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University of Diyala

College of Veterinary Medicine

Department of Internal and Preventive Medicine



**ISOLATION AND MOLECULAR
IDENTIFICATION OF
GIARDIA SPP. IN CHILDREN AND
EXPERIMENTAL STUDY IN RABBITS
INFECTED WITH *G.DUODENALIS***

A Thesis

**Submitted to the Council of the College of Veterinary Medicine/University
of Diyala in partial fulfilment of Requirements for The Degree of Master
of Science in Veterinary Medicine (Zoonosis)**

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ
يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۗ وَقُلْ رَبِّ زِدْنِي عِلْمًا
﴿طه ١١٤﴾

صدق الله العظيم

Dedication

**To those who cover me with boundless love My dear father
and my mother soul.**

To my beloved wife.....who support me every time.

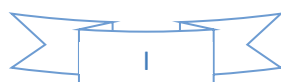
To my precious my children &brothers and sister.

To my friendswho help me every time.

**I present my modest effort with deepest and sincere gratitude
for their support.**

Mohymin.

2022



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Supervisors Declaration

We certify that this thesis has been prepared under my supervision at the Department of Internal and Preventive Medicine / University of Diyala entitled "**Isolation and Molecular Identification of *Giardia spp.* in Children and Experimental study in Rabbits Infected with *G. duodenalis***" as a partial fulfillment of the requirements for the degree of Master of Science in Veterinary Medicine (Zoonosis).

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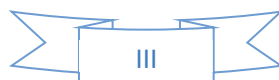
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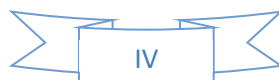
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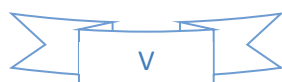
Abstract

Giardia duodenalis is one of the most prevalent human intestine protozoan parasites in the world, and infected a broad variety hosts of animals. The goal of the current study was to determine the infection rate and risk factors of *Giardia duodenalis* isolates from regional hospitals and private clinics in the Iraqi province of Diyala and the genetic analysis for infection.

The study is beginning of October 2021 to the end of April 2022, 100 children's stools were collected from hospitals and health centres in Diyala, Iraq. *Giardia duodenalis* cysts and trophozoites were show symptoms of diarrhoea, nausea, and abdominal pains, according to the examined under the microscope and PCR analysis, a 7 % infection rate was recorded.

The highest infection rate was 10 % in up 4-5 year category and the lowest infection rate 4.65% recorded in up one year category. While, the infection rate was highest in male samples 8.16% than female samples 5.88%.

Most peoples who were infected were living in the rural at ratio 9.25%, followed by 4.34 % in persons living in the urban, and other side the infection rate of Giardia infection was 6.89%,4.16% and 8.51% respecting to the tap water, Reverse Osmosis(RO) water and filter water respectively. After experimental infection in rabbits, the haematological analysis between control group and treated groups at one and two months revealed a statistically



significant difference ($P < 0.05$) decrease in the mean of the following RBC count, Hb, PCV, platelets and increase of WBC count, monocytes and lymphocytes, also the pathological changes were observed grossly and histopathological in intestine including hyperplasia in colonic glands and mucosa, increase no. of goblet cells, aggregation of parasites in mucosa, in liver showed acute cellular swelling of hepatocytes, congested central vein, in kidney showed cystic dilation of proximal tubules, apoptotic cells and infiltration of mononuclear cells.

In conclusion the laboratory animals experimentally infected with *Giardia* showed similar clinical signs, symptoms to infected child including of diarrhea, abdominal pain and flatulence.

