Case Report

Globalization effects on the reports of non-endemic parasitosis in Italy

Giorgia Bracaglia[1], Livia Mancinelli[2], Stefania Ranno[2], Federica Del Chierico[3], Hyppolite K Tchidjou[4], Ottavia Porzio[1], Andrea Onetti Muda[2],Lorenza Putignani[2,3,*]

[1]Laboratory Medicine, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant' Onofrio 4, 00165, Rome, Italy. Email: giorgia.bracaglia@opbg.net; ottavia.porzio@opbg.net

[2]Unit of Microbiology, Parasitology and Virology, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant' Onofrio 4, 00165, Rome, Italy. Email: livia.mancinelli@opbg.net; stefania.ranno@opbg.net; andrea.onettimuda@opbg.net

[3]Human Microbiome Unit of Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165, Rome, Italy . Email: federica.delchierico@opbg.net

[4]Unit of Immunology and Infectious Diseases, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant' Onofrio 4, 00165, Rome, Italy. Email: hyppolite.tchidjouku@opbg.net

* Correspondence: lorenza.putignani@opbg.net; Tel.: +39-066-859-2598/2980

Abstract: Protozoa and helminths are responsible for several intestinal parasite infections (IPIs). Generally helminth infections are very unsafe but scarcely reported in high-income countries, while protozoa and helminth co-infections are usually reported in children living in inadequate hygienic-sanitary conditions and in rural areas. However, the impact of growing globalization, intense travelling, international adoptions and high levels of immigrants and refugees has significantly incremented the incidence of oro-faecal parasitosis in non endemic areas. Although most IPs clear without treatment when population, even children, emigrate from endemic to different geographical areas, some IPIs such as strongyloidiasis may persist for decades as subclinical infections or as low-grade disease with nonspecific clinical manifestations, unless to reappear under impairment conditions.

Herein we report an unusual case of *Giardia lamblia* and *Trichuris* spp. chronic asymptomatic co-infection in a healthy adopted Romanian child, living in a Central Italy rural area, and an hidden case of *Strongyloides stercoralis* in an adopted Burundian child, resident in South Italy, long misdiagnosed as a recurrent undefined dermatitis. Our report suggests the need to review primary care practitioner guidelines and children's hospital procedures for appropriate IPIs screening and follow-up, hence providing new screening and prevention strategies, in agreement with international guidelines.

Keywords: International adoptions; asymptomatic and misidentified parasitosis; intestinal parasite infections, IPIs; *Giardia lamblia*; *Trichuris* spp.; *Strongyloides stercoralis*; IPIs screening guidelines.

 doi:10.20944/preprints201808.0343.v1

1. Background

Protozoa and helmints are responsible for several intestinal parasite infections (IPIs), especially in children [1], even occurring after ingestion of only few cysts or eggs, related with several factors such as age, patient's immune status and nutritional conditions. Parasites frequently detected in stools are protozoa (*e.g.*, *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp.) [2] and soil-transmitted helminths (STH) (*e.g.*, *Ascaris*, whipworms, and hookworms) [3]. Particularly, *G. lamblia* is a flagellate affecting people worldwide, especially children living in rural areas with inadequate hygienic conditions or attending school in social contaminated environments [4].

Risk of helminthiasis has been associated to wastewater and excreta contamination in agricultural settings, suggesting water treatments as key factors to reduce infestations [3]. Helminths are parasites infecting humans especially in developing countries which can cause several clinical troubles such as anemia, growth retardation and increased susceptibility to other infections [5].

In Italy, a study including 5323 patients has found that *G. lamblia* is the most common protozoan in both Italian and non-Italian patients, while worms, particularly *Hymenolepis nana*, *Strongyloides stercoralis* and *Trichuris trichiura*, are found only in 0.9% of cases, especially in foreign children [6].

Herein we report a *G. lamblia* and *Trichuris* spp. co-infection and a *S. stercoralis* misdiagnosed infection, two cases found in adopted children living in Italy and coming from endemic regions for IPIs. The aim is to discuss new alert algorithms for IPIs in our country by implementing pediatric screening programs for adopted and migrant children from endemic areas, within the context of specialized reference Centers for children parasitosis control .

