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DIENTAMOEBA FRAGILIS IS MORE PREVALENT THAN *GIARDIA DUODENALIS* IN CHILDREN AND ADULTS ATTENDING A DAY CARE CENTRE IN CENTRAL ITALY

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Summary:

Giardia duodenalis is a well recognised enteropathogen, while *Dientamoeba fragilis* is rarely detected and consequently it is not recognised as an important human pathogen. In 2002-2003, a survey has been carried out on enteroparasites in faecal samples of outpatients attending a day care centre in the town of Perugia (Central Italy). To improve the detection level, at least three samples from each patient were collected at different days and within two hours from defecation. The coproparasitological examination has been carried out by direct microscopic examination, faecal concentration, and Giemsa and modified Ziehl-Nielsen stainings of faecal smears. The genotypes of *Giardia duodenalis* isolates were determined by PCR of the β-giardin gene. Of 1,989 enrolled people (966 children, 1,023 adults), 165 persons (8.3 %; 153 adults, 15.0 %; 12 children, 1.2 %), were positive for parasites, but only 112 adults (73.2 % of those infected) and eight children (66.7 % of those infected) harboured *D. fragilis* and *G. duodenalis*. Both the Assemblages A and B were detected in 18 *G. duodenalis* isolates examined at the β-giardin gene. The higher prevalence of *D. fragilis* infections than that of *G. duodenalis* is probably related to the method used, a procedure, which is rarely followed in laboratories for the diagnosis of enteric parasites. These epidemiological data suggest that when faecal samples are examined after a period of time and without Giemsa staining, most *D. fragilis* infections goes undetected.

KEY WORDS : *Giardia duodenalis, Dientamoeba fragilis*, parasite, Giemsa stain, diagnosis, PCR.

Résumé : LA PRÉVALENCE DE *DIENTAMOEBA FRAGILIS* EST PLUS ÉLEVÉE QUE CELLE DE *GIARDIA DUODENALIS* CHEZ LES ENFANTS ET LES ADULTES EN TRAITEMENT AMBULATOIRE DANS L'ITALIE DU CENTRE

Giardia duodenalis est un parasite entérique bien connu, tandis que Dientamoeba fragilis, rarement détecté, n'est pas considéré comme un agent pathogène important chez l'homme. En 2002-2003, une investigation sur les entéroparasites a été conduite sur des échantillons de selles de patients en traitement ambulatoire dans la ville de Perugia (Italie du Centre). Afin d'augmenter le niveau de détection, un minimum de trois échantillons pour chaque patient a été collecté aussitôt après la défécation et pendant plusieurs jours. L'analyse coproparasitologique a été faite en utilisant différente méthodes : examen microscopique direct, concentration, coloration par le Giemsa ou le Ziehl-Nielsen modifié. Les génotypes de G. duodenalis ont été déterminés par PCR du gène de la β-giardine. Parmi les 1989 individus engagés (966 enfants, 1023 adultes), 165 personnes (8,3 %; 153 adultes, 15,0 %; 12 enfants, 1,2 %) étaient porteurs du parasite, mais seulement 112 adultes (73,2 % des infectés) et huit enfants (66,7 % des infectés) étaient positifs pour D. fragilis ou G. duodenalis. Parmi les 18 isolats de G. duodenalis caractérisés par PCR du gène de la β-giardine, sept ont été attribués à l'assemblage A, huit à l'assemblage B, et trois étaient mixtes. La prévalence plus élevée de D. fragilis (67 %) comparée à celle de G. duodenalis (33 %), observée dans notre étude, est probablement due à la méthode employée, très rarement utilisée par les laboratoires de diagnostic. Les données épidémiologiques suggèrent que la plus grande partie des infections dues à D. fragilis ne sont pas détectées quand les selles sont examinées après un certain temps et sans l'aide de la coloration au Giemsa.

