystis spp.	SSU rRNA	RD5-F	ATCTGGTTGATCCTGCCAGT	[39]
		BhRD-R	GAGCTTTTTAACTGCAACAACG	[39]
Entamoeba histolytica/dispar	SSU rRNA	Enta-F	ATGCACGAGAGCGAAAGCAT	[40]
		Eh-R	GATCTAGAAACAATGCTTCTCT	[40]
		Ed-R	CACCACTTACTATCCCTACC	[40]

Additional file 2: Table S2. Reference sequences of *Giardia intestinalis* used in this study to compare with the sequences obtained from the participants from Fortín Mbororé Village, Puerto Iguazú (Misiones, Argentina) in order to build the phylogenetic tree and determine the assemblages and sub-assemblages circulating in the community.

Species	GeneBank accession number	Assemblage	Sub-assemblage	Host	Country	Reference
Giardia intestinalis	AY655702	A	A I	*Bos Taurus*	USA	[41]
	AY072723	A	A II	Human	Italy	[37]
	AY072724	A	A III	Human	Italy	[37]
	AY072727	B	B III	Human	Italy	[37]
	AY072728	B	B IV	Human	Italy	[37]
	AY545647	D	D I	*Canis familiaris*	Italy	[38]
	AY545648	D	D II	*Canis familiaris*	Italy	[38]
Giardia muris	AF069565			Mouse	USA	[42]

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Additional file 3: Table S3. List of *Giardia intestinalis* positive sample from participants of Fortín Mbororé, Puerto Iguazú, Misiones (Argentina) identified by microscopy and qPCR that were also identified at the assemblage level using de β-giardin gene.

Code Sample	Assemblage	Sub-assemblage	Reference Sequence
F005	D	D II	AY545648
F016	A	AIII	AY072724
F057	A	AIII	AY072724
F075	B	BIV	AY072728
F077	A	AIII	AY072724
F105	A	AIII	AY072724
F112	A	AIII	AY072724
F118	A	AIII	AY072724
F121	B	BIV	AY072728
F144	A	AII	AY072723
F145	B	BIV	AY072728
F158	B	BIV	AY072728
F163	B	BIV	AY072728
F174	B	BIV	AY072728
F179	B	BIV	AY072728
F181	B	BIV	AY072728
F183	B	BIV	AY072728
F193	B	BIV	AY072728
F195	B	BIV	AY072728
F196	B	BIV	AY072728
F199	B	BIV	AY072728
F214	B	BIV	AY072728
F255	A	AIII	AY072724
F277	B	BIV	AY072728
F280	B	BIV	AY072728
F282	B	BIV	AY072728

ANEXO II. Apéndices del artículo de investigación

3.2. *The Relationship Between Soil-Transmitted Helminth Infections and Environmental Factors in Puerto Iguazú, Argentina: Cross-Sectional Study.*

Multimedia Appendix 1. Environmental index formulas.

Topographic Position Index (TPI)

$$TPI = Z_0 - \frac{\sum_{1-n} Z_n}{n}$$

Z_0 = elevation of the model point under evaluation

Z_n = elevation of grid within the local window

n = the total number of surrounding points employed in the evaluation

Vegetation Heterogeneity Index (VHI)

$$VHI = V_0 - \frac{\sum_{1-n} V_n}{n}$$

V_0 = Vegetation index value of the model point under evaluation

V_n = Vegetation index value of grid within the local window

n = the total number of surrounding points employed in the evaluation

Topographic wetness index (TWI)

$$TWI = \frac{\alpha}{tanb}$$

α = local upslope area draining through a certain point per unit contour length

tan b = local slope in radians.

The topographic wetness index is unitless.

Soil Adjusted Vegetation Index (SAVI)

$$SAVI = \frac{NIR - R}{NIR + R + L} * 1 + L$$

NIR = reflectance value of the Near Infrared band

R = reflectance value of the Red band

L = soil brightness correction factor. The value of L varies by the amount or cover of green vegetation: in very high vegetation regions, L=0; and in areas with no green vegetation, L=1. Generally, an L=0.5 works well in most situations and is the default value used. When L=0, then SAVI = NDVI.