2. Cases' Presentation

First case report We present a 8-years-old boy, adopted from Hungary by an Italian family, living near Simbruini Mounts (Lazio-Italy) since May 2012. Except for recurrent respiratory tract diseases, his medical history was negative for viral, bacterial and parasitic infections and all recommended vaccines were accomplished. He was brought to the attention of pediatricians of Bambino Gesù Children's Hospital (OPBG) of Rome, for routine screening evaluations, including a complete physical examination, which resulted normal with respect to age. Laboratory tests for HIV, HBV, HCV, tuberculosis and immunological status resulted negative. However, parasitological analyses resulted positive for *G. lamblia* and *Trichuris* spp. Therefore, the child was treated with mebendazole for 5 days and probiotics for 14 days. Three stool samples, collected at alternate days and fixed in 10% formalin, were analyzed, after concentration, for clinical microscopy workflow under a 100x light microscope with micrometric eyepiece, in accordance with Ritchie and stained with Lugol [7, 8]. Ten slides for sample were set-up for microscopy. A first slide revealed one *G. lamblia* cyst of 12x10 µm size (**Figure 1 A**) and a brown lemon-shaped egg of *Trichuris* spp. of 52x23 µm size (**Figure 1 B**). In the other slides a total of 15 cysts of *G. lamblia* , 6 eggs and 2 larval forms of *Trichuris* spp. were observed (**Figure 1 C-E**). Each faecal sample was also processed through rapid immunocromatographic test (ICT), Rida quick immunochromatographic dipstick tests *Cryptosporidium/Giardia/Entamoeba* Combi (R-Biopharm, Darmstadt, Germany), which resulted positive for *Giardia* antigen.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 August 2018 doi:10.20944/preprints201808.0343.v1

