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Genotyping Zoonotic Giardiasis In Kaloubia Province, Egypt

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ABSTRACT

Out of 100 fecal samples from Cattle, 24%gave positive results to Giardiasis, and also out of 100 stool samples from man occupationally contacts with cattle in different age and seasons, 32% were positives. The percentage of human Giardiasis among man in different ages (infants, preschool children and adolescents) in Kaloubia Province revealed that 11% 10%, and 8% respectively and lastly in adults 3% while the percentage of human Giardiasis among man in different seasons revealed that 12% , 10 and 8% during the summer season , autumn, and spring respectively and the lowest infectious rate was in winter 2% . In this assay by using heminested PCR-restriction fragment length polymorphism analysis for the detection and genotyping of *Giardia lamblia* on the basis of polymorphism in the triose phosphate isomerase (tpi) gene. According the taxonomy of Giardia, genotype indicate that the assemblages A and B classified as zoonotic type and also represented result may give us indication to the possibility of zoonotic potential of giardiasis.

INTRODUCTION

Giardiasis is an infection with the protozoan parasite *Giardia lamblia* which may act as a commensal or invade the tissues, giving rise to intestinal or extra-intestinal disease. The infection presents worldwide but the most prevalent and severe one is in tropical areas, where rates may exceed 40% under conditions of crowding, poor sanitation, and poor nutrition. the parasite exists as resistant cysts or more fragile trophozoites. Cysts are the infectious form found in the stool of asymptomatic carriers or patients with mild disease *(1)*.

Giardia is an important causative agent of infective diarrhea of children below five years in rural and less developed urban areas of developing countries. Infection is usually water-borne, using water from polluted water channels *(2)*.

The greatest zoonotic risk is from genotypes of *Giardia lamblia* in assemblage A, and to lesser extent genotypes in assemblage B. And a better assessment for this can only come from studies which examine the dynamics of Giardia transmission between hosts living in the same geographical area or localised endemic focus *(3)*. Zoonotic genotyping of *Giardia lamblia* isolates provides important information for establishing their phylogenetic relationship or for the epidemiological evaluation of the spreading of this parasite *(4)*.

This study was carried out to determine the cumulative incidence of *Giardia lamblia* infections in cattle. It also sought to assess the changes in infection pattern of animals diagnosed as shedding Giardia over time and determine risk factors that may be associated with *Giardia lamblia* infections, and also identify potentially zoonotic infections *(5)*.

MATERIAL AND METHODS

Sampling

The samples were collected randomly only one sample from the examined individual including apparently healthy and clinically diseased. Feces may contain only cysts, and/or

fragile *Giardia lamblia* trophozoite, may be present in diarrheic specimens. Cattle fecal samples, were collected from the Tukh centers abattoirs of Kaloubia province - Egypt. Fecal sample directly obtained from the rectum or immediately after defecation and placed in polyethylene bags, closed well labeled with a serial number, locality and date of collection. Data recorded from each cattle included: species, owner's name and the time the specimen collected. Closed fecal polyethylene bags were placed in plastic bags, then transported to the laboratory with a minimum of delay, fecal specimens should never be incubated or frozen before examination *(6)*. One hundred human stool specimens were collected from different age groups, 25 from each, infant, pre-school children, adolescents and adults, from Tukh centers abattoirs of Kaloubia province-Egypt. The stool was collected according to the described method *(7)*.

DNA extraction and polyvinylpyrrolidone (PVP) treatment

DNA extraction from whole feces and from stained smears on glass microscope slides, including further DNA purification using PVP, was performed by 1 ng DNA μl−1 from cultured *Giardia lamblia* trophozoites of reference strains VNB3 and AMC13 (assemblage A), AMC9 (assemblage B), 265KA1184. DNA from purified oocysts was also prepared by a similar method, *(8)*.

PCR amplification

The tpi gene sequences of *Giardia lamblia* assemblage A groups I and II (GenBank accession nos L02120 and U57897, respectively) and assemblage B (L02116 and AF069561) were aligned using the program BioEdit. Two sets of four primers were designed to amplify *Giardia lamblia* assemblages A and B. Amplification was performed in two phases. A duplex phase-I PCR was performed using a conventional thermocycler (Biometra T3; Anachem) and primers designed to amplify fragments of the tpi gene of *Giardia lamblia* of 576 bp from assemblage A (primers TPIA4F/TPIA4R) and 210 bp from assemblage B (primers TPIB4F/TPIB4R). The duplex reaction was performed in a 10 μl volume with 5 μl DNA in 1× PCR buffer, 2 mM MgCl2, 0.25 mM of each dNTP, 0.3 μM of each primer and 0.5 U Taq DNA polymerase (all reagents from Invitrogen). Samples were subjected to an initial denaturation of 94°C for 1min, 25 cycles of 94°C for 20 s, 50°C for 30 s and 72°C for 1min and a final extension at 72 °C for 5 min.

Two separate phase-II PCRs, with inner forward (IF) and reverse (IR) primers, were devised to amplify fragments of the *Giardia lamblia* tpi gene of 452 bp from assemblage A (primers TPIA4IF/TPIA4IR) and 141 bp from assemblage B (primers TPIB4IF/TPIB4IR). Both phase-II reactions were performed as real-time hot-start PCRs using a Light Cycler (Roche Molecular Biochemicals). The reaction comprised 10 μl of the phase I duplex-PCR product diluted 10 times in nuclease-free water (Sigma), 2 mM $MgCl_2$, 1 μM of each primer (IF/IR) and 2μl Master Mix (FastStart DNA Master SYBR Green I kit; Roche Molecular Biochemicals) in a volume of 20 μl. Cycling conditions were 95°C for 8 min followed by 40 cycles of 95°C for 15s, 58°C for 3s and 72°C for 10s, with a transition rate of 20°C s. Fluorescence readings were taken after each extension step and as a final melting analysis by treatment at 95°C for0 s, 68°C for15s followed by a transition at 0.1°C s−1 to 95°C. Melting temperatures (Tm) were derived from melting peaks using Light Cycler software version 3.5. Each test batch contained a maximum of 30 samples plus one positive control (AMC13- or AMC9-derived DNA) and one negative control *(9)*.

Gel electrophoresis and RFLP

RsaI restriction sites were identified from an alignment of the tpi gene of G. duodenalis assemblage A to distinguish between subgenotypes groups I and II. The predicted restriction fragments were 437 and 15 bp for group I and 235, 202 and 15 bp for group II.

PCR products were recovered from Light Cycler glass capillaries by centrifugation and RFLP analysis was performed by digesting 5 μl

PCR product with 5 U restriction enzyme in 1× enzyme buffer (Invitrogen) in a final volume of 30 µl for at least 4 h at 37 °C. Restriction fragments were separated in 3.2 % agarose / ethidium bromide gels by horizontal electrophoresis and examined by UV transillumination.

DNA sequencing

PCR products were purified using a StrataPrep PCR purification kit (Stratagene). Sequencing of PCR products (sense and antisense) was performed at the Advanced Biotechnology Centre, Imperial College, London, UK, using an ABI 377 automated DNA sequence and appropriate IF and IR primers.

RESULTS

Table 1. Infectious rate of *Giardia lamblia* in Cattle in Kaloubia Province

Numbers of examined animals	Infectious rate
100	24%

Table 2. Infectious rate of human *Giardia lamblia* in Kaloubia Province

Infants		Preschool children		Adolescents		Adults		Total	
N	%	N	%	N	%	N	%	N	%
25	11	25	10	25	8	25	3	100	32

Table 3. Seasonal infectious rate of human *Giardia lamblia* in Kaloubia Province

Seasons	Number of examined patients	Infectious rate (%)
Summer	25	12
Autumn	25	10
Spring	25	8
Winter	25	2
Total	100	32

Table 4. Results of PCR-RFLP analysis of the *Giardia lamblia* gene amplified from DNA extracted from whole feces (32 samples) and IF-stained fecal smears

DNA extracted from whole feces sample	Assemblage A	Assemblage B	Assemblage A and B	Percentage of positive tests (%)
Patients samples Assemblage A	6	0	0	18,7
Patients samples Assemblage B	0	7	0	21,8
Patients samples both A and B Assemblage	0	0	4	12,5

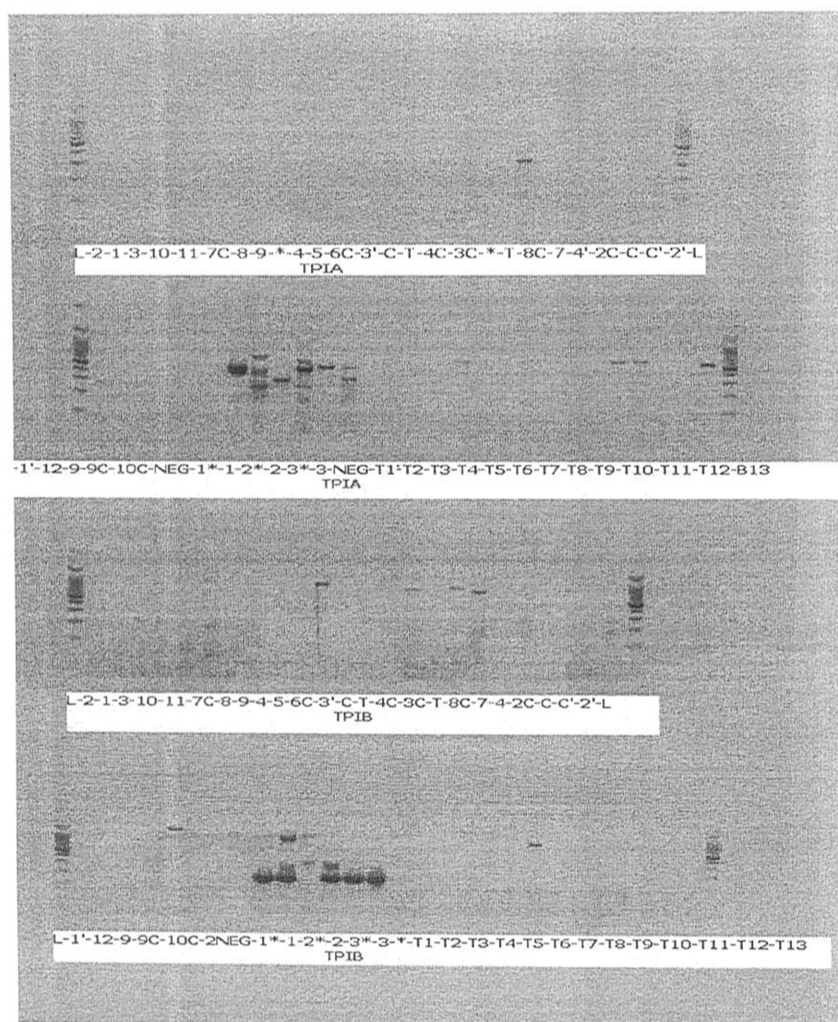

Fig. 1. RsaI digestion of TPIA-PCR products on an ethidium bromide- stained 3.2% agarose gel. (Upper group: Giardia assemblage A, lower group: Giardia assemblage B).

Table 5. Genotypic groupings of Giardia Lamblia *(10)*.

Assemblage (genotypic grouping)	Host range
Assemblage A (Group I)	Humans and other mammals; zoonotic
Assemblage A (Group II)	Mainly humans; zoonotic
Assemblage B (Group III)	Humans and other mammals; zoonotic
Assemblage B (Group IV)	Humans
Assemblage C/D	Dogs
Assemblage E	Livestock
Assemblage F	Cats

DISCUSSION

The coprologic examinations of Giardia lamblia among Cattle in Kaloubia Province revealed that out of 100 examined Cattle, 24 animals gave positive results, with an infectious rate of 24% (table 1). Our finding agrees with that reported by previous studies ***(5,11)***.

Table 2 showed summarized results of infectious rate of human Giardiasis. Out of 100 fecal samples 32 were positives, with infectious rate 32%. Also it is noticed that the infectious rate was high in infants (11%) followed by preschool children (10%), then adolescents (8%) and lastly in adults (3%).This indicates that *Giardia lamblia* is mainly a disease of infants ***(12,13)***.

The seasonal infectious rates of human Giardiasis were presented in Table 3, and revealed that the summer season was the highest infectious rate (12%), followed by autumn (10%), then spring (8%), and the lowest infectious rate was in winter (2%). From these results it may safely conclude that days and months of high temperature are of high infectious rate. High temperature is always associated with increased relative humidity, this condition favors the survival and maintenance of parasitic infectious agent. Our findings agree with those reported in Tokyo ***(14)***.

From the Table 4, it is evident that zoonotic potential in this represented study by applied PCR-RFLP which demonstrates that the PCR-RFLP procedure described here reliably identifies both *Giardia lamblia* strains of assemblage A and assemblage B, which has been advised to use this sensitive PCR-restriction fragment length polymorphism assay for detection and genotyping of *Giardia lamblia* in human stool, also the detection and genotyping of *Giardia lamblia* from human faeces by real-time PCR/RFLP analyses ***(9)***, as well as several previous studies have described the development of specific PCR techniques for the detection of *Giardia* cysts and assessed their sensitivities ***(15-17)***. In addition, the application of DNA-based 'tools' is helping to understanding zoonotic transmission and epidemiological issues, in particular, the nature of the etiological agents that are responsible for causing Giardiasis in mammals ***(3)***.

In this represented study assemblage A was detected in 6 samples, assemblage B was detected in 7 samples, and a mixture of assemblages A and B was detected in 4 samples. The isolates belonging to genotype A and B of *Giardia lamblia* are widespread in humans, in particular infection with genotypes A and B, which could be zoonotic ***(18)***. The use of molecular epidemiological tools has significantly changed our understanding of zoonotic transmission of Giardia spp. Systematic comparisons of assemblage A subtypes and assemblage B at various genetic loci and elucidation of parasite population of zoonotic transmission of *Giardia lamblia* ***(4)***. The development of next-generation subtyping tools, the integration of molecular analysis in epidemiological studies, and an improved understanding of the population genetics of *Giardia lamblia* in humans and animals ***(19)***.

On the other hand children with Giardia isolates from assemblage B were more likely to have symptomatic infections than children with isolates from assemblage A. Although considerable sequence variability was seen in the assemblage-B isolates, the assemblage-A isolates were relatively genetically homogeneous ***(20)***.

CONCLUSION

From this work we can conclude that Cattle infected with *Giardia lamblia* should be treated, because of close contact with human. Infants must be breast feeding, strict sanitation & also education of the general public personal hygiene, protective clothing and gloves must be used, and also sanitary disposal of human stool and improvement of living environment can help to prevent spreading of the infection. Dissemination of information regarding the risks involved in eating uncooked vegetables

and in drinking water of questionable purity. Supervision by health agencies of the health and sanitary practices of persons preparing and serving food in public eating places and general cleanliness of the premises involved. Periodical examination of food handlers as a control measure must be adopted. The taxonomy of Giardia genotype indicate that the assemblages A and B classified as zoonotic type and also represented result may give us indication to the possibility of zoonotic potential of giardisis specially in children and the application of genotyping may helping to understanding Zoonotic transmission and epidemiological issues.

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الملخص العربى

التصنيف الجينى للجيارديا المشتركة فى محافظة القليوبية ، جمهورية مصر العربية

عادل محمد عبد العزيز نويشى

قسم الأمراض المشتركة - كلية الطب البيطرى - جامعة بنها

من ١٠٠ عينة براز من الماشية أظهرت النتائج ٢٤ ٪عينة أيجابية لمرض الجيارديا. وأيضا أخذت ١٠٠ عينة براز من الانسان المخالط لتلك الماشية أظهرت النتائج ٣٢٪ عينة أيجابية. ولوحظ أيضا أن نسبة الاصابة كانت عالية عند الرضع (١١٪)، يليه الأطفال قبل سن المدرسة (١٠٪)، ثم المراهقين (٨٪)، وأخيرا في البالغين (٣٪). وقد لوحظ أيضا أن نسبة الأصابة في المواسم المختلفة في محافظة القليوبية أن موسم الصيف هو أعلى معدل أصابة(١٢٪)، تليها الخريف (١٠٪) ، فالربيع (٨٪)، وكان أدنى معدل فصل الشتاء بنسبة أصابة (٢٪). وبفحص ١٠٠ عينة من مختلف الفئات العمرية من الجنسين ، بأستخدام تحليل PCR والذى أفاد بتعدد الأشكال للكشف والتنميط الجيني للجيارديا لامبليا على أساس تعدد الأشكال في (TPI) ، وتبعا لفحص التمييز الجيني لنواع الجيارديا لامبلياA وB من بين عينات الحمض النووي والتى أظهرت العلاقة المرضية المشتركة للذين يعانون مؤكدا من مرض الجيارديا وأظهرت أيضا أحتمالية الأصابة التبادلية بين الحيوان و الأنسان المخالط.