Bare Soil Index (BSI) [https://ieeexplore.ieee.org/document/1370429]

$$BSI = \frac{(R + SWIR) - (NIR + B)}{(R + SWIR) + (NIR + B)}$$

R = reflectance value of the Red band

B = reflectance value of the Blue band

SWIR = reflectance value of the Shortwave Infrared band

NIR = reflectance value of the Near Infrared band

Enhanced Normalized Difference Impervious Surfaces Index (ENDISI)

$$ENDISI = \frac{B - \alpha * \left(\frac{SWIR_1}{SWIR_2} + MNDWI^2\right)}{B + \alpha * \left(\frac{SWIR_1}{SWIR_2} + MNDWI^2\right)}$$

$$\alpha = \frac{2 * B_{mean}}{\left(\frac{SWIR_1}{SWIR_2}\right)_{mean} + MNDWI_{mean}^2}$$

$$MNDWI = \frac{G - SWIR_1}{G + SWIR_1}$$

B = reflectance value of the Blue band

G = reflectance value of the Green band

$SWIR_1$ = reflectance value of the Shortwave Infrared 1 band

$SWIR_2$ = reflectance value of the Shortwave Infrared 2 band

The subscript “Mean” is the mean value of the image.

Multimedia Appendix 2. Report of the statistical models used.

A. Distribution of hookworm positive individuals

1. Full Model

The full multilinear regression model was fitted to explore the relationship between the response variable U_{pos} (distribution of hookworm positive individuals) and several candidate predictors. The model equation is given as:

U_{pos} = 0.946017 - 1.496673 * SAVI - 1.618511 * VHI + 3.053108 * VHIσ - 0.000636 * TWI - 5.127594 * ENDISI - 2.622317 * BSI + 0.056738 * BFDF - 0.069859 * PpR

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.946017	0.966848	0.978	0.3301
SAVI	-1.496673	1.167171	-1.282	0.2026
VHI	-1.618511	1.062754	-1.523	0.1308
VHIσ	3.053108	1.217828	2.507	0.0137 *
TWI	-0.000636	0.010840	-0.059	0.9533
ENDISI	-5.127594	0.903960	-5.672	1.28e-07 ***
BSI	-2.622317	2.092168	-1.253	0.2129
BFDF	0.056738	0.028251	2.008	0.0472 *
PpR	-0.069859	0.053992	-1.294	0.1986

Significancy codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

The results indicate that only the predictor ENDISI had a highly significant effect on U_{pos} ($p < 1.28e-07$). Other predictors such as VHIσ and BFDF also showed significant relationships ($p < 0.05$).

Model Statistics:

Residual standard error: 0.3038

Multiple R-squared: 0.5767

Adjusted R-squared: 0.5441

F-statistic: 17.71 on 8 and 104 DF

p-value: < 2.2e-16

2. AIC Stepwise Variable Selection

AIC stepwise variable selection was applied to identify the most significant predictors for the reduced model. The algorithm suggested retaining the following predictors: VHI, VHIσ and ENDISI.

These variables were found to have the greatest impact on the response variable U_{pos}.

3. Reduced Model

The reduced multilinear regression model was fitted using the selected predictors from the AIC stepwise process. The model equation is given as:

U_{pos} = -0.39445 - 2.31023 * VHI + 3.13706 * VHIσ - 4.03425 * ENDISI

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.39445	0.17632	-2.237	0.02734 *
VHI	-2.31023	0.76057	-3.037	0.00299 **
VHIσ	3.13706	1.07241	2.925	0.00420 **
ENDISI	-4.03425	0.50338	-8.014	1.4e-12 ***

Significancy codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

The results show that all selected predictors have significant effects on U_{pos}, as their p-values are less than 0.05.

Model Statistics:

Residual standard error: 0.303

Multiple R-squared: 0.5627

Adjusted R-squared: 0.5465

F-statistic: 34.75 on 3 and 108 DF

p-value: < 2.2e-16

4. Coefficient Confidence Intervals

The 95% confidence intervals for the coefficients of the reduced model are as follows:

(Intercept): [-0.74395, -0.04494]

SAVI_TPI_2hamean: [-3.81782, -0.80264]

SAVI_TPI_2hastdev: [1.01136, 5.26276]

Dens_Constr_2hamean: [-5.03203, -3.03646]

These intervals indicate the range of plausible values for each predictor coefficient.