MOTS CLÉS : *Giardia duodenalis, Dientamoeba fragilis, parasite, coloration de Giemsa, diagnostics, PCR.*

INTRODUCTION

Giardia duodenalis (synonymous *G. lamblia* and *G. intestinalis*) and *Dientamoeba fragilis* have been recognised as intestinal human pathogens since 1681 and 1918, respectively (Thompson, 2000; Johnson *et al.*, 2004). The cycle of *G. duodenalis* is characterised by two morphologically well distinct stages, the trophozoite, which causes the disease, and the cyst, which represents both the infective and the environmentally resistant stage (Thompson, 2000). For *D. fragilis*, on the contrary, only a trophic stage has been described (Johnson *et al.*, 2004). As a result, the diagnosis of *G. duodenalis* infection is relatively easy provided that more than one faecal sample, collected on consecutive days, is examined. The identification of cysts, and, more rarely, of trophozoites, can be performed by direct microscopic examination of fresh samples, by the use of fluorescein-conjugated monoclonal antibodies to detect the cysts under a fluores-

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cent microscope, or by the use of monoclonal antibodies to detect *G. duodenalis* antigens by an ELISA. These diagnostic tools have been developed after the establishment of *in vitro* culture of this pathogen. On the other hand, *D. fragilis* can be identified only by specific staining of smears prepared from fresh faecal samples. Therefore, this pathogen is identified only in a few specialised laboratories.

Recently, several PCR-based assays have been developed to detect *G. duodenalis* in faecal samples (e.g., Cacciò *et al.*, 2002). The lack of an *in vitro* system to obtain highly purified nucleic acids from *D. fragilis* has limited the number of molecular studies on this parasite and prevented the use of a molecular approach to increase the detection level of this pathogen in faecal samples. To date, only PCR-derived methods based on the small-subunit rRNA gene has been developed (Peek *et al.*, 2004; Johnson & Clark, 2000).

The aim of this work was to evaluate the prevalence of infection with these pathogens and other associated parasites, in outpatients attending the day care centre of S. Silvestrini Hospital of Perugia (Central Italy) and to identify the genotypes of *G. duodenalis* by molecular analysis.

MATERIALS AND METHODS

Investigated persons

The parasitological investigation has been carried out in the period 2002-2003. Of 1,989 enrolled people, 966 were children (1-14 years of age) and 1,023 were adults (older than 14 years of age). Faecal samples were screened for parasites for the following reasons: 1) request from general practitioners, 380 children and 665 adults; 2) severe diarrhoea, 546 children and 291 adults; and 3) protracted diarrhoea (> 13 days), 40 children and 76 adults. For each person, al least three faecal samples have been examined and information on the age, suspected diagnosis, and epidemiological data were collected.

For children, parasitic infections were suspected on the basis of: 1) unspecific intestinal disturbance (abdominal pain, sometimes associated with diarrhoea) in 63.4 % (241/380); 2) linear growth retardation in 9.7 % (37/380); 3) eosinophilia in 1.1 % (4/380); 4) contact with persons with intestinal parasites in 1.1 % (4/380); and 5) there was no a risk factor or a symptomatology, suggesting the need of coproparasitological examination for 24.7 % (94/380).

For adults, the suspected parasitic infections were based on: 1 unspecific intestinal disturbance in 56.9 % (373/656); 2) eosinophilia in 6.4 % (42/656); 3) contacts with persons with intestinal parasites in 2.7 % (18/656); 4) cutaneous rush and/or cutaneous lesions, without eosinophilia, in 19.1 % (125/656); and 5) asymptomatic persons in 14.9 % (98/656).

Parasitological diagnosis

The coproparasitological examination has been carried out by direct microscopic examination, faecal concentration and Giemsa staining of faecal smears. In addition, faecal samples from persons with acute or protracted diarrhoea were screened by a modified Ziehl-Nielsen staining for the detection of coccidia (*Cryptosporidium* sp., *Cyclospora cayetanensis* and *Isospora belli*) after formol-ethyl-acetate concentration. Faecal samples were also tested for bacterial enteropathogens and for Rotavirus and Adenovirus, and, for some children or adults, for the toxin A of *Clostridum difficile*.

Molecular characterisation of *G. duodenalis* isolates

DNA extraction was performed according to the method of da Silva *et al.* (1999). Briefly, an aliquot (0.4 ml) of concentrated faecal material was homogenised using the FastPrep 120 instrument (Savant, Thermo Electro Corporation, Woburn MA, USA). The DNA released from disrupted cysts was purified using the FastDNA kit (Qbiogene, Illkirch Cedex, France), and stored at 4° C.