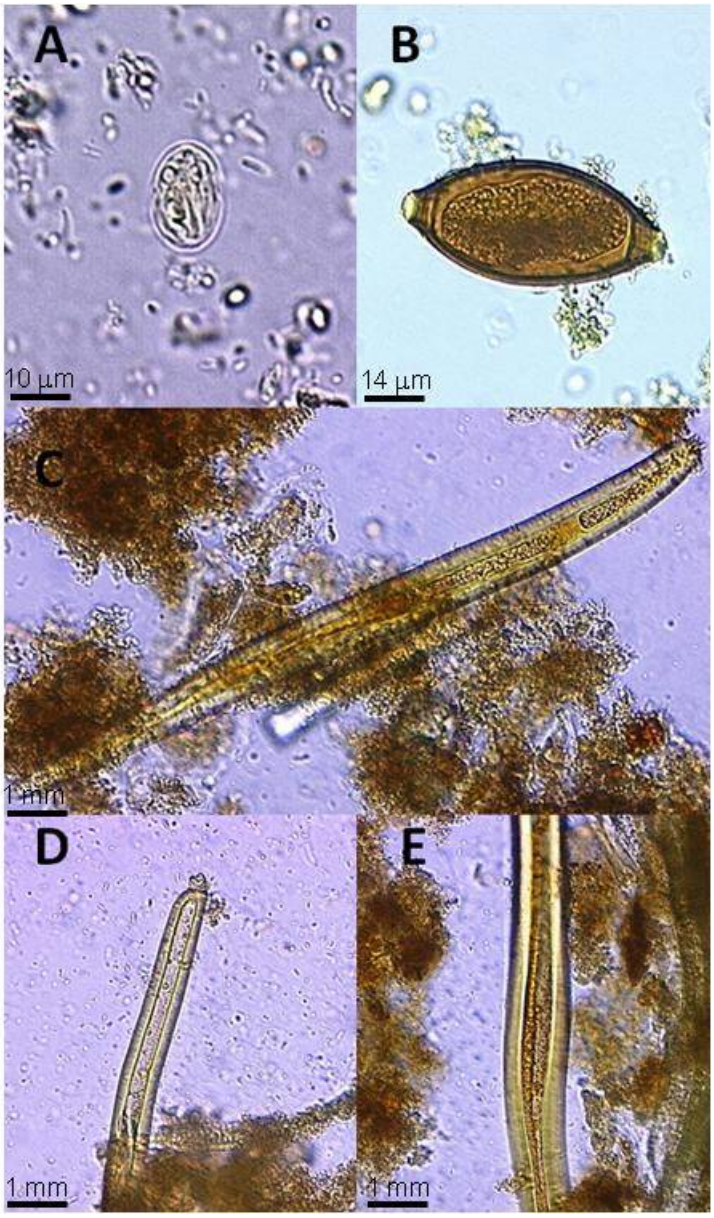

Figure 1. Microscope-based identification of *Giardia lamblia* and *Trichuris trichiura* in concentrated Lugol-stained samples. (A) Cyst of *G. lamblia*; (B) egg of *T. trichiura*; (C) adult worm of *T. trichiura*; (D) mouthparts of *T. trichiura*; (E) intestinal apparatus of *T. trichiura*.

All samples were processed, according to routine barcode-based automatic DNA extraction, to perform molecular assays on *Giardia* isolates (QIAsymphony SP/AS Instruments, Qiagen, Hilden, Germany). A *G. lamblia* molecular typing was performed by multilocus PCR analysis based on: i) a nested-PCR procedure, amplifying a DNA region of 130 bp (18rRNA small subunit, SSU) (**Figure 2 A**) [9] (*i.e.*, genotyping) ; ii) a semi-nested PCR procedure, amplifying a 432 bp region of the glutamate dehydrogenase gene (*gdh*) (**Figure 2 B**) [10] (*i.e.*, sub-genotyping). The PCR products were purified using centrifugal filter Amicon Ultra according to the manufacturer's instructions (Millipore, Carrigtwohill, Ireland) to proceed with amplicon characterization. Sequencing reactions were performed using BigDye terminator v.3.1 Sequencing kit, following the manufacturer's instruction (Applied Biosystems, CA, USA). *G. lamblia* sequences were corrected by base calling, edited using FinchTV 1.4 software (Geospiza, Inc, Seattle, WA, USA), and queried using BLAST

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 August 2018 doi:10.20944/preprints201808.0343.v1

(http://www.ncbi.nlm.nih.gov/BLAST) to infer similarities of forward/reverse consensus sequences to assign species/assemblage/sub-assemblage ranking . Nucleotide *gdh* sequences were deposited into GenBank (BankIts: KF317538; KF317539; KF317540). In order to underline phylogenetic relationships, software MEGA Version 5.05 was used and the best tree construction model test (model T92, Tamura 3-parameter) was performed for the dataset. (**Figure 2 C**). All three *G. lamblia* isolates resulted sub-genotyped as BIII assembly group.