B. Intentisity of hookworm infection

1. Full Model

The full multilinear regression model was fitted to explore the relationship between the response variable U_{int} (intensity of hookworm infection) and several candidate predictors. The model equation is given as:

U_{int} = -0.92734 + 1.32154 * SAVI - 2.31871 * VHI + 0.20155 * VHIσ - 0.02246 * TWI - 0.96314 * ENDISI + 3.88593 * BSI + 0.33735 * BFDF - 0.28546 * PpR

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.92734	1.42362	-0.651	0.516231
SAVI	1.32154	1.71859	0.769	0.443653
VHI	-2.31871	1.56484	-1.482	0.141429
VHIσ	0.20155	1.79318	0.112	0.910723
TWI	-0.02246	0.01596	-1.407	0.162313
ENDISI	-0.96314	1.33102	-0.724	0.470933
BSI	3.88593	3.08059	1.261	0.209978
BFDF	0.33735	0.04160	8.110	1.06e-12 ***
PpR	-0.28546	0.07950	-3.591	0.000505 ***

Significancy codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

The results indicate that the predictors BFDF and PpR had highly significant effects on U_{int} ($p < 1.06e-12$ and $p < 0.000505$, respectively). Other predictors, such as VHI, BSI, and SAVI, also showed significant relationships ($p < 0.05$).

Model Statistics:

Residual standard error: 0.4473

Multiple R-squared: 0.5167

Adjusted R-squared: 0.4795

F-statistic: 13.9 on 8 and 104 DF

p-value: 1.449e-13

2. AIC Stepwise Variable Selection

AIC stepwise variable selection was applied to identify the most significant predictors for the reduced model. The algorithm suggested retaining the following predictors: BFDF, PpR.

These variables were found to have the greatest impact on the response variable U_{int}.

3. Reduced Model

The reduced multilinear regression model was fitted using the selected predictors from the AIC stepwise process. The model equation is given as:

$U_{int} = 0.05234 + 0.35294 * BFDF - 0.20609 * PpR$

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.05234	0.04427	1.182	0.23965
BFDF	0.35294	0.03672	9.611	3.05e-16 ***
Pers_cuart	-0.20609	0.06904	-2.985	0.00349 **

Significancy codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

The results show that all selected predictors have significant effects on U_{int}, as their p-values are less than 0.05.

Model Statistics:

Residual standard error: 0.4612

Multiple R-squared: 0.4566

Adjusted R-squared: 0.4467

F-statistic: 46.21 on 2 and 110 DF

p-value: 2.712e-15

4. Coefficient Confidence Intervals

The 95% confidence intervals for the coefficients of the reduced model are as follows:

(Intercept): [-0.29053, 0.51181]
BFDF: [0.28702, 0.44048]
PpP: [-0.36347, -0.09373]

These intervals indicate the range of plausible values for each predictor coefficient.

ANEXO IV. Documentos de aprobación de Comité de Ética en Investigación en Humanos de la Provincia de Misiones y la Provincia de Salta.

MISIONES PROVINCIA | MINISTERIO DE SALUD PÚBLICA AiSalud

CEiP
Comité de Ética en Investigación Provincial

"2018 - Año del centenario de la reforma universitaria en el marco de la inclusión de las nuevas tecnologías de la información y comunicación para el conocimiento y educación de los jóvenes misioneros"

Posadas 28 de junio de 2018

DICTAMEN del CEIP

A LA INVESTIGADORA:

Nos dirigimos a Ud., con el objeto de informarle que el Comité de Ética en Investigación Provincial (CEIP), ha evaluado el Proyecto de Investigación titulado **"INTERVENCIÓN INTEGRAL COMUNITARIA PARA LA PREVENCIÓN Y EL CONTROL DE PARÁSITOS INTESTINALES EN PUERTO IGUAZÚ, MISIONES, ARGENTINA "** a ser llevado a cabo por Ud., dentro del ámbito provincial.

Tras la evaluación no hemos encontrado objeciones éticas ni metodológicas para realizar el trabajo de investigación. Las observaciones previamente mencionadas fueron modificadas correctamente.

Le recordamos que se debe considerar los Derechos y la Integridad de los seres humanos participantes en la investigación biomédica, ajustándose a la Declaración de Helsinki y sus modificaciones.

Los documentos evaluados son:

- Formulario de asentimiento para niño de 6 a 13 años, inclusive.
- Formulario de consentimiento para jóvenes de 14 a 17 años, inclusive.
- Formulario de consentimiento para adultos.
- Formulario de consentimiento para padres y/o tutores de niños entre 1 y 13 años de edad, inclusive.
- Planilla de caracterización de viviendas.
- Protocolo de investigación.

MISIONES PROVINCIA | MINISTERIO DE SALUD PÚBLICA AiSalud

CEiP
Comité de Ética en Investigación Provincial

"2018 - Año del centenario de la reforma universitaria en el marco de la inclusión de las nuevas tecnologías de la información y comunicación para el conocimiento y educación de los jóvenes misioneros"

Con atenta consideración.