A 511 bp fragment of the β-giardin gene was amplified using a nested PCR assay. The primary amplification was performed using 2 µl of DNA and the forward primer G7 and the reverse primer G759, as previously described (Cacciò *et al.*, 2002). Five µl of the primary PCR were used in the nested amplification with primers βGiarF (5'-GAACGAGATCGAGGTCCG-3') and βGiarR (5'-CTCGACGAGCTTCGTTGTT-3').

Reactions were carried out on a Perkin-Elmer GeneAmp 2400 (Applied Biosystems, Foster City, CA) thermocycler under the following conditions: after an initial denaturation cycle of five min. at 94° C; 35 cycles were run, each consisting of 30 sec. at 94° C, 30 sec. at 53° C and one min. at 72° C; followed by a final extension cycle of seven min. at 72° C. After purification with Qiaquick kit (Qiagen), PCR products were fully sequenced on both strands using the ABI Prism BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). The sequencing reactions were analysed on an ABI 310 automatic DNA sequencer (Applied Biosystems) and assembled with the program SeqMan II (DNASTAR, Madison WI).

RESULTS

Parasites have been detected in 165 persons (8.3 %), 153 adults (15.0 %) and 12 children (1.2 %), but pathogenic parasites, *i.e. D. fragilis*

and *G. duodenalis*, have been detected in only 112 adults (73.2 % of those infected) and eight children (66.7 % of those infected). The most common species was *D. fragilis*, which was detected in 75 adults (7.3 %) and six children (0.6 %). *Giardia duodenalis* was detected in 33 adults (3.2 %) and two children (0.2 %) (Table I). The relationship between the different groups of outpatients and their related infections and other associated parasites are shown in Table I. Among children for whom there was a request of their general practitioners, those with *G. duodenalis* had recurrent abdominal pain (1) or showed growth retardation (1); whereas, those (2) with *D. fragilis* infection had intestinal disturbances. Among adults belonging to the same group of persons, *G. duodenalis* was detected in 18 persons (4.8 %) with intestinal disturbances, in two with eosinophilia (4.8 %), in one (0.8 %) with itch and/or cutaneous lesions and in three without symptoms (3.1 %); *D. fragilis* was detected in 34 persons (9.1 %) with intestinal disturbances, in seven with eosinophilia (16.7 %), in nine (7.2 %) with itch and/or cutaneous lesions and in seven without symptoms (7.1 %).

No relationship was identified between the infection with *G. duodenalis* or *D. fragilis* and potential risk factors. Only 6 % of persons with *G. duodenalis* and 3 % with *D. fragilis* consumed non-controlled drinking water (of well origin), and the same percentage of persons had recently taken a mud bath treatment. For 11 % of persons with *G. duodenalis* and 12 % with *D. fragilis*, there was a recent travel to endemic countries. 26 % of persons with *G. duodenalis* and 22 % with *D. fragilis* lived in rural areas and/or had dogs at home.

	Request of general practitioners		**Severe diarrhoea**		**Protracted diarrhoea**	
	Children No. 380	Adults No. 656	Children No. 546	Adults No. 291	Children No. 40	Adults No. 76
G. duodenalis	2 (0.5 %)	24[1] (3.7 %)	0	5 (1.7 %)	0	4[2] (5.3 %)
D. fragilis	2[2] (0.5 %)	57[3] (8.7 %)	2 (0.4 %)	13[4] (4.5 %)	2 (5.0 %)	5 (6.6 %)
B. hominis	2 (0.5 %)	24 (3.7 %)	1 (0.2 %)	1 (0.3 %)	1 (2.5 %)	4 (5.3 %)
Non-pathogenic protozoa	0	11 (1.7 %)	0	0	0	0
Helminths	0	5[5] (0.8 %)	0	0	0	0

[1] In six persons in association with non-pathogenic protozoa (*Iodamoeba bütschlii, Endolimax nana, Entamoeba coli* and *Trichomonas hominis*).
[2] In one person in association with *Blastocystis hominis*.
[3] In 22 persons in association with non-pathogenic protozoa.
[4] In two persons in association with *Blastocystis hominis*.
[5] Two persons with *Enterobius vermicularis*, two persons with O*pistorchis felineus*, and one person with *Dicrocoelium dentriticum*.