List of contents

1.1. Introduction.....	خطأ! الإشارة المرجعية غير معرفة.
1.2. Aims of Study	خطأ! الإشارة المرجعية غير معرفة.
2. Literature Review.....	خطأ! الإشارة المرجعية غير معرفة.
2.1. Definition	خطأ! الإشارة المرجعية غير معرفة.
2.2. Transmission	خطأ! الإشارة المرجعية غير معرفة.
2.3. Taxonomy of Giardiasis.....	خطأ! الإشارة المرجعية غير معرفة.
2.4. Life Cycle.....	خطأ! الإشارة المرجعية غير معرفة.
2.5. Epidemiology of Giardiasis	خطأ! الإشارة المرجعية غير معرفة.
2.6. Diagnosis.....	خطأ! الإشارة المرجعية غير معرفة.
2.7. The Pathological Features	خطأ! الإشارة المرجعية غير معرفة.
2.8. Giardia Pathogenesis and Clinical Signs.	14
2.8.1 Giardia Pathogenesis and the Microbiome	خطأ! الإشارة المرجعية غير معرفة.
2.9. Giardia Immunity	18
3. Materials and Methods.....	20
3.1. Materials.....	20
3.1.1. Instruments and Tools.....	20
3.2: Methods.....	20
3.2.1. Area of Study.	20
3.2.2. The Experimental Animals	20
3.2.2.1. Collection of Samples	20
3.2.2.2. The Isolation and Identification Giardia.....	20
3.3. Morphological Identification	خطأ! الإشارة المرجعية غير معرفة.
3.3.1. Cyst counting.	خطأ! الإشارة المرجعية غير معرفة.
3.4. Experimental infection of rabbits.	خطأ! الإشارة المرجعية غير معرفة.
3.5.2. Total White Blood Cell Count (WBCC).	خطأ! الإشارة المرجعية غير معرفة.

3.5.3. Differential White Blood Cells Count.	24
3.5.4. Hemoglobin Concentration (Hb).	24
3.5.5. Packed Cell Volume (PCV).	25
3.5.6. The Absolute Values.....	25
3.6. Molecular Study.....	26
3.6.1. Protocol	خطأ! الإشارة المرجعية غير معرّفة.
3.6.2. Agarose gel electrophoresis of DNA	27
3.6.3. Prepare of the Agarose gel.....	27
3.6.4. Preparation of Samples	28
3.6.5 Red safe Nucleic acid staining solution.....	خطأ! الإشارة المرجعية غير معرّفة.
3.6.6 Diagnosis of primers Genes	خطأ! الإشارة المرجعية غير معرّفة.
3.6.6.1. Maxime PCR PreMix kit (i-Taq) 20µlrxn (Cat. No. 25025) see appendix	29
3.6.6.2. Diagnosis of Gene.....	خطأ! الإشارة المرجعية غير معرّفة.
3.6.6.3. Diagnosis of Gene.....	30
3.7. Histopathological Changes Examination.....	31
3.8. Statistical analysis	31
4. Results	32
4.1 Clinical Signs	32
4.2 Morphology and laboratory examination of Giardia sp.	33
4.3. Severity infection of Giardia sp.	34
4.4 Epidemiological Study.....	34
4.4.1 Distribution of Giardia sp. Infection in the Area of the Study.	34
4.4.1.2. Distribution of Giardia According Locality	35
4.4.1.3.The Infection Rate of Giardia Infection According to the Age groups ..	35
4.4.1.4. The Infection Rate of Giardia According to the Gender	36

4.5. Molecular Identification.....	36
4.5.1. Detection of Samples Using Conventional PCR	36
4.5.2 Molecular Identification Giardia by PCR from Children.....	37
4.5.3 Molecular Identification Giardia by PCR from Experimental Rabbits ...	40
4.5.4 Agar Gel Electrophoresis Results	41
4.5.5 Results of Sequences.....	42
4.5.6 Iraqi Isolate Submitted to the National Center for Biotechnology Information(NCBI)	42
4.5.7 Results of Human and Rabbit Sequences	43
4.6. Haematological Parameters.	47
4.6.1. Red Blood Cells Count (RBCC).....	47
4.6. 2. Hemoglobin Concentration (HB).....	48
4.6. 3. Packed Cell Volume (PCV)	48
4.6. 4. Mean Corpuscular Hemoglobin (MCH).....	48
4.6. 5. Mean Corpuscular Hemoglobin Concentration (MCHC)	49
4.6. 6. Mean Corpuscular Volume (MCV)	49
4.6. 7. Platelets	49
4.6. 8. White Blood Cells Count (WBCC)	49
4.6. 8.1 Heterophiles	50
4.6. 8.2.Lymphocytes.....	50
4.6. 8.3. Monocytes.....	50
4.6. 8.4. Eosinophils.....	51
4.7 Pathological examination:.....	51
4.7.1 Macroscopic changes:.....	51
4.7.2 Microscopic Examination:.....	54
5. Discussion	69