Victoria Periago.
SU DESPACHO

Dra. Cristina Martín
Presidente - CEIP

CEIP – COMITÉ DE ETICA EN INVESTIGACION PROVINCIAL

MINISTERIO DE SALUD PÚBLICA DE SALTA
DIRECCION DE RECURSOS HUMANOS
PROGRAMA DE DOCENCIA E INVESTIGACION
Comisión Provincial de Investigación en Ciencias de la Salud.
Comité de Ética de Investigación

FORMULARIO DE APROBACIÓN DE PROTOCOLO DE INVESTIGACIÓN Nº _16___

PROTOCOLO: N° 16 Versión Nº _3.0 (1 DE AGOSTO DE 2018)

CÓDIGO: Mundo Sano **Salta, 21 de agosto de 2018.-**

Expediente: N° 0100321-111265/2018-0

El Comité de Ética de Protocolos de investigación de la Comisión Provincial de Investigación en Ciencias de la Salud CO.Pi.C.SA., dependiente del Programa de Investigación de la Dirección de Recursos Humanos del Ministerio de Salud de la Provincia de Salta aprobado por Resolución Ministerial 1738 el 15 de Octubre de 2015, bajo la Ley 7544 de Investigaciones Biomédicas de la Provincia de Salta, ha **evaluado y aprobado** el protocolo cuyo:

TITULO: ES "CARACTERIZACIÓN EPIDEMIOLOGICA DE LA INFECCIÓN POR PARASITOS INTESTINALES DEL DEPARTAMENTO GENERAL JOSÉ DE SAN MARTÍN PROVINCIA DE SALTA.(ARGENTINA)".-

Y EL **CONSENTIMIENTO INFORMADO: Fecha** 21/08/ 2018 que se entrega sellado y firmado." **APROBANDO" la realización del estudio a cargo de: INVESTIGADOR PRINCIPAL**: Dra. María Victoria Periago, investigadora adjunta de CONICET, Fundación Mundo Sano, Diego Cruz, Fundación Mundo Sano.-

CENTRO O INSTITUCION DONDE SE REALIZA LA INVESTIGACION: Sede de Tartagal, Fundación Mundo Sano

Domicilio: San Martin 785, Tartagal, Provincia de Salta, Argentina.-

Cuyo **OBJETIVO PRINCIPAL: Determinar la prevalencia de Parásitos intestinales e intensidad de la infección por geohelmintos en niños y niñas entre 1 y hasta 15 años de edad en barrios periurbanos y/o comunidades rurales del Departamento General José de San Martin Provincia de Salta.-**

Siendo su **DISEÑO**: Estudio Piloto, para determinar la presencia de parásitos en la comunidad.

	SI	NO
Requiere la aprobación de ANMAT	[ ]	[X]

Dirección de Recursos Humanos
Sarmiento 625- Planta Alta
Te/fax. 0387- 4953469
Correo electrónico: investigadorrhhsalta@gmail.com

4. Los eventos adversos informados por el patrocinante o detectados por Uds. mismos.

5. Otras modificaciones al protocolo no se podrán aplicar sin ser antes evaluadas por este Comité, salvo en casos de riesgo de vida para el Paciente.

6. En caso de suspensión del protocolo, la comunicación deberá ser inmediata.

7. Comunicación de finalización de la investigación.

8. Comunicación de los resultados.

De este documento se emiten tres copias, una para el Programa de Investigación de la Dirección de Recursos Humanos donde se archivará toda la documentación relacionada, otro para el investigador y otro para el patrocinador en caso de corresponder

OBSERVACIONES _____

"El Comité de Ética en Investigación Comisión Provincial de Investigación en Ciencias de la Salud (CO.PI.C.SA.), aprobado por Resolución Ministerial N°1738/15 adhiere a los principios éticos y científicos que tienen su origen en:

La Declaración de Helsinki de la Asociación Médica Mundial, 1964 y sus modificaciones; las "Pautas Éticas Internacionales para la Investigación Biomédica en Seres Humanos establecidas por el Consejo de Organizaciones Internacionales de las Ciencias Médicas" (CIOMS 2002 y sus enmiendas); las Buenas Prácticas de Investigación Clínica de la reunión Internacional de Armonización (ICH-GCPs), "Declaración Universal sobre Bioética y Derechos Humanos" aprobada por la Conferencia General de la UNESCO el 19 de octubre de 2005; las "Guías operacionales para comités de ética que evalúan investigación biomédica (OMS 2000)" ; la "Declaración Universal sobre el Genoma Humano y los Derechos Humanos" aprobada por la Conferencia General de la UNESCO (11 de noviembre de 1997); la "Declaración Internacional sobre los Datos Genéticos Humanos" aprobada por la Conferencia General de la UNESCO el 16 de Octubre de 2003; el "Documento de las Américas sobre Buenas Prácticas Clínicas", (OPS, República Dominicana, 4/03/05); las "Guías de Buenas Prácticas Clínicas de la Conferencia Internacional de Armonización" (ICH E6); las "Guía de las Buenas Prácticas de Investigación Clínica en Seres Humanos", aprobada por Resolución 1490/2007 del Ministerio de Salud de la Nación, Guía para Investigaciones con Seres Humanos aprobada por Resolución N°1480/11 del Ministerio de Salud de la Nación; las normativa vigente emitidas por la Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT). Resolución 6677/10 y Disposición 10017- E /2017, por el Instituto Nacional Central Único Coordinador de Ablación e Implantes (INCUCAI), Ministerio de Salud de la Nación; la "Ley Nacional 25.236 de protección de datos Personales" y normas relacionadas a la protección de datos personales en investigación emanadas de la Dirección de Protección de Datos Personales, Ministerio de Justicia de la Nación, y el Código Civil y Comercial de la República Argentina.-

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MINISTERIO DE SALUD PÚBLICA DE SALTA
DIRECCION DE RECURSOS HUMANOS
PROGRAMA DE DOCENCIA E INVESTIGACION
Comisión Provincial de Investigación en Ciencias de la Salud.
Comité de Ética de Investigación

Aprobado en reunión de fecha _21_/ 08_/_2018_ según consta en el Libro de Actas Nº _66_ Folio 98 y 99_

COMISIÓN EVALUADORA

LIC. RICARDO JUAREZ	DIRECTOR DE RECURSOS HUMANOS - M.S.P.	
DRA. VALERIA OLIVA	MIEMBRO TITULAR: Programa de Investigación CO.Pi.C.SA CEI M.S.P.	
DRA. VERONICA LENCINA	MIEMBRO TITULAR Programa de Investigación Y Docencia CO.Pi.C.SA. CEI M.S.P.	Dra. VERONICA LENCINA M.P.6273 MEDICA ESPECIALISTA EN REUMATOLOGÍA
Dr. ENRIQUE FINETTI	MIEMBRO TITULAR – Programa de Investigación y Docencia CO.Pi. C.SA. CEI M.S.P.	
LIC. CESAR MONTELLANO	MIEMBRO TITULAR – Programa de Investigación y Docencia CO.Pi.C.SA.CEI M.S.P.	Lic. César Montellano Director de Gestión del Conocimiento Del Área Operativa LV "PADRE FRANCISCO"
ISMAEL ANZE	MIEMBRO TITULAR Programa de Investigación y Docencia CO.Pi.C.SA. CEI Monitor de Investigación y Ensayo Clínico M.S.P.	

El Consentimiento Informado y Formulario Informativo al participante del estudio que se adjunta, con firma y sello de este comité, es el que corresponderá fotocopiar para entregar a la población

Posterior a esta aprobación los investigadores o patrocinadores (según corresponda), deberán informar a este Comité:

1. Comunicación de inicio de la investigación (reclutamiento del 1º paciente)

2. informe sobre la marcha del protocolo dentro del año de inicio del mismo

3. El progreso del protocolo con los sujetos de investigación reclutados y datos parciales si los hubiese, una vez por año, (salvo que este Comité decida que el informe deba ser más frecuente).

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ANEXO V. Procedimiento Operacional Estándar para la Caracterización Socioeconómica y Encuesta de Caracterización.

PROCEDIMIENTO OPERACIONAL ESTÁNDAR

Para

Caracterización Socioeconómica

040

para

Fundación Mundo Sano

Autoría: María Victoria Periago
Coordinación Científica
Fundación Mundo Sano

Coordinación: Maria Victoria Periago
Coordinación Científica
Fundación Mundo Sano

Historial de Revisión

Número de Documento y Versión	Título	Intervalo de Revisión
040, versión 1	*Caracterización Socioeconómica*	*Anual*

Substituye: NA
Fundación Mundo Sano

1. Objetivo

Este procedimiento mide diferentes características socioeconómicas a través de un cuestionario con un esquema enfocado en variables relacionadas con las familias y características de la vivienda, y unos módulos complementarios opcionales enfocados en otras variables de salud, ruralidad o vectores.