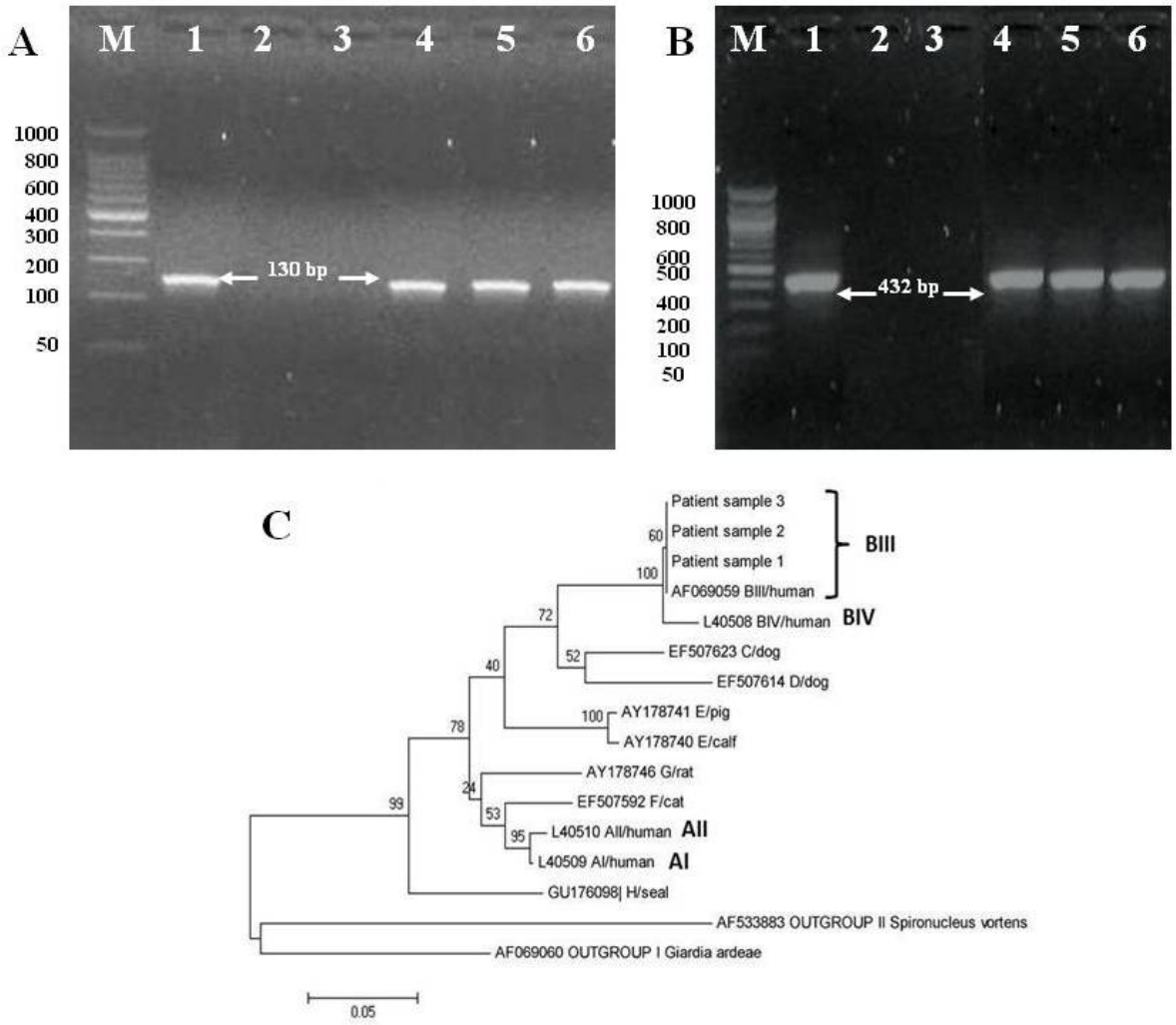

Figure 2. Molecular analysis of *Giardia lamblia*. (A) Gel representation of the SSU-18rRNA-based PCR for *G. lamblia* assemblage detection: in order (M) marker, (1) internal positive control, (IPC) (2) first PCR negative control, (3) nested-PCR negative control, (4) patient sample 1, (5) patient sample 2, (6) patient sample 3. (B) Gel representation of the *gdh*-based PCR for *G. lamblia* sub-assemblage detection: in order (M), marker, (1) IPC, (2) first PCR negative control, (3) nested-PCR negative control (4) patient sample 1, (5) patient sample 2, (6) patient sample 3,. (C) Neighbour-joining phylogenetic tree based on a ClustalW alignment of the *gdh* sequences from the three *G. lamblia* patient isolates. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown above branches.

On the other end, under routine laboratory procedures, *Trichuris* DNA extraction failed, as ascertained by STH-based amplification, possibly because the automatic DNA extraction was unsuitable for *Trichuris* spp. [11]. Therefore only clinical microscopy was exploited for STH diagnosis.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 August 2018 doi:10.20944/preprints201808.0343.v1

After therapy, the patient not returned at control as recommended. However, after one year, during a routine control, physical examination showed normal height-weight development and absence of disease. Parasitological exams showed absence of *Giardia* infection, but still presence of *Trichuris* spp. ova. However, consistently with literature data, clinicians decided to reserve treatment only in the case of further presenting symptoms [12].

Second case report. We also report on a 2-years-old child coming from Burundi, arrived in Italy in April 2012 and hospitalized for hypereosinonophilic syndrome and recurrent dermatitis in Potenza, where he was treated for itchy hives for several months with steroids and antihistamines, without remission of symptoms. All vaccinations were completed. At the arrive to our OPBG Hospital the child showed good conditions, except for skin lesions due to previous skin infection scarring. Haematological parameters revealed hypereosinonophilia (eosinophil count 3.66×103/μL, 42.1% reference values, RV), lymphopenia (lymphocyte count 2.48×103/μL, 28.5% RV) and neutropenia (neutrophils count 2× 103/μL, 23% RV).
Parasitological exams and routinely DNA-extraction procedures were performed by clinical microscopy and molecular biology pipelines. Only one, amongst the multiple analyzed slides, showed the presence of a *S. stercoralis* larva (**Figure 3 A**). *S. stercoralis* PCR was performed using specific STRO 1530 forward and STRO 1630 reverse primers [13], amplifying a 18rRNA SSU fragment of 114 bp, which resulted consistent with the size of the internal positive control (IPC) [13] (**Figure 3 B**). All microscopic and molecular data correlated with the presence of body erythematous lesions associated to a cutaneous larva migrans (**Figure 3 C**).

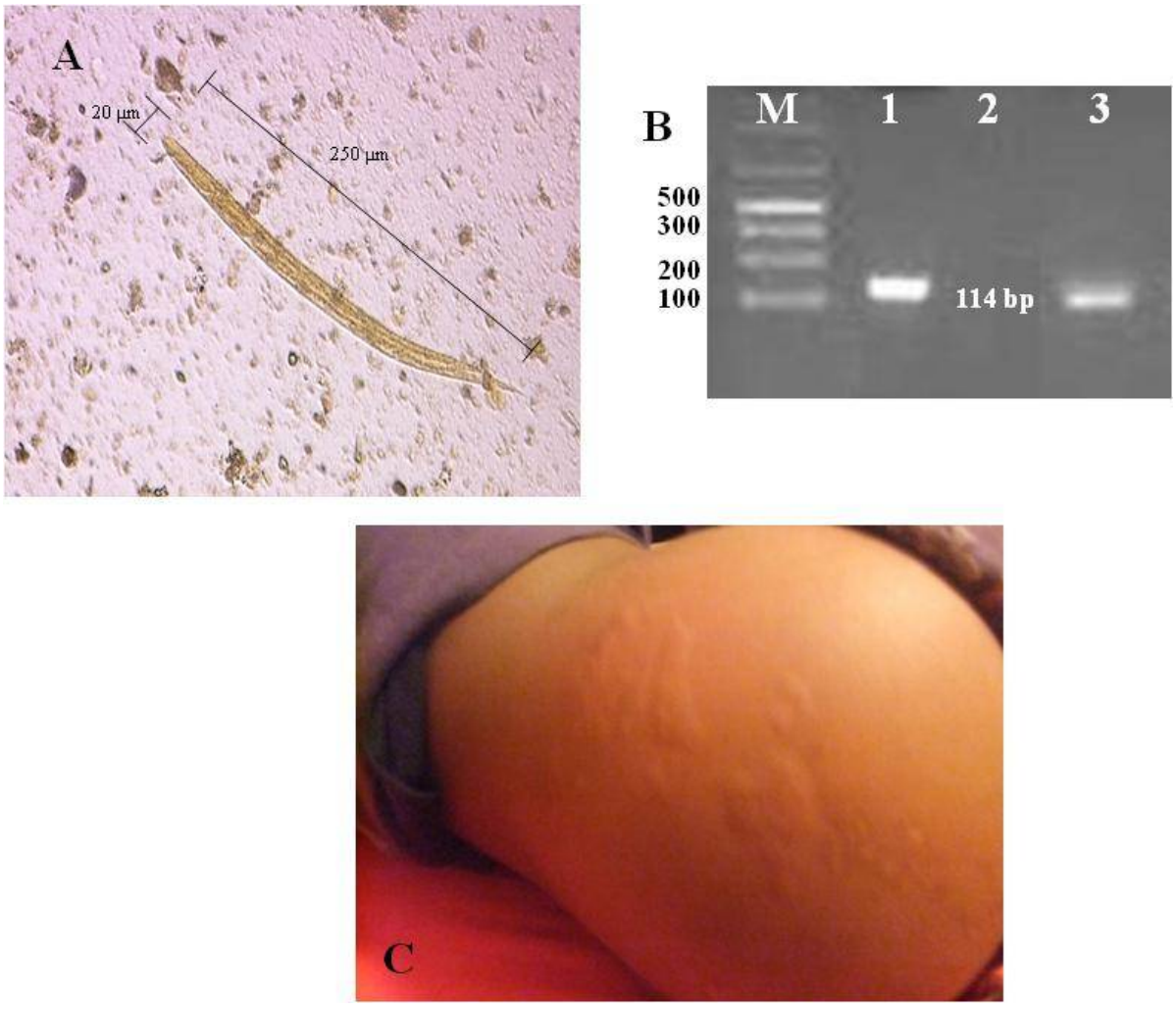

Figure 3. Microscope- and molecular-based identification of *Strongyloides stercoralis* coupled to clinical inspection of larva migrans detectable at cutaneous level. (A) Filariform (L3) larvae of *S. stercoralis* in Lugol staining. (B) Electrophoresis gel for *S. stercoralis* detection: (M) marker, (1) Patient sample, (2) Negative Control, (3) Internal positive control (IPC) for *S. stercoralis* DNA . (C) Cutaneous larva migrans.

Ethics Approval and Consent to Participate: Written informed consent was obtained from the patient's parents for inclusion in the study and for publication of this manuscript. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Bambino Gesù Children's Hospital Ethics Committees.

3. Review and Discussion

The presented two cases to focus on childhood health state in poor areas and during immigration/adoption flows, in order to realize appropriate disease control and prevention measures for adopted or migrant children. Helminthiasis and protozoan diseases cover a significant infectious disease burden and many are the parasites responsible for IPIs leading to persistent diarrhea and/or major complications. Epidemiological data on IPIs disease diffusion and prevalence in developing countries are numerous, while in high income countries such infections are rarely reported [14].

Microscopical analysis, coupled to DNA-based typing for *G. lamblia*, allowed us to describe a *G. lamblia* and *Trichuris* spp. co-infection in an adopted asymptomatic child. This was an unusual co-infection morbidity in Italy, apparently not previously reported for other patients, although frequently association between protozoa and helminths have been widely described in literature [1,6]. Indeed, in a study from Ancona Hospital, in 2006-2011, protozoa and helminth co-infections were reported in children, especially in strangers [6] and *G. lamblia* was detected with *Taenia* spp and *S. stercoralis* eggs, but not with other worms. Moreover, *T. trichiura* has never been reported with amoebae. *G. lamblia* is genetically considered a multi-species complex which includes a variety of morphologically similar genotypes differing for host specificity [15]. Molecular genotyping of isolates, coming from different hosts, has allowed so far the identification (ID) of seven assemblages (*e.g.*, A-G) [15]. The A and B assemblages are considered to be potentially zoonotic, because of their ID in both humans and animals and they include AI, AII, AIII, and BIII and BIV sub-assemblage groups, respectively. The PCR-based analysis for *G. lamblia*, from our stool samples, confirmed by microscopy-based positivity, and performed by exploiting both 18rRNA SSU and *gdh* gene targets, assigned assemblage B and sub-assemblage BIII. The BIII sub-assemblage ID [16], seems to suggest the hypothesis of a zoonotic transmission, supported by the patient behavior within his socio environmental conditions. Also infections by *Trichuris* spp. primarily affect people living in rural areas, particularly people in contact with domestic animals [17]; in the herein presented case the possible reservoir of parasite infection might had been the child's dog. In Italy, indeed, a previous study [18] reported the presence of mixed trichuroid infestation in an asymptomatic dog suggesting a zoonotic risk.

In the second case, we reported a *S. stercolaris* infection in a child from Burundi, treated for several months with antihistaminic and corticosteroid therapy, because of a suspected allergy reaction. When the child arrived to our hospital, parasitological exams showed the presence of one only larva of *S. stercolaris* in just one slide, regardless several stool collection and multiple slide sets for each microscopic session. Clearly, the wrong previous diagnosis and treatment was overcome only because the parasitologist team was particularly skilled to perform parasitological exams of second levels and because the clinician was specialized in parasitic infection detection and control. Symptoms were solved after specific albendazole treatment with complete eradication of STH

 doi:10.20944/preprints201808.0343.v1

parasite at control six months later, with remission of skin lesions due to *larva migrans* and resolution of haematological parameters.

4. Conclusions

This cases show the importance to deeply examine, by dedicated parasitological protocols, also healthy children with history of migration of international adoption, hence avoiding further aggressive treatments for hidden or neglected parasitosis. Our laboratory is focused on the evaluation of prevalence and incidence of pediatric parasitosis, paying remarkable attention to new IPIs, currently circulating in Italy as effect of globalization processes. In fact, in a previous study we have reviewed the prevalence of parasitic infections in Italy and in high income countries by effect of globalization phenomena [2]. In addition it has been demonstrated in a retrospective study on 504 adopted children that *G. lamblia* was detected in 87 out of 461 tested children, especially native of Eastern Europe [19], as in the case of our patient. When populations emigrate from parts of the world where intestinal parasites are endemic and resettle in countries where they do not exist or are rare, most infections will clear without treatment within a few years after immigration [20]. However, some IPIs agents may persist for decades as subclinical infections or as low-grade disease with nonspecific symptoms.

To prevent and to control neglected IPIs associated to environmental and/or sociological infestations (*i.e.*, anthroponotic and zoonotic transmission), it is now mandatory to design new advanced diagnostic and clinical prevention strategies, to be systematically employed during early life stages, when history of migration can actually interfere with the forthcoming child health status**.** Especially Paediatric Hospitals should put into effect new clinical protocols and diagnostic algorithms able to detect neglected parasitosis, often asymptomatic and misidentified or, even, hidden. Diagnostic workflows need now to combine serology, molecular biology and clinical microscopy handled by well trained laboratory staffs especially when medical history is not available or confused by scarceness of clinical data.

 doi:10.20944/preprints201808.0343.v1

215

216 **Author Contributions:** GB, SR and LP formulated the idea and wrote the manuscript; GB, SR, LM
217 and FDC performed the analyses. HT followed the clinical cases and outcomes of the patients. FC,
218 OP, provided critical comments to the methods and paper discussion. LP provided granting and
219 revised entirely the manuscript. All authors approved the final version of this manuscript

220

221 **Acknowledgments.** This work was supported by the Ministry of Health, Ricerca Corrente
222 (RC201302P002991) Bambino Gesù Children's Hospital, IRCCS, and by Dicofarm Grant to LP.

223

224 **Conflicts of Interest:** The authors declare that they have no competing interests.

225

226 **Abbreviations**

gdh	Glutamate dehydrogenase
IPC	internal positive control
IPIs	intestinal parasite infections
PCR	Polymerase chain reaction
SSU	Small subunit of 18rRNA
STH	Soil-transmitted helminths

227

References

[1] Brown, J.; Cairncross, S.; Ensink, J.H. Water, sanitation, hygiene and enteric infections in children. *Arch Dis Child.* **2013** Jun 12.

[2] Putignani, L.; Menichella, D. Global distribution, public health and clinical impact of the protozoan pathogen Cryptosporidium. *Interdiscip Perspect Infect Dis*. **2010**.

[3] Pham-Duc, P.; Nguyen-Viet, H.; Hattendorf, J.; Zinsstag, J.; Phung-Dac, C.; Zurbrügg, C.; et al. *Ascaris lumbricoides* and *Trichuris trichiura* infections associated with wastewater and human excreta use in agriculture in Vietnam. *P. Parasitol Int.* **2013**, *62*, 172-180.

[4] Barry MA, Weatherhead JE, Hotez PJ, Woc-Colburn L. Childhood parasitic infections endemic to the United States. *Pediatr Clin North Am*. **2013**, *60*, 471-485.

[5] Hotez, P.J.; Brooker, S.; Bethony, J.M.; Bottazzi, M.E.; Loukas, A.;Xiao, S. Hookworm infection. N Engl J Med. **2004**, *351*, 799-807.

[6] Silvestri, C., Greganti, G., Arzeni, D., Morciano, A., Castelli, P., Barchiesi, F., et al. Intestinal parasitosis: data analysis 2006-2011 in a teaching hospital of Ancona, Italy. *Infez Med*. **2013**, *21*, 34-9.

[7] Ritchie, L. An ether sedimentation technique for routine stool examination. *Bull U S Army Med Dep*. **1948**; *8*;326.

[8] Vandenberg, O., Van Laethem, Y., Souayah, H., Kutane, W.T., van Gool, T., Dediste, A. Improvement of routine diagnosis of intestinal parasites with multiple sampling and SAF-fixative in the triple-faeces-test. *Acta Gastroenterol Belg*. **2006**, *69*, 361-6.

[9] Read, C., Walters, J., Robertson, I.D., Thompson, R.C. Correlation between genotype of Giardia duodenalis and diarrhea. *Int J Parasitol.* **2002**, *32*, 229-231.

[10] Read, C.M., Monis, P.T, Thompson, R.C. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. *Infect Genet Evol.* **2004**, *4*, 125-130.

[11] Liu, G.H., Gasser, R.B., Su, A., Nejsum, P., Peng, L., Lin, R.Q., Li, M.W., et al. Clear genetic distinctiveness between human- and pig-derived *Trichuris* based on analyses of mitochondrial datasets. *PLoS Negl Trop Dis.* **2012**, *6*, e1539.

[12] Tahas, S.A., Diakou, A. Persistent *Giardia* spp. and *Trichuris* spp. infection in maras (Dolichotis patagonum) at a zoo in Greece. *J Zoo Wildl Med.* **2013**, 44, 389-394.

[13] Dorris, M., Blaxter, M. The small subunit ribosomal RNA sequence of *Strongyloides stercoralis*. *Int J Parasitol.* **2000**, *30*, 939-941.

[14] Peruzzi, S., Gorrini, C., Piccolo, G., Calderaro, A., Dettori, G., Chezzi, C. Prevalence of intestinal parasites in the area of Parma during the year 2005. *Acta Biomed*. **2006**, 77, 147-151.

[15] Thompson RC. Giardiasis as a re-emerging infectious disease and its zoonotic potential. *Int J Parasitol.* **2000**, *30*, 1259-1267.

[16] Souza, S.L., Gennari, S.M., Richtzenhain, L.J., Pena, H.F., Funada, M.R., Cortez, A., et al. Molecular identification of *Giardia duodenalis* isolates from humans, dogs, cats and cattle from the state of São Paulo, Brazil, by sequence analysis of fragments of glutamate dehydrogenase (gdh) coding gene. *Vet Parasitol.* **2007**, *149*, 258-264.

[17] Dunn, J.J, Columbus, S.T., Aldeen, W.E., Davis, M., Carroll, K.C. *Trichuris vulpis* recovered from a patient with chronic diarrhea and five dogs. *J Clin Microbiol*. **2002**; *40*; 2703-2704.

[18] Di Cesare, A., Castagna, G., Meloni, S., Otranto, D., Traversa, D. Mixed trichuroid infestation in a dog from Italy. *Parasit Vectors.* **2012**, *5*, 128. doi: 10.1186/1756-3305-5-128.

[19] Saiman, L., Aronson, J., Zhou, J., Gomez-Duarte, C., Gabriel, P.S., Alonso, M, et al. Prevalence of infectious diseases among internationally adopted children. *Pediatrics.* **2001**, *108*, 608-612.

[20] Pottie, K., Greenaway, C., Feightner, J., Welch, V., Swinkels, H., Rashid, M., Narasiah, L., Kirmayer, L.J., Ueffing, E., MacDonald, N.E., Hassan, G., McNally, M., Khan, K., Buhrmann, R., Dunn, S., Dominic, A., McCarthy, A.E., Gagnon, A.J., Rousseau, C., Tugwell, P. Coauthors of the Canadian Collaboration for Immigrant and Refugee Health Evidence-based clinical guidelines for immigrants and refugees *CMAJ.* **2011** Sep 6, *183*, E824-925. doi: 10.1503/cmaj.090313.