6. Conclusions and Recommendations	78
6.1. Conclusions.....	79
6.2 . Recommendations.....	80
7. References.....	80
Appendix 1	97



List of tables		
Table No.	Table Title	Page
2-1	Essentially a Giardia. groups of duodenum, their hosts, and their communities	7
3-1	Instruments and Tools utilized in the study.	97
3-2	The Equipment's used in this study.	98
3-3	The chemicals and solutions used in this study	98
3-4	The materials of PCR	99
3-5	Quick-DNA™ Blood MiniPrep Catalog Nos. D3024 & D3025	100
3-6	Primer of gene(Beta-giardin)	100
3-7	Mixture of the specific interaction for diagnosis gene	100
3-8	The optimum condition of detection	101
3-9	Specific primer of gene RH11	101
3-10	The Components of the Maxime PCR PreMix kit (i-Taq)	101
3-11	Mixture of the specific interaction for diagnosis gene	102
3-12	The optimum condition of detection	102
3-13	specific primer TPiA of gene tpi	102
3-14	Maxime PCR PreMix kit (i-Taq) 20µlrxn (Cat. No. 25025)	103
3-15	Mixture of the specific interaction for diagnosis gene	103
3-16	The optimum condition of detection	103
4-1	Severity of Giardia infection according to the cyst counting.	34
4-2	Show the infection rate of Giardia according to area of study.	35
4-3	Infection rate and distribution of Giardia spp. according to the house system.	35
4-4	Show the infection rate of Giardia according to the age groups categories.	36
4-5	Prevalence and distribution of Giardia according to the gender	36
4-6	Show the primers that used in this study and the positive and negative samples from human.	38
4-7	Include the total samples (human, rabbit) and their identity percentage with other genotype of Giardia duodnalis that were collected in this study.	43
4-8	Giardia duodnalis isolate 105N triosephosphate isomerase (TPI) gene, partial cds	44
4-9	Giardia duodnalis isolate 105N triosephosphate isomerase (TPI) gene, partial cds	45
4-10	Giardia duodnalis isolate 105N triosephosphate isomerase (TPI) gene, partial cds	46
4-11	Haematological analysis between control group and treated groups at one and two months of experimental study.	47

List of Figures		
Figure No.	Figure Title	Page
2-1	Life cycle of Giardia duodenalis in Patients	9
3-1	Shown Collection of stools and procedure of isolation and	21
3-2	Working the electrophoresis system	28
4-1	Experimentally rabbits clinically show, bloody diarrhea , abdominal distention	32
4-2	A: Giardia duodenalis stained new methylene blue stain show the cyst morphology 1000X B, Cyst of Giardia without any stain 1000X : C : Cyst of Giardia stained with iodine stain measuring 10 µm D: different stages of Giardia cyst 400X. The images are captured by a digital camera.	33
4-3	Band size of 500 base pairs, PCR product. Electrophoresis on agarose gels of 2% at 5 volts/cm ² yielded the final product. There will be a one-hour buffer of TBE strength one. N = 100, DNA ladder (7 human samples). 500 base pair beta giardin primers.	38
4-4	Band width is 292 base pairs in the PCR result. The final result was electrophoresis on agarose gels with a volt/cm ² potential of 2%. A one-hour buffer of one-time use TBE. N: A DNA ladder of 100 rungs (7 human samples) with the RH11 sequence, 292 base pairs in length..	39
4-5	Band size, in bp, for a 332 bp PCR result. The end result was an electrophoresis product on agarose gel 1.5% at 5 volt/cm ² . TBE buffer of 1x for 1:30 hours. DNA Ladder (100). the TPIA gene-specific primer was used.	40
4-6	Band size of 500 bp PCR product. The final result was electrophoresis on agarose gels with a volt/cm ² potential of 2%. A one-hour buffer of one-time use TBE. DNA Ladder (100) (Four Rabbit samples). Using 500 base pair (bp) beta-giardin primers.	41