2. Alcance

Los cuestionarios se realizan por miembros de la Fundación involucrados en los proyectos que pretenden estudiar la relación entre los parámetros colectados y las enfermedades cubiertas por dichos proyectos. Los cuestionarios se administran a aquella familias incluidas en los diferentes proyectos.

3. Definiciones y Abreviaciones

Término/Siglas	*Definición/Significado*
FMS	Fundación Mundo Sano
POE	Procedimiento Operacional Estándar

4. Roles y Responsabilidades

Rol	*Responsabilidad*
Operador de campo	Comprender y adherir a este POE. Desarrollar los procedimientos de acuerdo a este POE para realizar las encuestas a las familias que participan de los diferentes proyectos.
Responsable de Sede	Comprender y adherir a este POE. Coordinar la realización de las encuestas en terreno y asegurar que los operadores sepan rellenarlas y lo hagan de forma correcta.
Coordinador del Proyecto	Supervisar la realización de la actividad. Definir el uso de las encuestas según el proyecto, coordinar la utilización de las mismas con el Responsable de Sede, asegurar que se completen todas las encuestas necesarias y que los datos se colecten y analicen.

5. Entrenamiento

Título	Tipo de Trabajo	Frecuencia	Entrenamiento Requerido
Coordinador del Proyecto	Coordinador	Anualmente o después de una revisión	Leer y comprender
Responsable de Sede	Supervisor	Anualmente o después de una revisión	Leer y comprender
Operador de laboratorio	Operador	Anualmente o después de una revisión	Leer y comprender

6. Material

- Lapicera de tinta negra o azul.

7. Equipamiento

Ninguno.

8. Precauciones de Seguridad

Ninguna.

9. Procedimiento

Según el proyecto, se utilizará la encuesta de Características Socioeconómicas (Anexo 1) como base y se agregarán diferentes módulos según los objetivos del proyecto. Los módulos que pueden ser agregados son: Módulo Salud (Anexo 2), Módulo Rural (Anexo 3) y/o Módulo Vectorial (Anexo 4).

9.1. Los coordinadores de proyecto definirán en qué momento y a cuáles viviendas se le deberá aplicar las encuestas, así como cuáles módulos deben ser incluidos.
9.2. Los coordinadores de proyecto entrarán en contacto con el Responsable de Sede dónde se realizarán las encuestas para organizar el trabajo de forma que no sea una sobrecarga para la sede.
9.3. El Responsable de Sede coordinará el trabajo en terreno y asegurará de que las encuestas estén disponibles, se rellenen correctamente y se devuelvan a la sede completas para ser archivadas.
9.4. El Coordinador del Proyecto luego coordinará con el Responsable de Sede para obtener los datos en las encuestas y analizarlos.

10. Cálculos

Ninguno.

11. Referencias

Ninguna.

12.Formularios/Modelos/Anexos

Anexo 1. Encuesta de Caracterización Socioeconómica.
Anexo 2. Módulo Salud.
Anexo 3. Módulo Rural.
Anexo 4. Módulo Vectorial.

Mundo Sano

Planilla de Caracterización de Viviendas

Localidad: _____ Fecha: _____

ID vivienda: _____

Georeferencia:		Si		No
Foto:		Si		No

Perfil del encuestado – Jefe del Hogar*

Nombre _____ Sexo | | Hombre | | Mujer |

Fecha de Nacimiento _____ Edad _____

Antigüedad en la localidad _____ Saber leer y escribir | | Si | | No |

Escolaridad

	Ninguna		Inicial		Primario		Secundario
	Terciario		Universitario		**Otro:		

*El Jefe del Hogar es el principal sostén económico del hogar y si hay dos personas que ganan lo mismo, se toma el que "toma las decisiones" en cuanto a gastos en el hogar.

**En el caso de que haya ido a la primaria o secundaria hasta sólo cierto grado y no haya completado la primaria o secundaria, indicarlo en "otro".

Habitantes

º.- Listar los habitantes que viven con el encuestado. Incluir nombre y apellido, relación de parentesco con el encuestado, escolaridad, fecha de nacimiento y edad de cada uno. En el caso de la escolaridad colocar si actualmente va a la escuela. En caso que esté actualmente en la escuela, colocar el nombre de la misma, si actualmente no va, poner hasta qué grado fue.