Table I. – Parasitic infections detected in children and adults according to clinical suggestions.

Isolate code	**Host sex/age**	**Geographical origin**	**Genotype**	**Clinical symptoms**
ISSGD77	M/63	Italy	A2	Diarrhoea
ISSGD78	F/84	Italy	A2	Diarrhoea, vomit
ISSGD108	M/59	Italy	A2	Diarrhoea
ISSGD79	M/44	Italy	A3	Diarrhoea, abdominal pain
ISSGD107	M/28	Italy	A3	Diarrhoea
ISSGD42	F/11	Albany	A4	Not known
ISSGD40	M/8	Ukraine	A1 + A2	Not known
ISSGD65	F/22	Morocco	A + B	Diarrhoea, abdominal pain, vomit
ISSGD80	F/87	Italy	A + B	Diarrhoea, abdominal pain
ISSGD103	F/15	Albany	A + B	Diarrhoea, abdominal pain, fever
ISSGD111	M/47	Italy	B3	Diarrhoea, abdominal pain
ISSGD102	F/19	Ecuador	B4	Diarrhoea, itch
ISSGD105	M/20	Italy1	B4	Diarrhoea, abdominal pain
ISSGD106	F/4	Ecuador	B4	Diarrhoea, abdominal pain
ISSGD110	M/47	Italy	B4	Diarrhoea
ISSGD109	F/2	Italy	B1 + B3	Not known
ISSGD64	M/36	Italy[1]	B3 + B4	Diarrhoea, abdominal pain
ISSGD82	F/17	Ecuador	B3 + B4	Asymptomatic

[1] recent travel to Egypt.

Table II. – Assemblage and genotype of 18 isolates of *Giardia duodenalis* collected at the day care centre of Perugia, Italy, from 2002 to 2003.

The molecular typing of 18 *G. duodenalis* isolates shows that both the Assemblages A and B circulate in Italy and in other European and extra-European countries. In three persons, both the Assemblages A and B have been detected. Concerning the Assemblage A, four genotypes (A1-A4) have been identified, whereas for the Assemblage B three genotypes have been detected (B1, B3 and B4). No relationship was observed between the Assemblage or the genotype and the clinical pattern (Table II).

DISCUSSION

The prevalence of infection detected in the present study for both *G. duodenalis* and *D. fragilis* is in the range of those detected by other studies carried out in the same region (Table III). In the area under study, *G. duodenalis* can be considered to be very rare in children (0.2 %) and rare in adults (3.2 %), and *D. fragilis* to be rare in children (0.6 %), whereas it is quite frequent in adults (7.3 %). Considering that about 25 % of infected persons had a recent history of travel to developing countries, the prevalence of infection in the human population living in the investigated area should be lower. In fact, in the same period of time of this survey (2002-2003), a higher prevalence of infection with *G. duodenalis* and *D. fragilis* was observed in immigrants living in the Perugia area; indeed, *G. duodenalis* and *D. fragilis* have been detected in 28.9 % in 4.4 %, respectively, of adopted children, in 19.6 % and in 8.9 %, respectively, of immigrant children, and in 2.0 % and 12.2 %, respectively, of immigrant adults (data not shown).

In our opinion, the higher prevalence of *D. fragilis* infections compared to that of *G. duodenalis* is mainly due to the examination of faecal samples by Giemsa stain in a very short period of time after defecation, a

Italian region	Positive/examined (%)	Study populations	References
Several regions	6/618 (0.9)	Children with diarrhoea	Caprioli *et al.*, 1996
	0/135	Healthy children	
Emilia Romagna	5/197 (2.5)	Children of crèches	Canestri Trotti *et al.*, 1988
	4/107 (3.7)	Crèche attendants	
	5/99 (5.1)	Child cohabitants	
	51/752 (6.8)	Children and adults	Libanore *et al.*, 1992
	7/214 (3.3)	Adults with enteritis	Libanore *et al.*, 1991
	8/561 (1.4)	Children (6 mo – 2 yr of age)	Cevenini *et al.*, 1985
Piedmont	10/803 (1.2)	Children and adults	Libanore *et al.*, 1992
	3/71 (4.2)	Italian food handlers	
	3/77 (4.2)	Immigrant food handlers	Miotti *et al.*, 1992
	17/665 (2.6)	Children and adults	Ferrini *et al.*, 1994
Veneto	13/550 (2.4)	Institutionalised psychiatric persons	Gatti *et al.*, 2000
Liguria	9/1,186 (0.8)	Children and adults	Barbaro *et al.*, 2001
Umbria	15/1,536 (1.0)	Children	Crotti & Del Sante, 1997
	30/1,168 (2.6)	Adults	
	11/3,077 (0.3)	Children	Crotti, 2002
	81/2,971 (2.7)	Adults	
	1/130 (0.3)	Children	Crotti *et al.*, 2002
	3/193 (1.6)	Adults	
	0/293	Children	Crotti *et al.*, 2003
	5/171 (2.9)	Adults	
Marche	(5.5)	Immigrants	Giacometti *et al.*, 2000
	(5.0)	Institutionalised psychiatric persons	
	(4.6)	HIV-positive persons	
	(2.5)	Travellers	
Abruzzo	64/606 (10.5)	Children and adults[1]	Del Vecchio *et al.*, 1994
	31/207 (13.9)	Children and adults[2]	
	36/355 (10.1)	Children	Fazii *et al.*, 1998
	11/600 (1.8)	Children and adults	Ricci & De Michele, 2001
	3/139 (2.2)	Children	Giangaspero *et al.*, 2002
	3/161 (1.8)	Adults	
Campania	237/5,000 (4.7)	Children	Scotti *et al.*, 1996
Apulia	4/65 (6.2)	HIV-positive persons with diarrhoea	Brandonisio *et al.*, 1999
	2/89 (2.3)	Asymptomatic HIV-positive persons	

[1] Attending the day care centre.
[2] Hospitalised.

Table III. – Prevalence of *Giardia duodenalis* in different categories of children and adults according to epidemiological survey carried out in Italy from 1983 to 2002.

Italian region	Positive/examined (%)	Study populations	References
Veneto	(3.5)	Children and adults	De Canale *et al.*, 2003
Umbria	17/151 (11.3)	Children and adults	Crotti *et al.*, 2001
	27/394 (6.9)	Children and adults	Crotti *et al.*, 2003
	0/330	Children with acute or protracted diarrhoea	Crotti & D'Annibale, 2001
	6/193 (3.1)	Adults with acute or protracted diarrhoea	
	15/413 (3.6)	Adults attending a day care centre	
	4/82 (4.9)	Children with enteritis	Crotti & D'Annibale, 2001
	5/23 (17.4)	Adults with enteritis	
	4/22 (18.2)	Adults with abdominal disturbances	
	5/12 (41.7)	Asymptomatic adults or adults with eosinophilia, itch, cutaneous lesions	

Table IV. – Prevalence of *Dietamoeba fragilis* in different categories of children and adults according to epidemiological survey carried out in Italy.

procedure, which is rarely followed in laboratories for the diagnosis of enteric parasites. These epidemiological data suggest that in other surveys, where faecal samples are examined after a period of time and without Giemsa staining, most of *D. fragilis* infections are undetected. The prevalence of these two parasites in the different groups of outpatients suggests that they mainly cause unspecific intestinal disturbances, frequently protracted in time, whereas their role in severe enteritis is less important.

In Italy, the prevalence of *G. duodenalis* in children of different ages ranges from 0 % to 10.1 % with an average of 2.7 % (321/11,776), whereas in adults the prevalence ranges from 1.6 % to 5.1 % with an average of 2.7 % (157/5,782) identical to that detected in children (Table III). The molecular typing of *G. duodenalis* isolates confirm previous studies carried out in Italy and in other countries, where only the Assemblages A and B were detected. Although the investigated sample was small, the clinical symptoms varied from very weak to moderately severe, yet there was no relation between the severity of the infection and the Assemblage or genotype of *G. duodenalis* (Table II). The potential link between the genetic background of the parasite and the severity of giardiasis was the subject of two investigations carried out in the Netherlands and in Australia, which, however, have conflicting results. In fact, Homan & Mank (2001) found a strong correlation between persistent diarrhoeal complaints and Assemblage B in a sample from the Dutch population, whereas Read *et al.* (2002) found a strong correlation between diarrhoea and Assemblage A in Australian children under five years of age. Whether these contrasting results could be explained by age differences remains an open question.

There are very limited data on the prevalence of *D. fragilis* in the Italian population, because most laboratories either ignore the existence of this parasite, or do not use specific precautions to examine the samples before the trophozoites disintegrate, or do not use the appropriate staining procedures (Table IV). In this study, faecal samples were examined by Giemsa staining immediately after collection.

The number of reports on *D. fragilis* from Europe and North America is also very limited, suggesting that there is a general problem to detect this parasite in faecal samples. In fact, the diagnostic methods employed have a profound effect on the successful detection of *D. fragilis* and consequently on the accuracy and interpretation of such reports (Johnson *et al.*, 2004). In addition, since the pathogenic role of this parasite is not fully recognised, *D. fragilis* is generally not included in the panel of parasitic pathogens that are screened in private and public laboratories.

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REFERENCES

Barbaro G.B., Cavallino A.M., Gianotti A., Patrone C. & Robello A. Esami coproparassitologici: analisi di una casistica quinquennale raccolta nel territorio del Ponente Genovese. *GIMMOC*, 2001, *5*, 18-20.

Brandonisio O., Maggi P., Panaro M.A., Lisi S., Andriola A., Acquafredda A. & Angarano G. Intestinal protozoa in HIV-infected patients in Apulia, South Italy. *Epidemiology and Infection*, 1999, *123*, 457-462.

Cacciò S., De Giacomo M. & Pozio E. Sequence analysis of the β-giardin gene and development of a polymerase chain

reaction-restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. *International Journal for Parasitology*, 2002, *32*, 1023-1030.

CANESTRI-TROTTI G., VISCONTI S., VACCA F. & ZONI D. Sulla presenza di *Giardia intestinalis* in asili nido della città di Bologna nel 1987. *Giornale Italiano Malattie Infettive Parassitarie*, 1988, *40*, 638-640.

CAPRIOLI A., PEZZELLA C., MORELLI R., GIAMMANCO A., ARISTA S., CROTTI D., FACCHINI M., GUGLIELMETTI P., PIERSIMONI C. & LUZZI I. Enteropathogens associated with childhood diarrhea in Italy. The Italian Study Group on Gastrointestinal Infections. *Pediatric Infectious Disease Journal*, 1996, *15*, 876-883.

CEVENINI R., VAROLI O., RUMPIANESI F., MAZZARACCHIO R., NANETTI A. & LA PLACA M. A two-year longitudinal study on the etiology of acute diarrhea in young children in Northern Italy. *Microbiologica*, 1985, *8*, 51-58.

CROTTI D. Parassitosi intestinali autoctone nella seconda metà degli anni '90: considerazioni critiche diagnostiche. *Microbiologia Medica*, 2002, *17*, 7-13.

CROTTI D. *Dientamoeba fragilis*: e se potesse anch'essa essere una zoonosi? *Notizie AMCLI*, 2003, *11*, 7-8.

CROTTI D. & D'ANNIBALE M.L. *Dientamoeba fragilis* e dientamoebosi: aspetti di parassitologia clinica e diagnostica di laboratorio. *Parassitologia*, 2001, *43*, 135-138.

CROTTI D., D'ANNIBALE M.L., FONZO G., MEDORI M.C. & UBALDI M. Diarree acute e protratte nella popolazione del territorio perugino: diagnosi microbiologica delle enteriti ed aspetti clinico-epidemiologici relativi al 2001. *Infezioni in Medicina*, 2002, *2*, 81-87.

CROTTI D., D'ANNIBALE M.L. & MEDORI M.C. Diagnostica microbiologica ed epidemiologia locale delle enteriti acute e protratte: risultati relativi all'anno 2002. *Giornale Italiano Malattie Infettive*, 2003, *9*, 205-212.

CROTTI D. & DEL SANTE M. Parassitosi intestinali autoctone nella popolazione del Perugino: prevalenze e considerazioni diagnostico-epidemiologiche. *Giornale Italiano Malattie Infettive*, 1997, *3*, 83-88.

DA SILVA A.J., BORNAY-LLINARES F.J., MOURA I.N.S., SLEMENDA S.B., TUTTLE J.L. & PIENIAZEK N.J. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. *Molecular Diagnosis*, 1999, *4*, 57-64.

DE CANALE E., TESSARI A., CAMPION L. & ROSSI L. *Dientamoeba fragilis*: is it really fragile? Approach to specimen handling and rapid microscopic diagnosis. *Parassitologia*, 2003, *45*, 19-22.

DEL VECCHIO A., FAZII P. & CASENTINO L. Studio della prevalenza della giardiasi in un gruppo di pazienti sintomatici pescaresi. *Microbiologia Medica*, 1994, *9*, 240-241.

FAZII P., SCASSA E. & COSENTINO L. Epidemiologia della giardiasi nel pescarese. *Parassitologia*, 1998, *40* (S1), 54.

FERRINI A., ORSO GIACONE G. & ZANELLA D. Parassitosi intestinali: aspetti epidemiologici in una U.S.S.L. del Piemonte. *Microbiologia Medica*, 1994, *9*, 245.

GATTI S., LOPES R., CEVINI C., IJAOBA B., BRUNO A., BERNUZZI A.M., DE LIO P., MONCO A. & SCAGLIA M. Intestinal parasitic infections in an institution for the mentally retarded. *Annals of Tropical Medicine and Parasitology*, 2000, *94*, 453-460.

GIACOMETTI A., CIRIONI O., FORTUNA M., DRENAGGI D., VECCIA S., D'ERRICO M.M. & SCALISE G. Giardiasis: a parasitic disease of continued topicality. Study of prevalence among a selected adult population. *Infezioni in Medicina*, 2000, *8*, 82-86.

GIANGASPERO A., PAOLETTI B., TRAVERSA D. & IORIO R. *Giardia* spp. in dogs and humans in Abruzzo region (Central Italy). *Parassitologia*, 2002, *44* (S1), 81.

HOMAN W.L. & MANK T.G. Human giardiasis: genotype linked differences in clinical symptomatology. *International Journal for Parasitology*, 2001, *31*, 822-826.

JOHNSON E.H., WINDSOR J.J. & CLARK C.G. Emerging from obscurity: Biological, clinical, and diagnostic aspects of *Dientamoeba fragilis*. *Clinical Microbiology Review*, 2004, *17*, 553-570.

JOHNSON J.A. & CLARK C.G. Cryptic genetic diversity in *Dientamoeba fragilis*. *Journal of Clinical Microbiology*, 2000, *38*, 4653-4654.

LIBANORE M., ROSSI M.R., CIRELLI C., ROCCHETTI A., SCAGLIONE L. & GARAVELLI P.L. Parassitosi intestinali nel ferrarese e nell'alessandrino: aspetti epidemiologici differenziali nel periodo 1986-90. *Microbiologia Medica*, 1992, *7*, 122.

MIOTTI T. & ROSSO S. Prevalenza di parassitosi intestinali in un campione di lavoratori italiani ed extracomunitari impiegati nel settore alimentare in Torino. *Microbiologia Medica*, 1992, *7*, 124-125.

PEEK R., REEDEKER F.R. & VAN GOOL T. Direct amplification and genotyping of *Dientamoeba fragilis* from human stool specimens. *Journal of Clinical Microbiology*, 2004, *42*, 631-635.

READ C., WALTERS J., ROBERTSON I.D. & THOMPSON R.C. Correlation between genotype of *Giardia duodenalis* and diarrhea. *International Journal for Parasitology*, 2002, *32*, 229-231.

RICCI E. & DE MICHELE M.B. Valutazione dell'incidenza di parassitosi sostenute da *Giardia intestinalis* in una popolazione compresa tra la Valle Peligna e l'alta Val Pescara dopo l'emigrazione di diversi nuclei familiari (crica 200 persone) provenienti dall'Albania avvenuta negli anni 90 (1997-1999). *Rivista Medica di Laboratorio*, 2001, *2*, 15-16.

SCOTTI S., PETTOELLO MANTOANI M., POLITO G., CARLOMAGNO F., COPPOLA A. & DI MARTINO L. Infezioni da *Giardia duodenalis* in pediatria: nostra casistica. *Infezioni in Medicina*, 1996, *4*, 35-40.

THOMPSON R.C.A. Giardiasis as a re-emerging infectious disease and its zoonotic potential. *International Journal for Parasitology*, 2000, *30*, 1259-1267.

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