4-7	Band size, in bp, for a 332 bp PCR result. The end result was an electrophoresis product on agarose gel 1.5% at 5 volt/cm ² . TBE buffer of 1x for 1:30 hours. DNA Ladder (100).tpi gene	42
4-8	Grossly appearance of intestine and liver in 2nd group shows accumulation of food in large intestine with enlargement and slightly congested of liver.	52
4-9	Grossly appearance of intestine in 2nd group shows swelling and accumulation of food in large intestine with adipose tissue.	52
4-10	Grossly appearance of intestine and liver in 3rd group shows irregular edges, congested with slightly enlargement.	53
4-11	Grossly appearance of intestine in 3rd group shows enlargement of intestine with filled by the food.	53
4-12	Histological section of intestine in 1st group show normal parenchyma of intestine (X10; H&E stain).	54
4-13	Histological section of liver in 1st group show normal parenchyma of liver tissue (X20; H&E stain).	54
4-14	Histological section of kidney in 1st group show normal parenchyma of glomeruli and tubules (X10; H&E stain).	55
4-15	Histopathological section of intestine in 2nd group shows: a: Eosinophils b: heavy infiltration of mucosa and submucosa by mononuclear cells and polymorphic cells mostly eosinophilic c: atrophy and necrotic of intestinal gland (X40; H&E stain).	56
4-16	Histopathological section of intestine in 2nd group shows: a: heavy increase in no. and size of mucosa goblet cells with basophilic mucine b: sloughing of mucosa c: hyperplastic of crypts of intestinal gland (X40; H&E stain).	56
4-17	Histopathological section of liver in 2nd group shows: a:	57

	Increase in number of kupffer cells b: hepatocytes swelling and degenerated c: irregular dilation of sinusoids d: Focal aggregation of dark basophilic mononuclear cells (X40; H&E stain).	
4-18	Histopathological section of liver in 2nd group shows: a: degenerated hepatocyte b: dilated blood vessels and filled with blood c: mononuclear cells infiltration (X40; H&E stain).	58
4-19	Histopathological section of liver in 2nd group shows: a: infiltration of hepatocytes by mononuclear cells b: thick fibrous tissue c: cystic dilation of bile duct with heavy infiltration by mononuclear cells and fibroblast d: Acute cellular swelling of hepatocytes (X20; H&E stain).	58
4-20	Histopathological section of liver in 2nd group shows: a: dilated sinusoids b: increase in the no. of Kupffer cells c: heavy mononuclear cells infiltration mostly lymphocytes and macrophages in portal and parenchyma d: acute cellular swelling of hepatocytes (X40; H&E stain).	59
4-21	Histopathological section of kidney in 2nd group shows: a: dilated and congested blood vessels b: acute cellular swelling of tubules c: interstitial haemorrhage d: cystic tubules dilation (X20; H&E stain).	60
4-22	Histopathological section of kidney in 2nd group shows: a: moderated haemorrhage b: ballooning dilation of proximal tubules c: mononuclear cells infiltration in interstitial layer (X40; H&E stain).	60
4-23	Histopathological section of kidney in 2nd group shows: a: interstitial infiltration of mononuclear cells b: fibroblast (spindle shape) c: increase size of glomeruli with increase glomerular tuft and narrowing human space d: all proximal tubules swelling and degenerated (X40; H&E stain).	61
4-24	Histopathological section of intestine in 3rd group shows:	62

	a: thick pseudomembrane covered the mucosa b:hyperplasia in colonic glands c: mononuclear cells infiltration d: eosinophilic materials e: damaged and destroyed gland (X20; H&E stain).	
4-25	Histopathological section of intestine in 3rd group shows: a: multiple areas of necrosis in mucosa and submucosa with dead neutrophils b: increase tissue thickening in fibromuscular c: congested of blood vessels (X40; H&E stain).	62
4-26	Histopathological section of intestine in 3rd group shows: a: increase in no. and size of goblet cells in mucosa layer b: lumen dilated contains several parasites c: congested of blood vessels d: lymphocytic cells infiltration in submucosal layer e: few basophilic lymphocytes infiltration (X40; H&E stain).	63
4-27	Histopathological section of intestine in 3rd group shows: a: mucosa and submucosa heavily infiltrated by large number of mononuclear cells most of them lymphocytes b: edema in submucosa layer c: lymphatic cells infiltration in submucosa layer (X20; H&E stain).	63
4-28	Histopathological section of intestine in 3rd group shows: a: hyperplasia of mucosa b: apoptotic cells c: Parasite aggregation present in the mucosa d: some lymphocyte infiltrated in submucosa (X40; H&E stain).	64
4-29	Histopathological section of liver in 3rd group shows: a: edema b: central lobular hepatic cells necrosis c: acute cellular swelling (degeneration) of hepatocytes d: mononuclear cells infiltration (X10; H&E stain).	65
4-30	Histopathological section of liver in 3rd group shows: a: degenerated hepatocyte b: dilation in the sinusoids c: lymphocytes infiltration in the hepatocyte (X10; H&E stain).	65
4-31	Histopathological section of liver in 3rd group shows: a:	66

	increase in the number of Kupffer cells b: lymphatic sells kupffing around central vein c: acute cellular swelling d: dilated blood vessels (X20; H&E stain).	
4-32	Histopathological section of liver in 3rd group shows: a: congested central vein b: degenerated hepatocytes c: Portal inflammation of mononuclear cells character extended over the limiting plate and infiltration the lobular parenchyma (piecemeal necrosis). (X10; H&E stain).	66
4-33	Histopathological section of kidney in 3rdgroup shows: a: Cystic proximal tubules dilation b: haemorrhage c: extensive interstitial infiltration of mononuclear cells (X20; H&E stain).	67
4-34	Histopathological section of kidney in 3rdgroup shows: a: acute cellular swelling of proximal tubules b: apoptosis cells c: interstitial infiltration of mononuclear cells mostly macrophage and lymphocytes (X20; H&E stain).	68

List of Abbreviation

Abbreviations	Full name
(EtBr) stain	Ethidium bromide BioReagent
BG	Beta –Giardin
DNTP	Deoxynucleoside Triphosphate
GDH	Glutathione Dehydrogenase
GT	Genotyping
IFN	Interferon
IgA	Immunoglobulin A
NNDSS	Notifiable Diseases Surveillance System
RFLP	Restriction Fragment Length Polymorphism
SSU-rRNA	Small Subunit Ribosomal Ribonucleic acid
TBE	Tris/Borate/EDTA
TNF	Tumor Necrosis Factor
TPI	Triose Phosphate Isomerase
VSP	Modified Version Proteins
WBCs PCR SPSS	White Blood Cells Polymerase Chain Reaction Statistical Package for the Social Sciences

1.1. Introduction

One of the most prevalent intestinal parasites that affects the human and a wide variety of other animals is *Giardia* according to Feng and Xiao, (2011). Since Antonie van Leeuwenhoek's initial discovery of the parasite more than three hundred years ago (Dobell 1920), that 6 different *Giardia* species have been identified. Among them the *Giardia spp.* (*Giardia agilis*, *Giardia ardeae*, *Giardia psittaci*, *Giardia muris*) infect the animals ranging from amphibians to the rodents, also the birds, whereas the *Giardia duodenalis* (syn. *Giardia intestinalis* and *Giardia lamblia*) has a wide range of hosts that includes humans and domestic, farmed, Giardiasis is a serious zoonotic illness that affects both human and veterinary health and is brought on by *Giardia duodenalis* (Ryan and Cacci, 2013).

According to examinations into outbreaks and case control studies, Giardiasis can be transmit from the human to human (anthroponotic) or from animals to people (zoonotic) according to Xiao and Fayer, (2008).

Giardia can spread through the oral route after coming into touch with infected individuals either directly or indirectly (Feng *et al.*, 2011). Members of this genus have been responsible for several outbreaks connected to consuming or surface water sources that have impacted whole towns (Robertson *et al.*, 2010).

Understanding the host range of various *Giardia sp.* genotypes, the possibility for inter transmission, risk possible factors in the exposure of the pathogen, and environmental variables is vital for determining the rate of infection of Giardiasis. This is crucial for establishing the reproductive capability of *Giardia* infections in livestock and the burden of disease caused by parasites with animal origins in humans. With the development of molecular typing technologies, the epidemiological of *Giardia* has only lately been comprehensively examined.(Feng & Xiao, 2011).

Giardiasis is expected to affect 280 million people annually worldwide, with infection rates being greater in poorer nations (Feng and Xiao, 2011; Ryan and Caccio, 2013; Squire and Ryan, 2017).

Infections can become severe and persistent in newborns, the elderly, and those with impaired immune systems, despite the fact that they frequently resolve on their own in immunocompetent adults (Feng and Xiao, 2011). Domestic animals like sheep and cattle are recognized as a key contributor to zoonotic sources of infection since *Giardia* species and genotypes that infect people have also been found (Xiao & Fayer, 2008). The amount of zoonotic transmission and the incidence of illnesses in human and animal populations, however, varied throughout various geographical locations of the world (Feng *et al.*, 2007).

Typically, Giardiasis is considered a self-limiting clinical condition marked by the watery diarrhea, cramping in the abdomen, bloating, loss of weight, and nutritional deficiencies (Einarsson *et al.*, 2016). But silent infections happen more often than symptomatic illnesses. (Feng *et al.*, 2011; Rayani *et al.*, 2014; Wegayehu *et al.*, 2016). El-Hady *et al.*, (2019) reported that clinically diarrhoea was the first complaint that affected all cases, secondly abdominal colic 84 (90.3 %), then failure to thrive affecting 32 (34.5 %) cases, also abdominal distension affecting 26 (28 %) cases, finally vomiting affecting 6 (6.6 %) of cases.

On the other hand, the disease occurs in a wide variety of hosts including monkeys (*G. intestinalis* syn *G. lamblia*), dogs (*G. canis*), cats (*G. cati*), cattle (*G. bovis*), goats (*G. capare*), horses (*G. equi*), rabbits (*G. duodenalis*), mice and rats (*G. muris*) and guinea-pigs (*G. caviae*) (Alhayali *et al.*, 2020), in addition to its veterinary importance for morbidity, death, and production losses (Robertson *et al.*, 2014).

In terms of pathology, the disease has pathological changes includes presence the trophozoites of *Giardia spp.* in the lumen of the gallbladder and

attach to the mucosal epithelium, presence of trophozoites of *Giardia* in the lumen of the gallbladder (Alhayali *et al.*, 2020). Also, (Scott *et al.*, 2004) who mentioned that the biopsies of various site of the duodenal and jejunal mucosa may display patch distribution of *Giardia* on villi and intervillous spaces. Also, Buret and Cotton (2004) showed that the trophozoites colonize the lumen of the small intestine without invading host tissue or entering the blood stream. presence of the parasites manifested by chronic inflammatory response including slightly hyperemic blood vessels, lymphocytes, plasma cells and macrophages in filtration in mucosal and submucosal layer with degeneration in the epithelial layer (Cotton *et al.*, 2011).

Since there are few studies in Iraq and other countries in the world about the experimental infection of *Giardia* and study of haematological and histopathological changes in rabbits therefore the study was conducted due to the importance of *Giardia* sp. in human and rabbits.

1.2. Aims of Study

. The study aimed to:

- (1) Isolation and molecular identification of *Giardia* sp. in children with effect of age and gender on infection rate in Diyala governorate.
- (2) Study the pathogenicity, histopathological changes and haematological changes in rabbits infected experimentally.