	Nombre y apellido	Relación de parentesco con el jefe de hogar	Escolaridad/ Nombre de la Escuela*	Sabe leer y escribir				Fecha de Nacimiento	Edad
1					Si		No		
2					Si		No		
3					Si		No		
4					Si		No		
5					Si		No		
6					Si		No		
7					Si		No		
8					Si		No		
9					Si		No		
10					Si		No		

Variables socioeconómicas

Mundo Sano

Planilla de Caracterización de Viviendas

2.- ¿Cuál es el medio principal de vida del grupo familiar? (Se puede marcar más de uno)

- ☐ Cría de animales
- ☐ Cultivo
- ☐ Empleado informal
- ☐ Empleado en relación de dependencia
- ☐ Sector Público
- ☐ Jubilación/Pensión
- ☐ Beneficiario de planes sociales
- ☐ Forestales
- ☐ Turismo (hostelería/gastronomía)
- ☐ Otra (por favor, especifique): _____

Preguntas relacionadas con la salud

3.- ¿Usted o sus hijos tienen cobertura de salud por alguna de las siguientes opciones?

- ☐ Obra Social
- ☐ Prepaga a través de obra social
- ☐ Prepaga sólo por contratación voluntaria
- ☐ Programas o planes sociales estatales de salud
- ☐ No tiene obra social, prepaga o plan estatal
- ☐ Otra (por favor, especifique): _____

4.- ¿Algún miembro de su familia sufrió o sufre de una enfermedad infecciosa? En caso afirmativo continuar con la pregunta 4a.

☐ Si, especificar cuándo: _____ ☐ No

4a.- ¿Recibió atención médica? En caso afirmativo pasar a la pregunta 4b

☐ Si ☐ No

4b.- ¿Con qué medicamento fue tratado?

- ☐ Albendazol
- ☐ Benznidazol
- ☐ Mebendazol
- ☐ Antibiótico
- ☐ Ivermectina
- ☐ Anfotericina
- ☐ Metronidazol
- ☐ Glucantime
- ☐ Otro (por favor, especifique): _____

5.- ¿Usted o alguno de sus hijos suele caminar descalzo?

☐ Si ☐ No

6.- ¿Usted y sus hijos se lavan las manos antes de comer?

☐ Si ☐ No

7.- ¿Usted y sus hijos se lavan las manos después de defecar?

☐ Si ☐ No

Características de la vivienda

8.- ¿De qué está hecho el techo de su vivienda?

- ☐ Chapa
- ☐ Ramas
- ☐ Tablones de madera
- ☐ Adobe
- ☐ Hojas de palmera
- ☐ Otra (por favor, especifique): _____

9.- ¿De qué está hecho el piso de su vivienda?

- ☐ Tierra
- ☐ Madera
- ☐ Cemento
- ☐ Ladrillo
- ☐ Otra (por favor, especifique): _____

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10.- ¿De qué están hechas las paredes de su vivienda?

- ☐ Tierra
- ☐ Madera
- ☐ Cemento
- ☐ Ladrillo
- ☐ Otra (por favor, especifique): _____

10.- ¿Cuántos cuartos de su vivienda son utilizados para dormir?

- ☐ 1
- ☐ 4
- ☐ 2
- ☐ 5 o más
- ☐ 3

11.- ¿Ve roedores dentro o alrededor de su vivienda?

- ☐ Si
- ☐ No

Características del peridomicilio

12.- ¿Tiene corral o chiquero? Si afirmativo, pasar a las pregunta 12a.

- ☐ Si
- ☐ No

12a.- ¿De qué está hecho el corral o chiquero?

- ☐ Enramado
- ☐ Palo a pique
- ☐ Otra (por favor, especifique): _____

13.- ¿Tiene animales domésticos o de granja? En caso afirmativo, pasar a la pregunta 13a.

- ☐ Si
- ☐ No

13a.- Marcar los animales que tiene y colocar las cantidades aproximadas en paréntesis. Si tienes animales para consumo pasar a la pregunta 13b.

- ☐ Perro
- ☐ Gato
- ☐ Gallina
- ☐ Pato
- ☐ Pavo
- ☐ Ovejas
- ☐ Cabras
- ☐ Cerdos
- ☐ Vacas
- ☐ Caballos
- ☐ Burros
- ☐ Conejos
- ☐ Otro (por favor, especifique): _____

14.- ¿Carnea en su casa? En caso afirmativo, pasar a la pregunta 14a y 14b.

- ☐ Si
- ☐ No

14a.- ¿Dónde suele carnear?

- ☐ Colgado de árbol u otro lugar en el peridomicilio
- ☐ Colgado de un palo de la casa
- ☐ Otra (por favor, especifique): _____
- ☐ Gato

14b.- ¿Cómo dispone de las tripas y deshechos?

- ☐ Las re-utiliza para hacer comida
- ☐ Las usa crudas para alimentar a los perros
- ☐ Las usa hervidas para alimentar a los perros
- ☐ Otra (por favor, especifique): _____

Acceso a servicios públicos

15.- ¿Tiene inodoro o letrina? En caso afirmativo pasar a las preguntas 15a.

- ☐ Si
- ☐ No

15a.- ¿Qué tipo de desagüe tiene?

- ☐ Red pública/cloaca
- ☐ Cloaca
- ☐ A cámara séptica y pozo ciego
- ☐ Sólo a pozo ciego

☐ A excavación a tierra ☐ Otro (por favor, especifique): _____

16.- ¿Cómo dispone de los pañales sucios? (puede marcar más de uno)
☐ No tiene niños con pañales ☐ Dentro de letrina/inodoro
☐ En la basura ☐ La entierra
☐ Otra (por favor, especifique): _____

17.- ¿Con qué cocina? (puede marcar más de uno)
☐ Fogón a leña ☐ Cocina a gas con garrafa
☐ Cocina a gas con gas natural ☐ Horno de barro
☐ Otra (por favor, especifique): _____

18.- ¿De dónde obtiene electricidad? (puede marcar más de uno)
☐ No tiene ☐ Red eléctrica
☐ Grupo electrógeno o motor ☐ Panel solar
☐ Otra (por favor, especifique): _____

19.- ¿Cómo realiza la eliminación de residuos? (puede marcar más de uno)
☐ Recolección municipal ☐ A cielo abierto
☐ Quema ☐ Entierra
☐ Otra (por favor, especifique): _____

Preguntas relacionadas con el agua y la higiene

Si la vivienda posee tanque de agua. Realizar la pregunta 24. Si no, marcar que la vivienda no posee tanque de agua.

20.- ¿De dónde obtiene el agua para beber? Si no bebe agua embotellada o si no tiene agua corriente pasar a la pregunta 20a.
☐ Pozo ☐ Bomba
☐ Aljibe ☐ Camión cisterna
☐ Lluvia ☐ Envasada
☐ Corriente dentro de la vivienda ☐ Corriente fuera de la vivienda
☐ Tanque de agua ☐ Otro (por favor, especifique): _____

20a.- ¿Le realiza algún tratamiento al agua para hacerla potable? En caso afirmativo, ¿Qué método utiliza?
☐ No le realiza ningún tratamiento ☐ Hervido
☐ Lavandina ☐ Filtro
☐ Otro (por favor, especifique): _____

21.- ¿De dónde obtiene el agua para cocinar?
☐ Pozo ☐ Bomba
☐ Aljibe ☐ Camión cisterna
☐ Lluvia ☐ Embotellada
☐ Corriente dentro de la vivienda ☐ Corriente fuera de la vivienda
☐ Tanque de agua ☐ Otro (por favor, especifique): _____

22.- ¿De dónde obtiene el agua para bañarse o lavarse las manos?
☐ Pozo ☐ Bomba
☐ Aljibe ☐ Camión cisterna
☐ Lluvia ☐ Embotellada

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☐ Corriente dentro de la vivienda
☐ Corriente fuera de la vivienda
☐ Tanque de agua
☐ Otra (por favor, especifique): _____

23.- ¿Tiene una estructura para lavarse las manos/los platos? En caso afirmativo pasar a la preguntas 23a, b y c.

☐ Si
☐ No

23a.- ¿La estructura cuenta con jabón/detergente?

☐ Si
☐ No

23b.- ¿La estructura se encuentra cerca de la letrina/baño?

☐ Si
☐ No

23c.- ¿La estructura se encuentra cerca del lugar de preparación de alimentos?

☐ Si
☐ No

24.- ¿Dónde está dispuesto el tanque de agua? Si el tanque está a nivel del piso, pasar a la pregunta 24a.

☐ No tiene tanque de agua
☐ Tanque elevado
☐ Tanque a nivel del piso
☐ Otro (por favor, especifique): _____

24a.- ¿Mantiene el tanque a nivel del piso tapado a lo largo del día?

☐ Si
☐ No

25.- Observaciones sobre el barrio (anotar el tipo de desagüe observado en la manzana, si es a cielo abierto, con canaletas o entubado y anotar si existe algún relleno sanitario en el barrio.

Observaciones: