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Parasitological and Molecular Detection of *Ascaridia* spp. in Local Chicken in Diyala Province, Iraq

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Parasitology

By

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2022 A. D. **1443 A. H.**

بِسْمِ اللّهِ الرَّحْمنِ الرَّحِيمِ

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I certify this thesis entitled (**Parasitological and Molecular Detection of *Ascaridia* spp. in Local Chicken in Diyala Province, Iraq**) was carried out by (**Zainab Fadhil Rahman**) has been prepared under my supervision at the College of Veterinary Medicine / University of Baghdad in a Partial Fulfillment of the requirements for the degree of Master of Science in Veterinary Medicine / Parasitology.

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Declaration

I hereby declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that this work has not been previously and is not concurrently submitted for any other degree at the University of Baghdad or other institutions.

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Name: Zainab Fadhil Rahman

Date: / / 2022

Dedication

I dedicate this thesis, my Simple and Modest Effort to …

Allah Almighty

Our master Muhammad peace is upon him …

For my mother and father

To my loyal and faithful friends

Zainab

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I thank God Almighty for his protection, guidance and providence during the period of my study

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Abstract

The current study was conducted from the beginning of October 2020 to March 2021 on 120 local chicken (*Gallus gallus domestica)* in Diyala province.to investigate the prevalence of *Ascaridia* spp., molecular diagnosis and characterization of lesions in parasite positive chicken. The total prevalence rate of *Ascaridai spp*. was (41.66%). The study showed significant ($P \leq 0.05$) difference between males and females. Females showed highest rate of infection was 50% (33/66) comparing to males 31.48% (17/54). The results showed that there was non-significant difference between adults and young chickens, where the infection rate in adults was 50% (24/48) and in young chickens was 36.11(26/72). A highly significant difference ($P \leq 0.01$) was reported in the slaughtered chicken according to months. October recorded the highest rate of infection of (55%), while the lowest rate was (30%) recorded in December.

The infected small intestine and liver subjected to histopathological study by conventional microtomy that revealed on losses of epithelia, villi atrophy, necrosis of sub-mucosal gland, cellular reaction mainly by mono nuclear cells and inflammatory cells. Furthermore, liver showed granulomatous, hemorrhagic area and necrotic foci in hepatic parenchyma, necrosis in bile duct tissue and portal vein thrombus, Along with gross pathological signs included presence of parasite in small intestinal lumen, hemorrhagic spots, thicken intestinal wall, congestion and paleness in liver.

Also, this study objective was to detect *Ascaridia* spp. by the molecular diagnosis and detection the presence of 18SrRNA gene (724bp). The technique included genomic DNA extraction from adult worms isolated from small intestine of naturally infected

local chickens with using tissue DNA extraction kits. This gene was amplified by using specific primers.

PCR technique results gave amplicon size at 724 bp. Ten positive PCR products randomly were selected and sent for sequencing and for phylogenetic analysis.

The sequences registered in NCBI GenBank and it is the first record of *A. galli* in Diyala province in local chickens by using conventional PCR. The registered accession numbers (MW732174.1), (MW732175.1), (MW732176.1), (MW732177.1), (MW732178.1), (MW732179.1), (MW732180.1), (MW732181.1), (MW732182.1) and (MW732183.1) showed high identity 99-100% with (EF180058.1) USA isolate as well as closely related to local isolates with accession numbers (MK918847.1, MK918636.1, MK918635.1, MK919081.1) from Iraqi with 99% identity to our isolates.

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List of Abbreviations

Abbreviation	Full name
AR	Antigenic resistance
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
BZs	Benzimidazoles
C.V	Central vein
ddH2O	Double-distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
EDTA	Ethylene di amine tetra acetic acid
EHA	Elemental Hair Analysis
EU	European union
FBZ	Fenbendazole
FECRT	Fecal egg count reduction test
FLBZ	Flubendazole
HCL	Hydrochloric acid
HTS	High- throughput sequencing
KCL	Potassium chloride
Kg	Kilogram
L	Lymphoid
LEV	Levamisole
MNCs	Mononuclear cells
mEq	Milliequivalents per liter
Mm	Millimeter
NCBI	National Centre For Biotechnology Information
Ng	Nanogram

NH	New Hampshire
P	Probability
PCR	Polymerase Chain Reaction
Pmol	Pico mole
Rpm	Round per minute
rRNA	Ribosomal ribonucleic acid
SAS	Statistical analysis system
SK	Skalborg
T	Thrombus
TBE	Tris / Borate / EDTA
USA	United States
UV	Ultraviolet
x^2	Chi-square
µl	Microliter
µm	Micrometer

Chapter One

Introduction

1. Introduction

There are many helminth parasites affecting and causing production losses to the poultry industry, including cestodes, trematodes and nematodes **(Abdullah, 2013: Sivajothi and Reddy, 2016).** Among them, *Ascaridia galli* is a most common nematode of domestic fowl and causing ascaridiosis in the hens, turkeys, geese and some other birds **(Radfar *et al.,* 2012)**. Studies have suggested that *Ascaridia* is the most common nematode in all types of production systems and has a worldwide distribution **(Rabbi *et al,* 2006; Abdelqader *et al.,* 2008).** This parasitic disease cause reduce growth, decrease egg production, emaciation and anemia also cause death **(Kaufmann**, **2011)**.

Life cycle of the nematode is direct but earthworms can ingest eggs and act as a transport host. Birds can get an infection by ingestion of infective eggs directly with contaminated food and water or indirectly by consumption of transport host. After ingestion, the eggs are mechanically transported to the duodenum and hatch within 24 hours. After hatching larvae penetrates the intestine for histotrophic phase, return to the lumen and finally get matured **(Soulsby, 1982; Tarbiat *et al*., 2015).** Ingestion of such eggs can not cause any clinical disease in the human as nematode will be destroyed by peptic digestion **(Ramadan and AbouZnada, 1991; Bharat *et al.,* 2017).**

Although presence of parasite worm in the hen's egg is not considered as hazard for public health, it can cause potential consumer complaint. While this erratic migration, parasite may lead the mechanical transmission of bacterial, parasitic, or viral 24 enteric organisms (like *E. coli*, *Salmonella* spp., *Campylobacter* spp., *Cryptosporidium* spp., *Giardia intestinalis*, *Rotavirus*, and *avian Influenza virus*) into

the egg **(Eigaard *et al*., 2006; Roussan *et al*., 2012; Zambrano *et al*., 2014; Okorie-Kanu *et al*., 2016)**

Diagnostic methods used for identification *Ascaridia* based on molecular characteristics by using conventional PCR (**Qazaz, 2020**), post mortem and fecal samples (**Taylor *et al*., 2007**).

Due to there is no previous studies related to the on morphological and molecular diagnosis of *Ascaridia galli* in chickens in Diyala Province, the study was designed to including the following aims:

1. Morophological study of *Ascarida* spp. isolated from intestines in naturally infected domestic chickens slaughtered in Diyala Province.
2. Study the effects of age, sex and months on infection rate.
3. Study the histopathological effects in naturally infected chickens.
4. Identification of *Ascaridia* spp.by PCR technique from domestic chickens.
5. Genotyping by sequences and phylogenetic tree.

Chapter Two

Literature review

2. Literature's review

2.1. History

The identification of *Ascaridi agalli*, previously known as *A. lineata or A. perspicillum*, dates back to the 18th century (**Schrank, 1788**). However, it was not until the early 20th century when systematic research started on this species. *A. galli* infection increased following change to traditional housing **(Jansson *et al.*, 2010)**. The problem with aviary system concerning *A. galli* is that it promotes fecal-oral contact, which is the main route of nematodes transmission. This created a good opportunity for *A. galli* to become a center of attention **(Jansson *et al.*, 2010; Höglund and Jansson, 2011).**. There is a growing body of literature suggesting that certain helminthes eggs including those of *A. galli* are highly resilient to adverse conditions **(Tarbiat *et al.*, 2015).**

2.2. Classification

The genus *Ascaridia* was classified according to **zipcodezoo (2012)** as follows:

Kingdom: Animalia**- Linnaeus, 1758-animals**

Subkingdom: Bilateria**- (Hatschek, 1888) Cavalier-Smith, 1983**

Super phylum: Aschelminthes

Phylum: Nematoda **- (Rudolphi, 1808) Lankester, 1877-Round worms**

Class: Secernentea **– Von Linstow, 1905**

Subclass: Rhabditia

Order: *Ascaridida*

Suborder: Ascaridina

Super Family: Heterakoidea

Family: Ascaridiidae

Genus: *Ascaridia* **Dujardin, 1845(1844)**

Species: *A. galli*

2.3. Morphology of *Ascaridia galli*

2.3.1. Adult

The adult worms live in the lumen of the intestines, but are occasionally also found in the crop, gizzard and rarely in the oviduct or body cavity **(Fioretti *et al*., 2005; Bharat *et al*., 2017).** The body is semitransparent, cylindrical and has a creamy-white color. Like all other nematodes, *A. galli* is dioecious with distinct sexual dimorphism, and females are longer than males with a length of 72-116 mm and a straight posterior terminal, whereas males are around 51-76 mm and possess a curved posterior terminal

(Ashour, 1994). In the anterior end, both sexes have a prominent mouth with three distinct lips, bearing teeth like denticles on their edges **(Hassanain *et al*., 2009).** The entire body is covered with a thick cuticle, which is striated transversely throughout the length of the body (**Figure 2.1**).

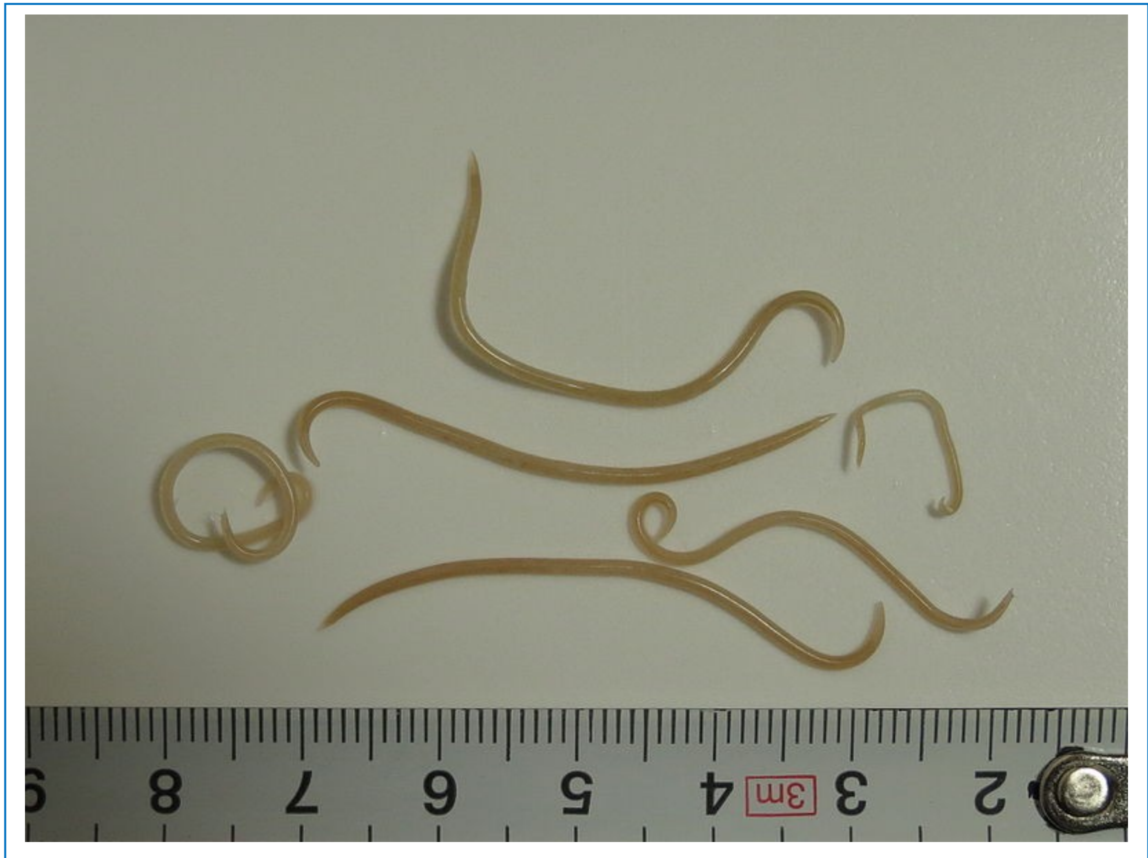


Figure (2.1): Morphology of male and female *Ascridia galli* **(Jacobs *et al*., 2003)**

2.3.2. Eggs

The eggs are oval in shape, measure 68-90 µm in length and 40-50 µm in width, and covered with a resistant three-layered shell; the vitelline membrane which is the inner permeable layer, chitinous layer which is the thick one, albuminous outer layer

which is thin one (**Wharton, 1980)**. The eggshell is important to protect the developing larva against harsh environmental conditions (**Figure 2.2**) (**Tarbiat *et al*., 2015**).

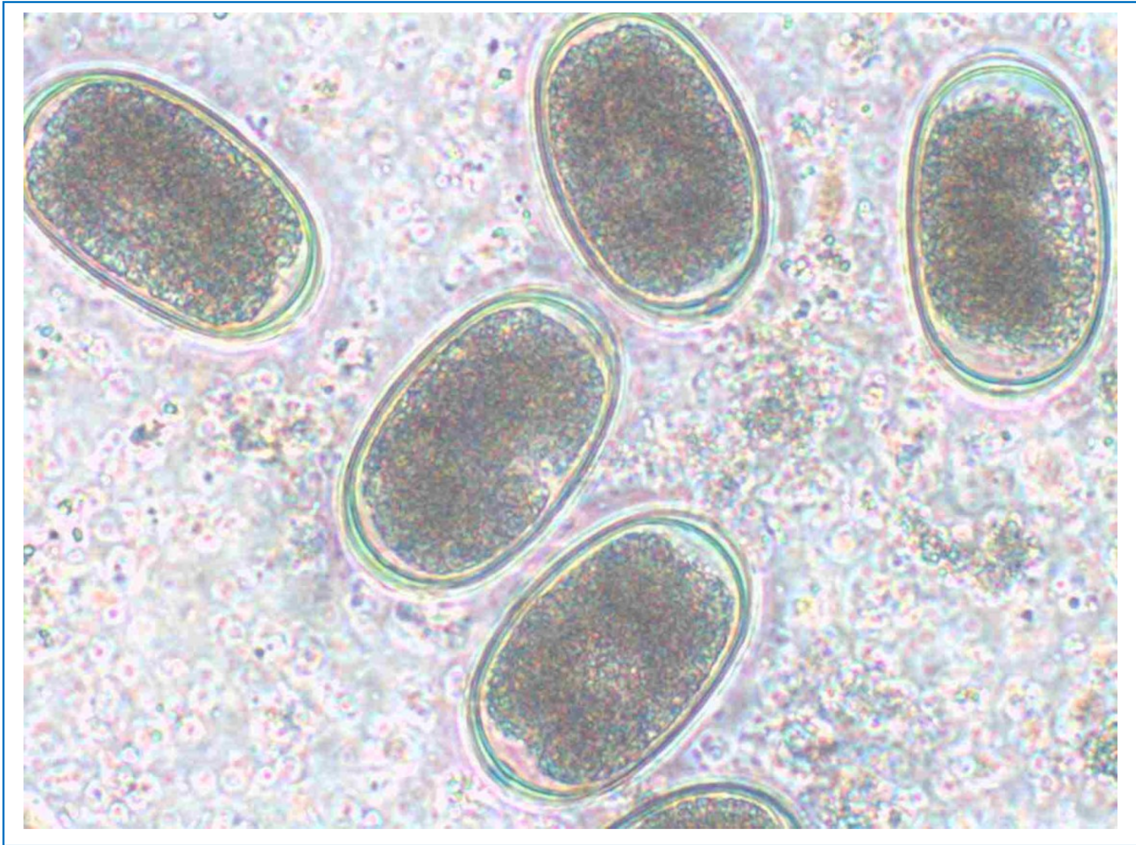


Figure (2.2): Morphology of eggs of *Ascaridia galli* **(Tarbiat *et al*., 2015)**

2.4. Life cycle

The life cycle of *A. galli* is direct involving two principal populations; the sexually mature parasite in the gastrointestinal tract and the infective stage (L 2) the in form of a resistant egg in the environment (**Prastowo and Ariyadi, 2019**). When laid, the eggs are covered with three layers: the inner permeable layer called the vitelline membrane, a thick chitinous resistant shell, and finally a thin albuminous outer layer. The eggs are passed with the feces of the host and develop in the environment, reaching the infective

stage (L2) in 10 to 20 days or longer depending on temperature and relative humidity (**Taylor *et al.*, 2007**). Occasionally earthworms can ingest *A. galli* eggs and transmit these to chickens, but this is not the principal route of transmission (**Luna-Olivares *et al.*, 2012)**. When ingested by the earthworms, the eggs of *A. galli* hatch in the intestine but are voided within 48-96 hours. Thus, unless the earthworms, they are eaten by chickens within 96 hours, the earthworms do not represent a potent risk factor for transmitting *A. galli* infections **(Ferdushy *et al.*, 2012).**

The life cycle is completed when the infective eggs ingested by new hosts through contaminated water or feed and the eggs that containing the L2-larvae that are mechanically transported to the duodenum. The larvae are protected by the three layers covering the eggs until they reach the duodenum or jejunum, where they hatch within 24 hours.; During hatching the mature coiled larvae escape from any parts of the egg shell moving out to the lumen of the intestine. The larvae enter the histotropic phase where they embed themselves into the mucosal layer of the intestine. The histotropic phase has duration of up to 54 days before the final maturation in the lumen, and it is, the histotropic phase is a normal part of the life cycle, where its duration is dose-dependent and closely related to the phenomenon of arrested development (**Ackert and Tugwell, 1948; Höglund and Jansson, 2011**).

Normally the life cycle does not include a migratory phase, but occasionally larvae are found in the liver or in the pleuroperitoneal cavity. After the histotropic phase, the worms settle down in the lumen of the duodenum, the prepatent period varies from 5-8 weeks (**Figure 2.3**) **(Taylor *et al.*, 2007).**

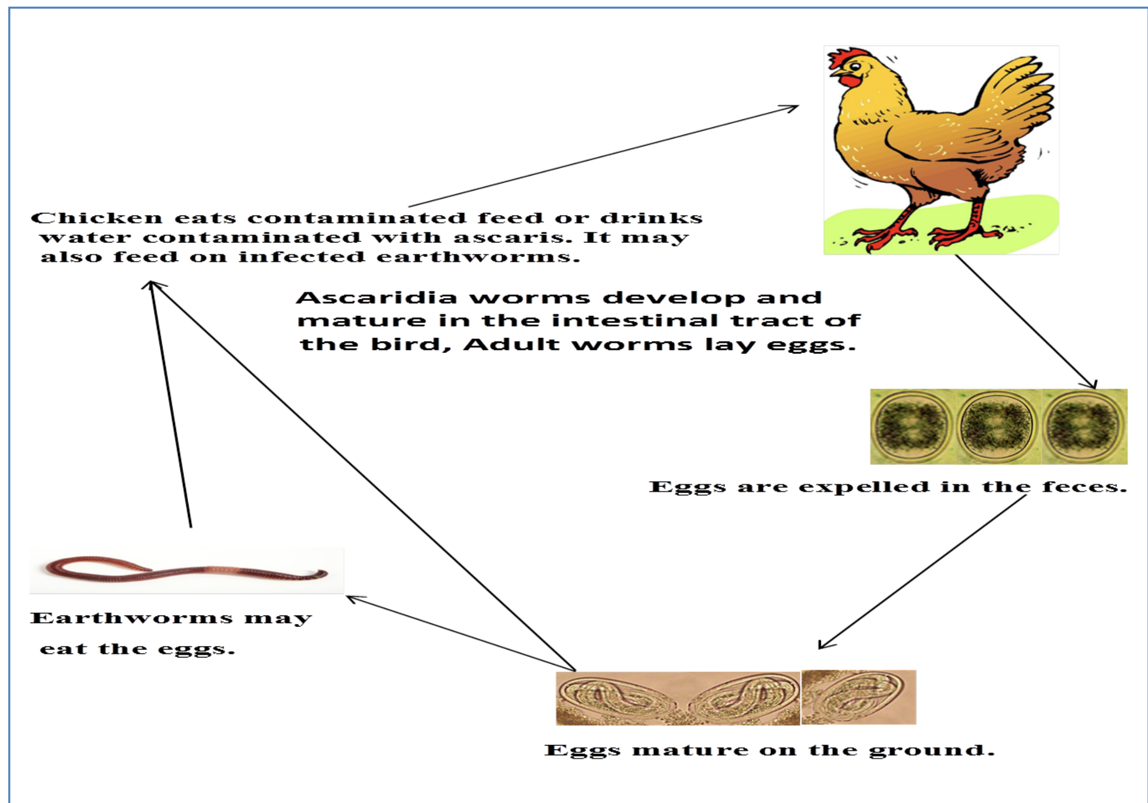


Figure (2-3): Life cycle of *Ascaridia galli* **(Ferdushy *et al.,* 2012)**

2.5. Epidemiology

The optimum temperature for eggs growth is 25°C where the eggs up to the infective stage within 7 days with a percentage of up to 88% **(Al-Ghazal, 1988**). After 22 hours between -12°C to -8°C temperatures, the eggs may die (**Ackert, 1931**); however, the eggs can survive a winter with moderate frost (**Cruthers *et al*., 1974**). Temperatures above 43°C are lethal for eggs at all stages (**Ackert, 1931; Reid, 1960; Permin *et al.,* 1998**). In deep litter systems, the eggs probably can remain infective for years depending on the temperature, humidity, pH and ammonium concentration (**Permin *et al.,* 2006**). The minimum time required to reach the infective stage is five days at 32-34°C when the eggs are incubated in water (**Ferdushy *et al.*, 2012**).

Eggs have low resistance to environmental factors outside the host. **Ruff (1988)** displayed that, eggs are killed at any stage of development at a temperature of 43ºC for 30 minutes; but maintain their vitality for more than three months in shady places. However, eggs can be destroyed quickly in dry and warm weather, even if at a depth of 15 cm below the soil surface exposed to sunlight **(Soulsby, 1982)**. The moisture is necessary for the growth and development of eggs, as it has been was found that 58% of the eggs are resistant to drought for three hours ,10% of them are resistant for 12 hours **(Al-Ghazal, 1988)**. **Al-Ghazal (1988)** found that Savlon was highly influential when used in low concentration (1.5-2.5%) for an hour and phenol more influential than Savlon when used without dilution. dilution Other studies found that the eggs are resistant to salinity, and that the adding of sodium chloride at a concentration of 0.2 mEq/L does not significantly affect the development or in the hatching process; but the concentration of 0.5 mEq/L can inhibit it. the effect of formalin on eggs is effective, in concentration of 10% for 48 hours and 60% of the eggs reached the infective stage ,while when using phenol 5% for a period of 24 hours, 22% of the eggs have reached the infective stage **(Salih and Abdul-Raheem, 2009).** Furthermore, and same researcher noted that the pH has little effect on the development of eggs and hatching and the best media for the growth of the eggs is the neutral and mean slight basal (7-7.5) **(Al-Ghazal, 1988).**

The *A. galli* eggs infrequently can be ingested by earthworms and transmit these to chickens, which consider not main route of spread (**Anderson, 2000**). The eggs of *A. galli* will be hatched after ingested by the earthworms, in the intestine but are voided within 48-96 hours. Therefore, the earthworms do not signify a possible hazard

influence for transmitting *A. galli* infections (**Augustine and Lund, 1974**). Therefore, the duration between the worm taking eggs parasite and eating from a bird of worms must not exceed this period four days or less and hatching egg parasite gets mostly during two days off to eat and put the larvae to ground and destroy in a short period **(Augustine and Lund, 1999)**.

In a study conducted by **Fossum *et al.* (2009)** showed that the occurrence of parasitic and bacterial disease was significantly lower in chickens housed in cages compared with those housed in barns or free-range. Infection with gastrointestinal parasites in poultry, including *A. galli*, occurs through the fecal-oral route (**Soulsby, 1982**). That explains the low occurrence of helminth infection in conventional cages since they separate birds from their feces. However, animal welfare concerns resulted in an EU-wide ban on conventional battery cages for laying hens from 2012 **(Gerzilov *et al.*, 2012).**

Comparisons of parasite infection and resistance in four commercial layers-lines, New Hampshire, ISA Brown, Skalborg and a cross of Skalborg (SK) and New Hampshire (NH) showed differences between the breed lines signifying that the establishment and survival of *A. galli* in the intestine of layers is due to the genetic factors (**Schou *et al.*, 2003**). Therefore, to prevent this disease resistance, improving genetic is the alternative way for free-range organic poultry producers for resistance to *A. galli* **(Gauly *et al.*, 2001; Permin and Ranvig, 2001; Gauly *et al.*, 2002)**. A comparison of resistance to *Ascaridia galli* infections in Danish Landrace breeds and Lohman Brown, both lines were showed a self-cure mechanism. However, in the Danish

Landrace during primary infection, there was a significantly higher worm burdens and egg excretion were seen **(Permin and Ranvig, 2001).** *Ascaridia galli* infections in brown (Lohmann Brown) and white (Lohmann LSL) chickens(artificially infected with 250 embryonated eggs) showed differences in the fecal egg counts in both groups, where eggs count were high in white hens than brown hens **(Gauly *et al.*, 2002).**

2.6. Prevalence of *Ascaridia galli*

Despite the description of *A. galli* in 1788, the extent of its prevalence was not the focus of many studies until recently. One possible reason could be that since mid-20th century many commercial laying hens have been housed in conventional battery cages, which lower the exposure of birds to feces and parasite eggs. The reported prevalence of *A. galli* parasite vary in different production systems **(Permin *et al.*, 1999)** with majority of chicken raised in extensive production system showing high prevalence of *Ascaridia galli* parasites as compared to intensive production systems where 64% was observed in free range organic systems,42%in deep –litter systems and 5% in convential cages in Denmark . These changes for the EU laying hen population was associated with re-emerging infections with *A. galli* **(Jansson *et al.*, 2010; Thapa *et al.*, 2015).** Likewise, reports from other European countries indicated similar results in free-range including organic (67–88%) in Germany **(Kaufmann and Gauly, 2009; Kaufmann *et al.*, 2011)** and 84% in England **(Sherwin *et al.*, 2013).** In Europe, many studies undertaken in former East Germany by **Schobries *et al.*** (**1989**) who revealed that 7% of the chickens carried *A. galli*. In Nigeria, 41% of the commercial chickens kept on deep litter systems harbored this parasite, whereas only 11% of free-range chickens had *A. galli* infections

(Oyeka, 1989). Zeller (1990) examined fecal samples collected from commercial poultry farms in Bavaria where 13% of the birds were infected with *A. galli*. **Yadav and Tandon (1991)** stated that *A. galli* was the most prevalent nematode in domestic fowl in India with a prevalence of 60%. In Thailand, 22% of the chickens were harboring *A. galli* **(Kunjara an Sangvar, 1993)**. In Switzerland, **Morgenstern and Lobsiger (1993)** recorded the prevalence of *A. galli* ranged from 2% to 20% in commercial chickens. Chickens in Pakistan were examined by **Khan *et al.*** (**1994**) and 12% proved to be infected with *A. galli* While in Western Cameroon, the parasite was identified in 52% of the local chickens **(Mpoame and Agbede, 1995)**. **Permin *et al.*** (**1997**) identified that 55% of chicken fecal samples were infected with the parasite. In traditional exploitations, prevalence of ascaridiasis is high in maintain birds in soil and in alternative systems. On the African continent studies indicated that the prevalence rate of this parasite in chickens ranging between 2-20% (**Permin *et al.*, 1999**). In Danish gallinaceous birds, **Madsen (1952)** found a prevalence of *A. galli was* about 4% Also, the prevalence is high in other countries, such as Austria, where the 64.1% of laying hens analyzed eliminate *A. galli* eggs in their feces (**Hohenberger, 2000).**

Also, this parasite had been recorded in Egypt 18% by **(Khashabah and Yousif, 2004)**. In Sudan, prevalence rate of *A. galli* was higher in more exotic chickens (46.53%) than in homegrown (10.18%), **(Karar *et al.*, 2005)**. For more explaining in central Spain traditional free-range systems, a mean prevalence was 21.8% (7.6% to 95%), (**Martı´n-Pacho *et al.*, 2005.** In different poultry production systems *A. galli* is the most widespread helminth species (**Kaufmann *et al.*, 2011**). **Thapa *et al.* (2015)** estimated the prevalence of *A. galli* in organic laying hens to be 97% in the Netherlands,

61% in Austria, 54% in Belgium and 50% in Italy. In a study conducted in Bangladesh, **Roy (2009)** reported that infections with *A. galli* were 75% in indigenous and 51% in exogenous chickens.

In Iraq, ascaridiasis from more diseases caused by nematode of *Ascaridia galli* in poultry which infect many types of birds as well as chicken **(Altaif, 1972; Al-khateeb *et al.,* 1982; Al-Khalidi *et al.*, 1988; Al-Mayahi, 1994; Muhsin, 2008)**. The percentage of *A. galli* infection in domestic chickens in the city of Baghdad, Nineveh, Basra was 42% 57% and 31%, respectively **(Al-Khateeb *et al.,* 1982**), and 40.1% in Basra **(Al-Khalidi *et al.,* 1988)**.

2.7. Pathogenesis and clinical signs

Penetration of the newly hatched larvae into the jejunal mucosa may cause hemorrhagic enteritis and anemia. This is often associated with severe diarrhea, loss of appetite and general weakness **(Ikeme, 1971).** In an analysis of chicken intestine, **Luna-Olivares *et al*. (2015)** reported that *A. galli* infection was associated with reduced length of villi, loss of muscle tone and the intestinal walls, hemorrhagic patches in the duodenum, scar tissue on the intestinal epithelium, Extensive destruction and erosion of glandular epithelium, and proliferation of mucus-secreting cells which may result in the adhesion of the villi have been reported by (**Ikeme, 1971).** Thickening of the tunica muscularis of the infected hens has also been reported **(Dänicke *et al*., 2009).** In addition *A. galli* damages the intestinal mucosa, which results in blood loss and comprised immunity leading to secondary infections **(Permin *et al.,* 1999).** More recently, **Hinrichsen *et al.* (2016)** reported that there was an association between combined

helminthes infection (*A. galli* and *Heterakis* spp.) and increased rate of mortality in Danish organic hens. However, other similar reports are limited. Infected chickens suffered from of behavioral changes. These include lower activities and higher food intake, with increase in nesting habits and reduction in ground pecking and during both the patent period and prepatent period **(Gauly *et al.*, 2007).**Symptoms of heavily infected chickens include drooping of wings, ruffled feathers, bleaching of the head, emaciation and diarrhea that followed by intestinal obstruction and anemia in very heavy infections **(Ackert and Herrick, 1928**).

2.8. Economic losses

Reports on the effects of gastrointestinal helminthes on egg production and reduced growth are generally scarce, both in commercial and backyard chickens. Many previous (**Reid and Carmon, 1958; Ikeme, 1971**) and recent **(Permin and Ranvig ,2001; Jacobs *et al*.,2016; Galli *et al*., 2018; Sharma *et al*., 2019; Stehr *et al*., 2019**) studies demonstrated infection with *A. galli* has been associated with reductions in overall growth and egg production in chickens. In central Zambia, a study by **Phiri *et al.* (2007)** showed that there was a reduced weight gain in young birds harboring multiple helminth species on free-range chickens. Concurrent *A. galli* and *Escherichia coli* **(Permin *et al.*, 2006)** or *Pasteurella multocida* **(Dahl *et al.*, 2002)** infections were showed to have a significant impact on egg production and weight gain. In the other hand, a study by **Sharma *et al.* (2018)** got different conclusions, when hens were exposed to different levels of *A. galli,* pointing out that, food conversion ratio, food intake and egg production were not affected. Furthermore, after analyzing the egg

quality parameters e.g., shell breaking strength, albumin height, and shell thickness with different exposure levels to *A. galli* no differences were observed between groups.

2.9. Diagnosis

Ascaridia galli infections can be diagnosed, either microscopically by identifying the eggs in the faeces using a simple flotation method or by using a modified McMaster method (**Henriksen and Aagaard, 1976; Jacobs *et al*, 2016).** Also, diagnosis can easily be done by post mortem examination for identifying the worms directly in the intestine (**Foreyt, 1994; Permin and Hansen, 1998).** Furthermore, presence hemorrhagic spots in the small intestine walls, in young bird we find immature worms, adults can be seen easily in small intestine. (**Atifi, 2011).**

2.10. Molecular techniques

Many parasitic nematode species cannot be identified using traditional morphological or morphometric techniques. Molecular diagnosis of parasitic nematode is a highly sensitive tool that differentiate nematode parasite of animals, as well as studying the host range, genetic variation, virulence, and resistance **(Mckand, 1999).**

Polymerase chain reaction-based methods have the benefit of allowing the particular identification of parasitic deoxyribonucleic acid from nanogram to picogram quantities of material, which helps to avoid misdiagnosis **(Gasser *et al*., 1993).**

The goal of genome-guided analysis is to identify genes or molecules whose inactivation by one or more drugs will selectively kill parasites but not harm their host because most parasitic nematodes are difficult to produce or maintain outside of their

host **(Lee *et al.,* 2008)** and this approach has indeed yielded effective targets for nematicides **(Campbell *et al.*, 2011).** So, molecular methods become a golden solution to the species – specific nematode identification **(Salma *et al.*, 2017).** In one of the earliest studies, **Roos *et al.* (1990)** investigated DNA polymorphisms in the genome of BZ susceptible and resistant population of *H. contortus*. They found that BZ resistance was associated with an amino acid substitution at position 200 in the β-tubulin gene, and showed that genetic assays can in fact be used successfully to detect AR to BZs. The development of PCR assays was allowed for detecting the AR at lower levels than in classical methods (e.g. FECRT) **(Saiki *et al.*, 1988; Elard *et al.*, 1999).**

Different genes used in molecular technique as markers for species identification like nuclear internal transcribed spacer (ITS) 2, cytochrome oxidase (cox1), 12SrRNA and nicotinamide adenine dinucleotide dehydrogenase (NADH) as target genes **(Webster *et al* ., 2012).** The small sub unit (SSU) 18SrRNA gene is one of RNA genes the most commonly used genes in phylogenetic analysis and an important marker for random target polymerase chain reaction in environmental biodiversity screening. Sequence data from these genes is widely used in molecular analysis to re construct the evolutionary history of organisms and its slow evolutionary rate make it suitable to reconstruct ancient divergences (**Meyer *et al.*, 2010).**

The first phylogenetic studies based on 18S RNA sequences were published by **(Field *et al.*, 1988**). In general, rRNA gene sequences are easy to access due to highly conserved flanking regions allowing for the use of universal primers **(Meyer *et al.*, 2010).** Their repetitive arrangement with in genome provides excessive amounts of

template DNA for PCR, even in smallest organisms. The 18S gene is a part of the ribosomal functional core in all living beings. The gene was celebrated as the prime candidate for reconstruction the metazoan tree of life and in fact, 18S sequence later provided evidence for the splitting of Ecdysozoa and lophtrochozoa thus contributing to the most recent revolutionary change in our understanding of metazoan relationships **(Meyer, 2010).** Multi gene analysis are currently thought to give more reliable results for tracing deep branching events in metazoans but 18S still is extensively used in phylogenetic analysis **(Meyer, 2010).**

In China, **Hao and He (2017)** determined genetic diversity in the mitochondrial cox1 and NAD4 genes of *Ascaridia galli*. **Cerutti *et al.* (2008)** identified *A. galli* with cox1 in Italy. **Katakam *et al.* (2010)** discovered a genetic variation in the cox1 gene of *A. galli* in Denmark. **Bazah (2019)** characterized *Ascaridia galli* in Egypt. **Qazaz (2020)** and **Faraj and Amery (2020)** showed that *Ascaridia* species characterized using 18S ribosomal RNA gene in Bagdad city. **Watcharakranjanaporn *et al.* (2021)** used NADH dehydrogenase sub unit 4gene for develop species-specific primer for *A. galli* which gave an amplification of 198bp and to investigate the epidemiological situation of gastro intestinal tract parasitic infections in Bangladesh. **Li (2013)** used three mitochondrial DNA genes (mDNA) demonstrated existence high intraspecific sequence variations among *A. galli* isolate from different geographical regions in china.

Major advancement in development of sequencing techniques from Sanger sequencing to high- throughput sequencing (HTS) techniques, which leading in recent years for their potential application in exploration of AR (**Sanger *et al.*, 1973; Heather and Chain, 2016**). Genome sequences and transcriptomic data of both animal and plant

parasites species are available on online data banks such as NCBI. Wormbase (www.wormbase.org) and helminth net of which the later provides specific data- mining and comparative analysis tools to study helminths (**Martin *et al.*, 2015**). These have provided major insights into the biology of some parasitic nematodes. For a review of the recent advances in both candidate-gene and whole-genome approaches to discover AR refer to **(Kotze *et al.,* 2014).** Although each of these resources improves accessibility to existing data and can help users with their analysis of their own data, little is known about the genome of *A. galli* **(Martis *et al.*, 2017).** Only a few molecular studies on *Ascaridia galli* have been conducted to date, and they have all focused on individual genes **(Höglund and Jansson, 2011).**

2.11. Host genetics and immunity

In poultry, slight is identified about genetic resistance to parasite infections. It has been demonstrated that worm burden and egg excretion can be significantly lower in Lohmann Brown hens compared to Danish Landrace (**Permin and Ranvig, 2001**). Use of genetic resistance in poultry in disease control was limited previously due to the routine extensive application of chemotherapeutics **(Malatji, 2017).** Further advantages of genetic resistance are emphasized by the emergence of virulent and drug –resistance pathogens and restrictions on the use of antimicrobials **(Malatji, 2017).** Good hygiene, practices, in combination with breeding of genetically resistance animals are now considered a relatively slight risk strategy to diseases control **(Wigleey, 2004)**. The authors suggested therefore the possibility of breeding for resistance to *A. galli* in

chickens. **Schou *et al.* (2003)** correspondingly stated that genetic factors are involved in *A. galli* survival and establishing in the GI tract of hens of different breeds.

The primary protection against infections in chicks is through maternal antibodies that are transferred via egg (**Brambell, 1970**). In chickens immunoglobulins are classified as IgY, IgA and IgM of which IgY is found primarily in egg yolk (**Leslie and Clem, 1969**). Even though maternally derived antibodies can provide partial protection against some bacterial and viral infections (**Ahmed and Akhter, 2003),** there is so far no evidence that maternally derived antibodies protect chickens against *A. galli* (**Rahimian *et al.*, 2017).** A strong immune response and intensive inflammatory reaction in the intestinal mucosa has been reported upon experimental infection of adult hens with *A. galli* (**Marcos-Atxutegi *et al.*, 2009**). Infected chickens with *A. galli* showed significant lower immunological response to vaccinations against other infectious diseases when vaccinated at ages (4, 10, 13) weeks against Newcastle disease **(Pleidrup *et al.*, 2014)** Yet, the immune response does not protect the host against re-infection with *A. galli* (**Andersen *et al.*, 2013; Norup *et al.*, 2013).**

2.12. Control strategies

2.12.1. Farm management and prevention

Encountered diseases in a poultry farm are usually influenced by the type of production system. In commercial egg production, management practices essentially determine the magnitude of parasite infections. Given the ubiquitous presence of *A. galli* infection in most EU member countries and its potential negative impact both on

production and animal welfare, effective control remains the utmost priority **(Gauly *et al.*, 2005).**

2.12.2. Biosecurity, cleaning and disinfection of barns

Strict biosecurity routines such as disinfecting delivery vehicles before entering the production sites, using disinfection boxes and footwear exchange for workers entering the barns, having a shower room and clean overalls headgear and footwear supplies will reduce the risk of introduction of new infective agents to farms and between farms (**Berg, 2002; Jansson *et al.*, 2010**). Biosecurity measures, cleaning and disinfection between consecutive flocks and anthelmintic are central to parasite control in poultry. However, current methods of roundworm control have proven to be insufficient in barn and free- range egg production, including organic farming **(Höglund and Jansson, 2011)**. Hygienic measures between consecutive flocks also aim to interrupt transmission of pathogens, prevent re-infection and gradually minimize the infection level on the farm to an acceptable level. Complete cleaning of the barn with high- pressure hot water is recommended before placement of new pullets to reduce the level of parasite egg contamination. This together with a downtime period between consecutive flocks would theoretically reduce the infection level and delay the spread of the infection within the flock (**Katakam *et al.*, 2014**).

The free-living stages of most parasite species including *A. galli* need high relative humidity to develop to the infective stage and must survive until they are ingested by the host. Therefore, keeping the floor and the litter dry is of utmost importance. If frequent removal of the litter is not possible, it has been suggested to remove the soiled and wet

parts of the litter bed (**Permin and Hansen, 1998; Bachaya *et al*., 2015**). **Maurer *et al.* (2009)** indicated that there were no significant differences in helminth egg reduction in relation to different litter managements practices (adding, replacing or no management). However, they reported that in the group where fresh litter was added on top of the old litter, the FEC results were lower compared to the group with unmanaged litter. Reports on the effect of different disinfectants against helminths eggs are limited to field observations **(Höglund and Jansson, 2011)**. However, they should be incorporated into the overall sanitation routine. Several years ago, been researchers implied that 1% dilution of chlorocresol, effectively eliminated all *A. galli* eggs in vitro (**Tarbiat *et al.*, 2015**). Whether this can be achieved in barns under commercial conditions remains to be determined. Other techniques such as lime-wash were suggested after cleaning and disinfection of the barn (**Permin and Hansen, 1998**). Overall, few systematic experimental studies are available from the field.

2.12.3. Anthelmintics

Anthelmintics are anti-parasitic drugs containing substances that are active against helminths including nematodes. These compounds either kill or remove the worms from any organs and tissues they may be present. They are used to prevent clinical and subclinical symptoms, production losses and to minimize associated costs. Anthelmintic drugs are available in various forms such as add- on feed, oral suspensions, pre-mixtures for water or feed administration pour-on preparations, and injectable solution depending on target hosts and parasite species **(Barragry, 1984).** Anthelmintic drugs approved in the EU for commercial poultry are usually administered in feed or in drinking water to

the flock rather than to individual birds. The three major broad-spectrum drug classes of anthelmintics used in veterinary medicine are benzimidazoles (BZDs), macrocyclic lactones such as ivermectin, and tetrahydropyrimidines such as levamisole (LEV) (**Jacobs *et al.*, 2016**), However, there are many compound with narrow spectrum are also efficient against *A. galli* like derivative of piperazine used in poultry as feed or drinking water additives, scarily as tablets or injectables **(Horton-Smith and Long, 1956).** Throughout the world, Medical plants appearing anthelmintic activity in vitro with low AR includes: *Allium sativum*, *Aloe secundiflora*, *Anacardium occidentale, Bassialati folia*, *Cassia occidentalis L, dacitri folia L.I, Piper betle* and *Tribulus terrestris., Bassia latifolia, Piper betle, Morindacitrifolia L.I, Cassia occidentalis L.* and *Aloe secundiflora,* while in vivo, medicinal plant includes : *Anacardium occidentale, Caesalpinia crista, Ocimumgratissimum, Piper betle, Pilostigma thonningi* and *Psoreliacoryli foliathese*. Medicinal plants seem to high anthelmintic activity in poultry and may subrogate conventionally utilized synthetic drugs, and their employment can moderate the resistance to drug in the populations of endemic pathogen and reducing the residues of drug in poultry meat **(Raza *et al*., 2016).**

Chapter Three

Materials and Methods

3. Materials and Methods

3.1. Materials

3.1.1. Laboratory equipment and apparatus

Laboratory equipment used in this study are listed in the table (3-1):-

Table (3.1): Laboratory equipment and apparatus utilized in current study

No.	Materials	Origin
1	AURA TM PCR Cabinet	Italy
2	Bio TDB-100, Dry block thermostatbuilt	Germany
3	Balance	Germany
4	Combi-spin	Latvia
5	Conical flask	USA
6	Electrophoreses	USA
7	Glass tube	Jordan
8	Incubator	China
9	Light microscope Olympus	Japan
10	Microspin	Germany
11	Microspin 12, High-speed Mini-centrifuge	Germany
12	Microwave	China
13	Micropipette variable volumes 0.5-10 µl 2-20 µl 10-100µl 20-200 µl 100-1000 µl	Germany
14	Multigene Optimax Gradient Thermal Cycler	Labnet
15	Oven	China
16	PCR thermocycler	USA

17	Plastic caps	Iraq
18	Rotary microtome	Germany
19	Slides and cover slide	China
20	Surgical instrument	China
21	Tips	Korea
22	Document system	USA
23	UV transmission	France
24	V-1 plus, Personal Vortex for tubes	Germany
25	Vortex centrifuge	Korea
26	Water bath	China
27	Water distillator	China
28	Nanodrop spectrophotometer	USA
29	Histokinette	Germany
30	TEC2900 Embedding Centre	Italy
31	TEC2900 Cryo Console	Italy
32	TEC2900 Thermal Console	Italy
33	Refrigerator	Japan

3.1.2. Reagents and chemicals

Reagents and chemicals used of the study as following:

Table (3.2): Reagents, solutions and chemicals utilized in this study

No.	Material	Origin
1	6X Loading dye	Korea
2	Agarose	USA
3	Canada balsam	England
4	Distal water	Iraq
5	Eosin stain	Germany

6	Ethyl alcohol 70% Ethyl alcohol 80% Ethyl alcohol 90% Ethyl alcohol 100%	England
7	Formalin 10%	England
8	Glycerol	Iraq
9	Hematoxylin stain	Switzerland
10	Lactophenol	Iraq
11	Ladder 100 plus bp	Korea
12	Normal saline 0,9	China
13	Paraffin	Germany
14	Red safe staining solution	Korea
15	TBE buffer 10 X	USA
16	Xylene	England

3.1.3. Kits

The Kits used in the study are listed in **Tables (3.3) and (3-4):-**

3.1.3.1. DNA Extraction kit

Table (3.3): Contents of DNA extraction kit used in current study

Material	**Origin**
Buffer CL	South Korea
Buffer BL	
Buffer WA	
Buffer WB	
Buffer CE	
Spin Columns	
Collection Tubes	
RNase A (Lyophilized)	
Proteinase K (Lyophilized)	

3.1.3.2. PCR-Premix kit

Table (3.4): Contents of PCR-Premix kit used for DNA amplification

Material	Origin
Top -Taq DNA Polymerase	South Korea
dNTP (dATP, dCTP, dGTP, dTTP)	
Tris-HCL (pH 9.0)	
KCL	
$MgCl_2$	
Stabilizer and tracking dye	

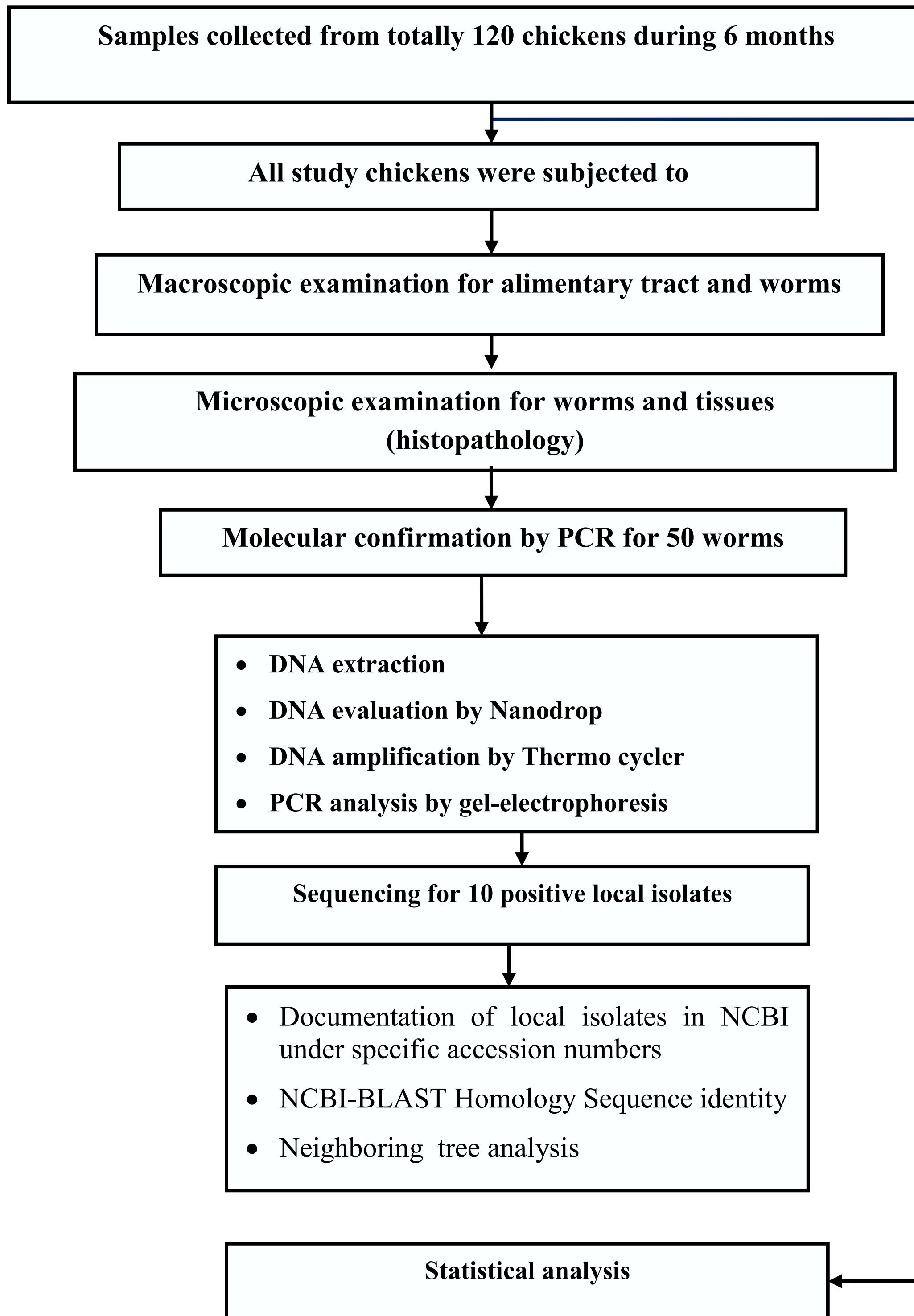
3.1.3.3. Primers

The set of primers was taken from recently published Iraqi study (**Faraj and Al-Amery, 2020**), Primers were manufactured and provided by Macrogen Company / South Korea, (**Table 3.5**).

Table (3.5): PCR primers with their nucleotide sequences and amplicon size

Target gene	Primer		Produce size (bp)	Reference
		Sequence 5′-3′		
18S rRNA	F	AGTGCTTAACGCGGGCTTAT	724	(**Faraj and Al-Amery, 2020**)
	R	AAAGCACGCTGATTCCTCCA		

3.3. Study design



3.3. Methods

3.3.1. Study animals

The survey was conducted in different area in Diyala Province (Baqubah, Shahraban, Jalawla and Khanaqen). The domestic chicken was brought from the markets and rural free-range chickens (*Gallus gallus domesticus*).

3.3.2. Collection and examination of parasite

A total 120 local chickens of both sexes and different age were bought from many regions in study areas from the beginning of October 2020 until the end of March 2021. The chickens were slaughtered and the abdomen region of each one was sectioned throughout the ventral mid-line using knife. The alimentary canal was removed from abdominal cavity and preserved in a container inside ice-box that labeled with required information, like the age and sex of each animal in addition to date of sample collection. All collected samples were transported to the Laboratory of Parasitology Department at the College of Veterinary Medicine, University of Baghdad (Baghdad, Iraq).

The small intestines were eviscerated after ligate both ends and opened longitudinally with sharp scissor, the contents of small intestine were scrapped into the petri-dishes that filled with the physiological saline. The recovered adult worms , washed by the physiological saline to remove the attached debris and transferred into plastic caps to be fixed in 70% ethyl alcohol for 24 hours. A hot mixture of 70% ethyl alcohol and glycerol (1:1) where used to straight the parasite. The worms were cleared with agent Lacto-phenol. Under light microscope, the adult worms were examined and identified based on their morphological keys mentioned in a number of references

(**Soulsby, 1982; Permin and Hansen, 1988; Ramadan and Abouzanda, 1992).** Males and females were measured to estimate their lengths using a ruler. For molecular procedures, worm samples preserved in 70% ethyl alcohol **(Katakam *et al.,* 2010; Urbanowicsz *et al* ., 2018).**

3.3.3. Histopathology

After recording the gross changes, samples of small intestine and liver of naturally infected chicken tissue were fixed with 10% neutral buffered formalin, dehydrated by passing successfully in ascending concentration of ethyl alcohol, cleared in xylene and embedded in paraffin. Sections of 5μm thickness were prepared and stained with H&E stain for microscopic examination **(Luna, 1968).**

3.4. Molecular examination

3.4.1. DNA extraction

Genomic DNA of *Ascaridia galli* was extracted from 50 worms, according to the manufacturer instructions. Briefly, a total 25 mg of grounded tissue sample from each worm were transferred into 1.5 ml tube using a spatula followed by the addition of 200 μl of Buffer CL, 20 μl Proteinase K and 5 μl RNase, vortexed vigorously and incubated at 56ºC for 30 minutes. Then, a total 200 μl of Buffer BL were added into upper sample tube, mixed thoroughly, and re-incubated at 70ºC for 5 minutes. The tubes were centrifuged at 13,000 rpm for 5 minutes to remove unlysed tissue particles, and then, a total 400 μl of the supernatant were transferred into a new 1.5 ml tube. Afterward, 200 μl of absolute ethanol were added into the lysate, mixed well by vortex and centrifuged at 13,000 rpm. Carefully, the mixture was applied in a 2 ml collection tube without

wetting the rim and centrifuged at 13,000 rpm for 1 minute. The filtrate was discarded and the tube was placed into the Spin Column. For washing, 700 µl of Buffer WA were added to the Spin Column and centrifuged for 1 minute at 13,000 rpm. The flow-through and the collection tube were discarded. Then 700 µl of Buffer WB was added to the Spin Column and centrifuge for 1 min at 13,000 rpm. The flow-through and the Collection tube were discarded. Then, the Spin Column was placed into a new 1.5 ml tube, and 100 µl of Buffer CE were added directly onto the membrane. After that, the tubes were incubated for 1 min at room temperature and centrifuged for 1 minute at 13,000 rpm to elute the DNA. Finally, the eluted DNA were kept frozen at -20ºC until further uses.

3.4.2. Estimation of genomic DNA concentration and purity

The concentration of the extracted gDNA was checked by Nano drop, and the purity was detected at (260/280 nm) absorbance. Briefly, the Nanodrop program was opened to select the appropriate application (Nucleic acid, DNA).Dry wiping and cleaning of the measuring bases have been done many times. Then carefully pipette 3ul ddH_2O onto the surface of the bottom measurement base. The sampling pedal is lowered and clicked OK to empty the Nanodrop and then clean the plugs. Then the substrates and DNA samples have cleaned for measurement.

3.4.3. Primer preparation

The primer pair used in this study was dissolved using sterile ddH_2O.Stock solution (100 pmol/ µl) was prepared by adding ddH_2O to the vial containing lyophilized primer while working stock of 10 pmol/ µl was made by mixing 10 µl of the stock primer and 90 µl of ddH_2O.

3.4.4. PCR Reaction Mixture

Samples of PCR- Mastermix were prepared according to manufacturer instructions at a final volume of 25µl (**Table 3.6**)

Table (3.6): Contents of the PCR reaction mixture

Contents of reaction mixture	Volume (µl)
Taq PCR PreMix	5
Template DNA	1.5
Forward primer (10 pmol/µl)	1
Reverse primer (10 pmol/µl)	1
Nuclease free water	16.5
Total volume	25 µl

3.4.5. Polymerase chain reaction (PCR) condition

Conventional PCR was used to amplify the targeted DNA using specific primers. PCR typically consists of three consecutive steps (denaturation, annealing, and elongation) of repeated cycles to get PCR product (amplicon). The PCR thermal -cycling conditions are mentioned (**Table 3.7**).

Table (3.7): PCR condition for amplification of 18S rRNA gene

Step	Temperature (°C)	Time (Second)	No. of Cycles
Initial Denaturation	95	5 minutes	1
Denaturation	95	45	35
Annealing	57	45	
Extension	72	45	
Final extension	72	7 minutes	1

3.4.6. Agarose gel electrophoresis

Electrophoresis is widely used to determine DNA pieces after the process of extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the agarose gel **(Sambrook *et al*., 1989)**. Briefly, a total 1.5 g agarose gel were measured using the sensitive balance and dissolved within the microwavable flask containing 100 ml 1×TBE buffer to obtain agarose gel solution at a concentration of 1.5%. Then, the flask was shacked vigorously and placed into the microwave for 1-3 minutes with frequent shaking to avoid that solution heats up. After that, the agarose gel solution was left at room temperature to cool to about 50°C. A total of 3µl Red-Safe dye were added to agarose gel solution that poured in the tray after fixation of comb in proper position, left to be solidified for 15 minutes at room temperature, and then, the comb was removed gently from the tray. The gel was fixed in the electrophoresis chamber that filled with 1×TBE buffer. Followed by the addition of 5µl of Ladder Marker (100-1000bp) were added to the first well comb, and 10µl of each PCR-product was added to the other well comb wells of agarose gel. Agarose gel electrophoresis was run at 70 Volt for 1.5 hour. Finally, the electric power turned-off, and agarose gel was removed from the gel tray to visualize DNA fragments under an ultraviolet (UV) transilluminator.

3.4.7. Sequencing and sequence alignment

After confirming the amplification via conventional PCR, 10 of positive PCR products were sent for sequencing following the Sanger method using ABI3730XL, automated DNA sequences by Macrogen Corporation – South Korea. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program, which is available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov and bio Edit program. Results were compared with data obtained from GenBank published EXPASY program which is available at the NCBI online.

3.5. Statistical analysis

Statistical analysis system (SAS, 2012), program was used to detect difference factors in the parameters employed in the current study. Chi-square (x^2) test was also applied to compare significances between percentages in this study.

Chapter Four

Results and Discussion

4. Results and Discussion

4.1. Macroscopic examination

Most of the worms isolated from infected intestines were adult *Ascaridia galli*. The adult worms were cylindrical in shape and semitransparent yellowish-white in color (**Figures 4.1, 4.2**). The body length measurement revealed that females; 69mm (40 - 82mm), were longer than males; 43.4mm (36-55mm), (**Appendix1**). Sexual dimorphism characteristics in ascarids were identical to that mentioned in other studies (**Ashour, 1994; Bowman, 2009**).

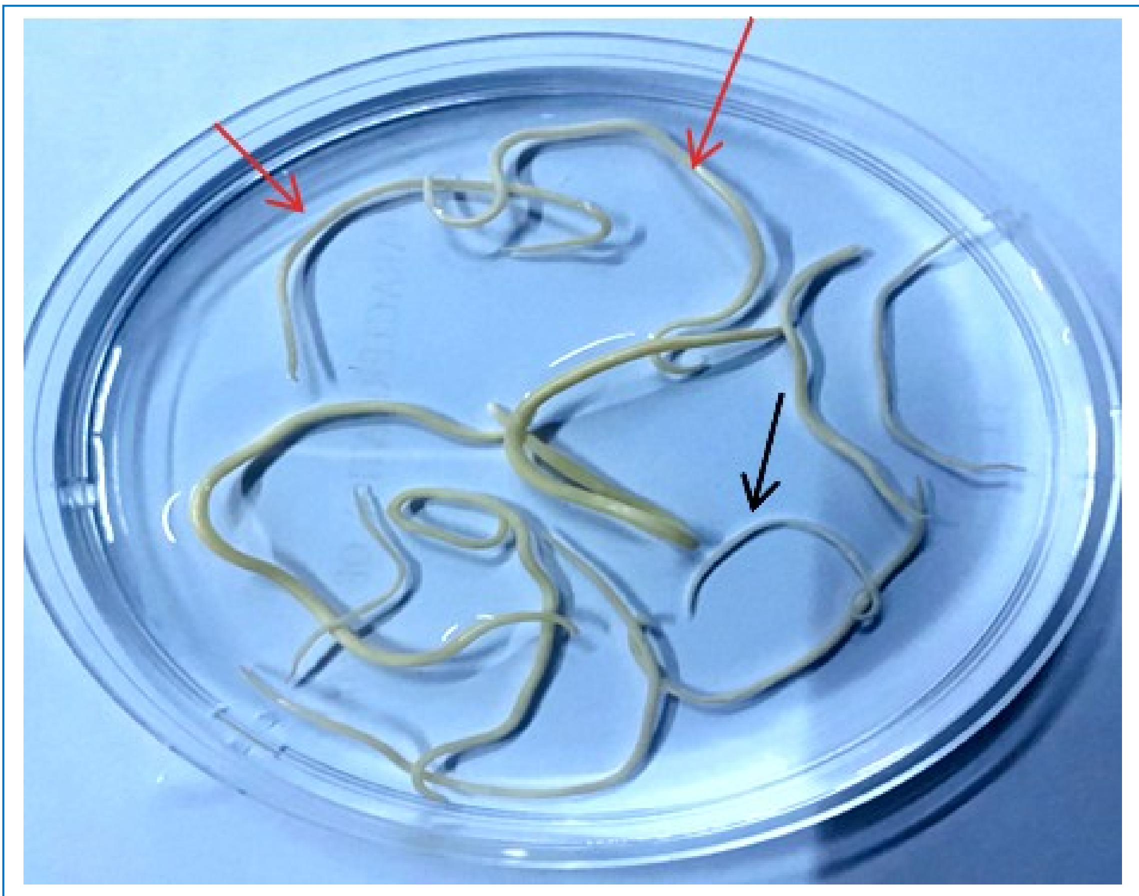


Figure (4.1): Adult females (red arrow) and males (black arrow) of *Ascaridia galli* isolated from small intestines of infected local chicken

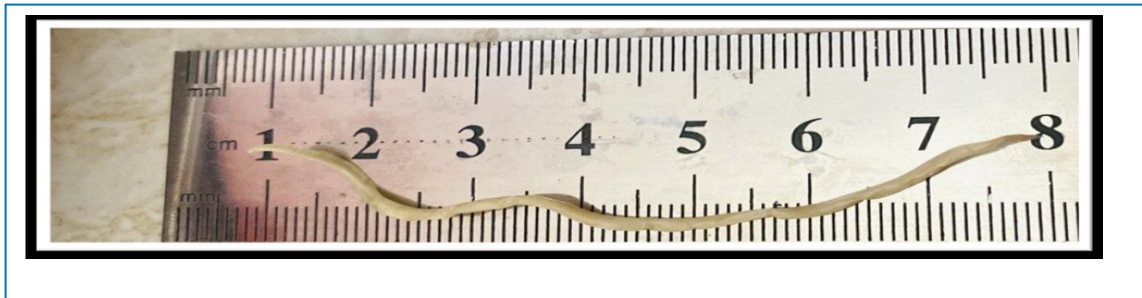


Figure (4.2): Female of *A. galli*

4.2. Microscopic examination

Microscopic examination of adult *A.galli* revealed the textbook characteristic features of the parasite. The anterior end characterized by, the presence of the mouth surrounded by three lips, and club shaped esophagus without distal bulb (**Figure 4.3**). The entire body was surrounded by a transversally striated cuticle, In male posterior end was pointed and curved with the presence of two equal spicules that protruded out at the anal opening with presence three pairs of caudal papillae (**Figure 4.4**). Also, there was circular pre- anal sucker that ventrally located (**Figure 4.5**). In female, the posterior end was blunt and straight with presence of anal opening before the posterior extremity (**Figure 4.6**). Additionally, the vulva is situated a short distance anteriorly to the middle of the body (**Figure 4.7**). These features were identical to that mentioned by other studies (**Kassai, 1999; Bowman, 2009**).

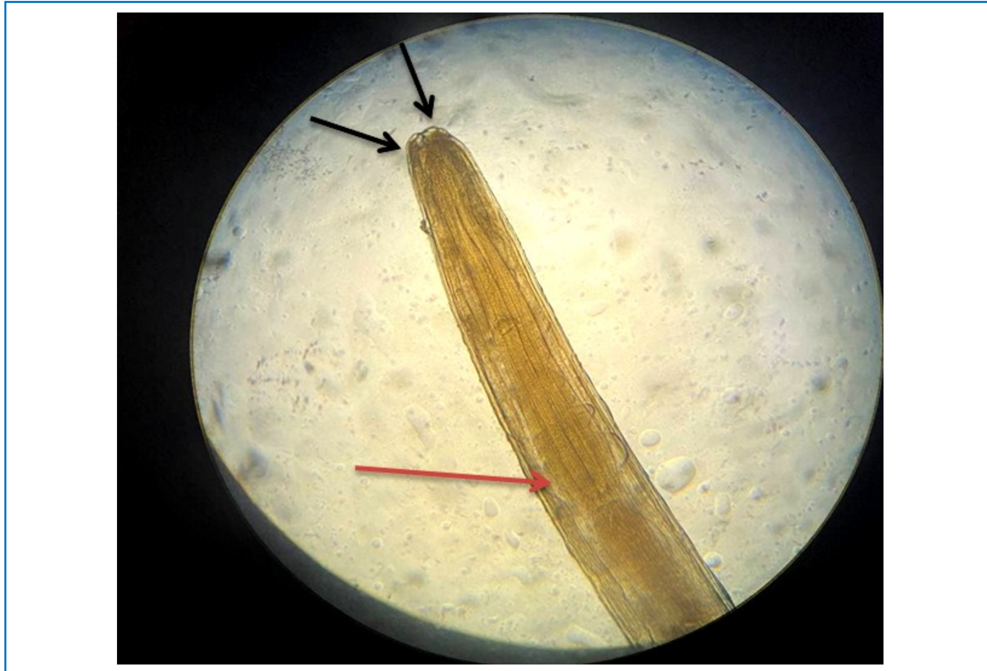


Figure (4.3): Anterior end of *A. galli* showing three lips (Black arrow), and the esophagus club in shape (Red arrow)

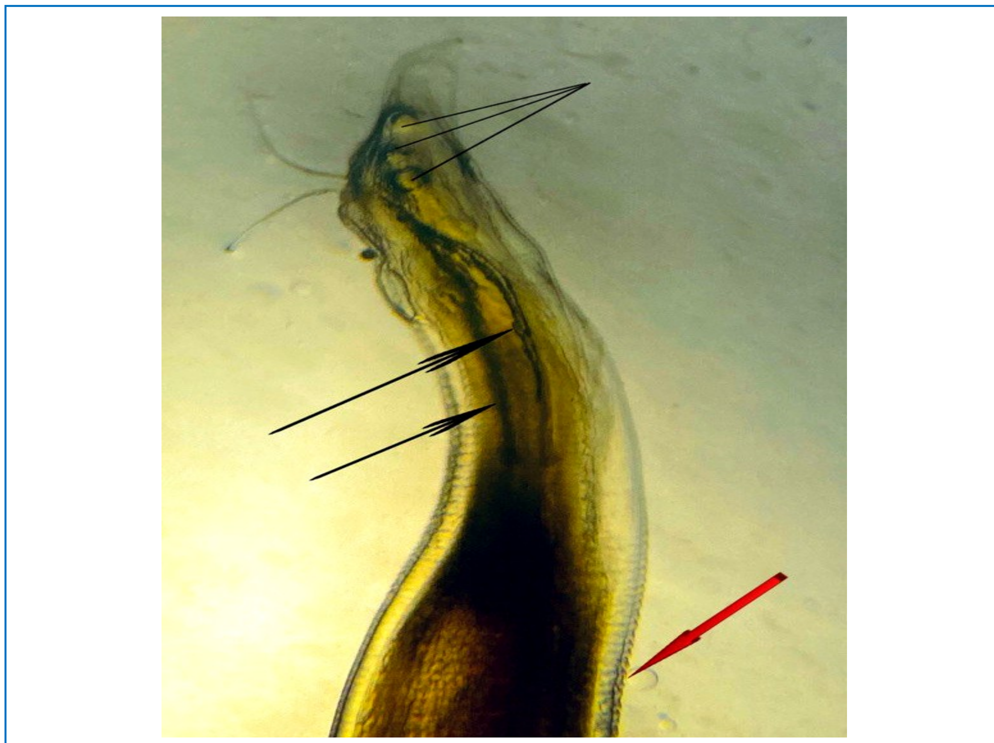


Figure (4.4): Posterior end of *A. galli* male shows well developed spicules (Black arrows) and caudal papillae (lines), with striated cuticle (Red arrow) (10×)

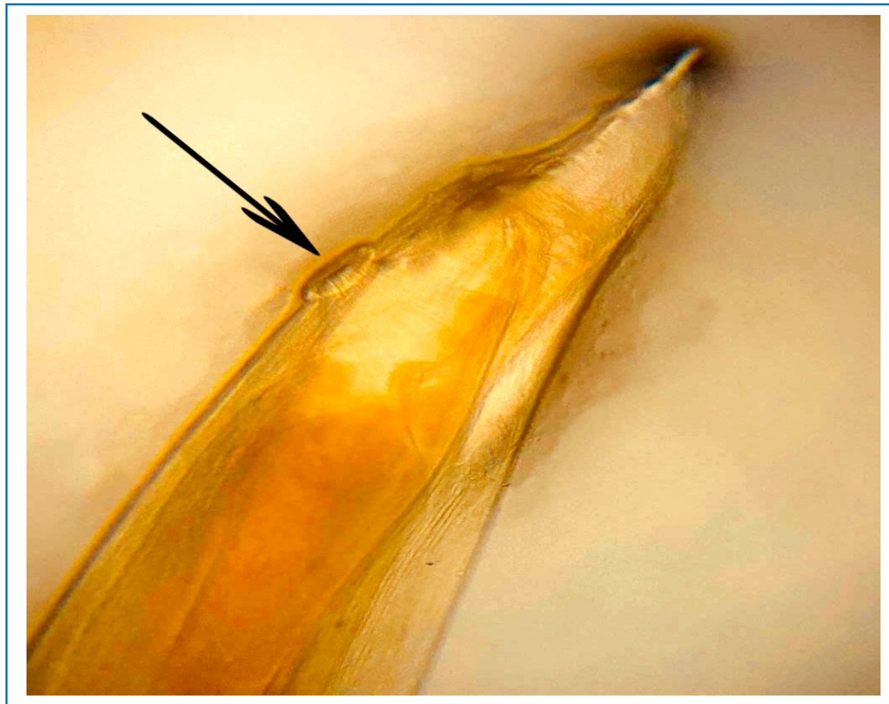


Figure (4.5): Posterior end of adult male *A. galli* shows pre -anal or pre-clocal sucker (Black arrow) (10×)

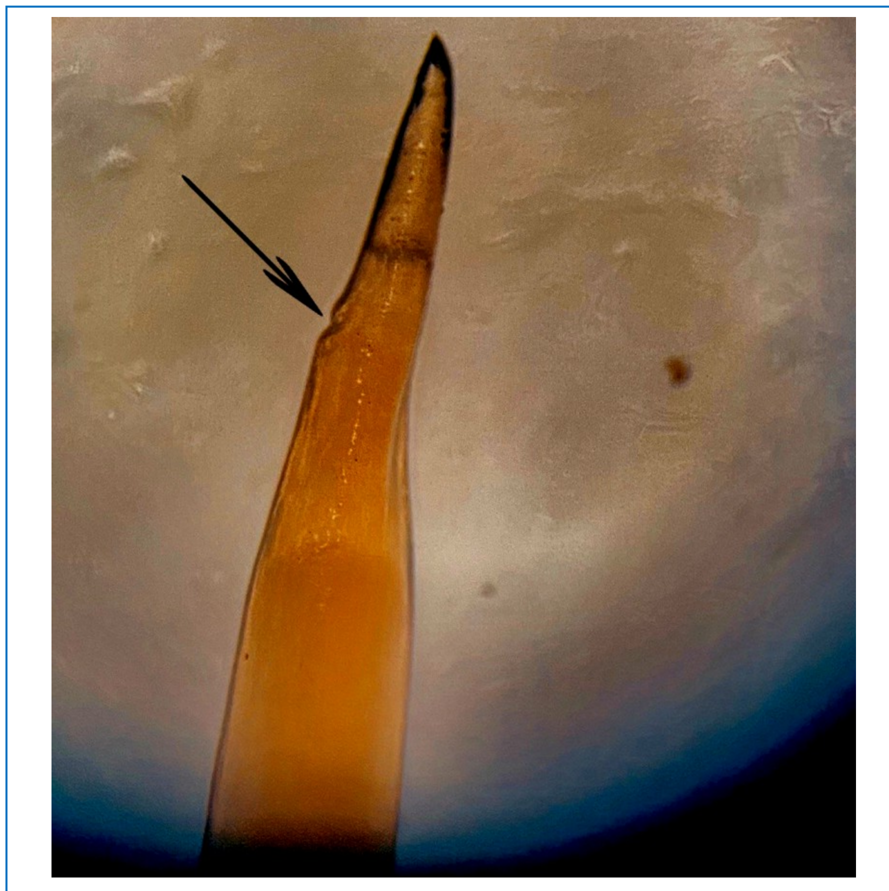


Figure (4.6): Posterior end of adult female *A. galli* showing the anus (Black arrow) (10×)

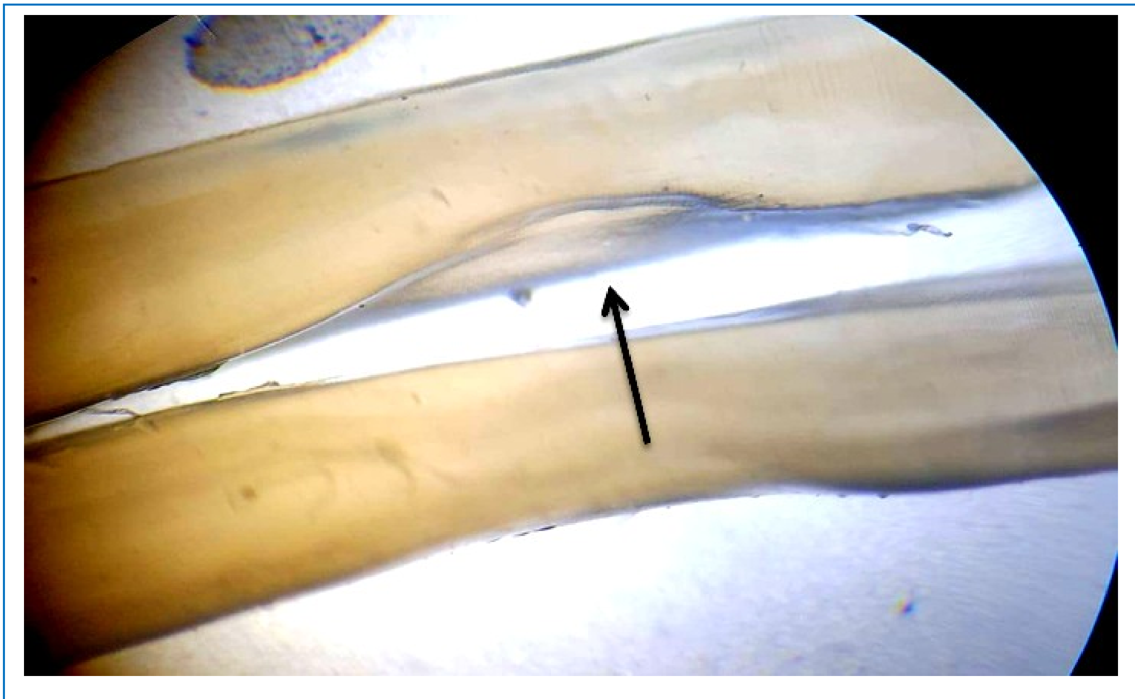


Figure (4.7): Adult female *A. galli* shows vulvar region (Black arrow) (10×)

4.3. Prevalence of *Ascaridia galli* in local chicken

The current study showed that rate of infection in local chickens naturally infected with *A. galli* reached 41.66% (50/120), (**Table 4.1**).

Table (4.1): Prevalence rate of *A. galli* in local chicken

Total No.	Positive	
	No.	%
120	50	41.66

However, other studies recorded different results. In Al- Diwaniya, the prevalence rate of *A. galli* was 31.95% among gastrointestinal helminths in local chicken **(Hamza, 2009)**. In Najaf province, overall prevalence rate of ascardiosis was 58.92% **(Al-Rubaie *et al*., 2009)**. In Baghdad, prevalence rate of *Ascaridia galli* was 36.9% **(Shathar, 2010).** In Salah Al-den, it was 52.9% (85) **(AL-jaumeili and Aljoburi, 2015).**

Worldwide, the prevalence rate of *A. galli* was 25.63% in Kenya (**Kaingu *et al*., 2010)**, 25.7% in Pakistan (**Sayyed *et al.*, 2000)** and 21.44 % in Mardan (**Zada, 2015).** This difference may reflect that management factors play a role in the spread of nematode infections within chicken fields. **Skallerup *et al*. (2005)** established that the environmental surroundings must be considered among the most important determining factors for transmitting infective eggs in natural helminthes infections. Therefore, factors other than wild bird for example farm to farm contamination via vehicle, machine, equipment or people might also have contributed as a source of infections, especially for *A. galli*. The discrepancies among the result of the present and earlier works in other countries could belongs to different reasons such as geographical location of the research area, method of detection, sample size, age and sex of the birds.

4.4. Prevalence rate of *Ascaridia galli* in local chicken according to sex

The study showed significate (P≤ 0.05) difference between males and females. Female showed highest rate of infection 50% (33/66) when compared to males 31.48% (17/54), (**Table 4.2**).

Table (4.2): Prevalence rate of *A. galli* in local chicken according to sex

Sex	Total No.	Positive	
		No.	**%**
Males	54	17	31.48
Females	66	33	50
Total	120	50	41.66
Chi-Square (χ^2)	--	--	5.120 *
* (P≤0.05)			

This may relate to the number of females slaughtered during the period of study, and this simulating a previous studies in India by **Salam (2015)** that have reported the higher rate infection in the females 63.7% than males 48.7%.and in Pakistan by **Yousaf *et al.* (2019)** that found higher percentage in females 22.45% than males 17.22%.Also, **Khanum *et al.* (2021)** found that prevalence rate was higher in females 83.3% than males77.8%. It was documented that females are extra susceptible to infection with helminthes as compare to males **(Ekpo *et al.,* 2010)**. Moreover, high infection rate in females may be due to hormonal condition, stress during egg production and feeding habit **(Bachaya *et al.*, 2015).**

4.5. Total infection rate of *A. galli* in local chicken according to age

The results showed non-significant difference recorded between adults that revealed 50% (24/48) infection rate and young's that recorded 36.11% (26/72) (**Table 4.3**).

Table (4.3): Prevalence rate of *A. galli* in local chicken according to age

Age	Total No.	Positive	
		No.	%
Adults	48	24	50
Young's	72	26	36.11
Total	120	50	41.66
Chi-Square (χ^2)	--	--	1.067 NS
NS: Non-Significant			

Parallel to our findings and according to age, **Tawaya *et al.* (2020)** showed non-significant difference between age group. This could be attributed to the immunity

against infection, IgY secretion increased when infection rate increased **(Gauly *et al*., 2005; Marcos-Atxuutegi *et al*., 2009).** Furthermore, higher infection rate in adult chicken can be attributed to their repeatedly exposed to larvae, coupled with management system that might have contributed for higher infection rate in older than younger.

4.6. Prevalence rate of *A. galli* in local chicken according to months

This study showed a highly significant difference ($P \leq 0.01$) in the infection rate in slaughtered chickens according to the months. Although, the infection was registered at all months of study, the high rate of infection (55%) was recorded in October and lower infection rate was for December (30%), (Table 4.4).

Table (4.4): Prevalence rate of *A. galli* in local chicken according to months

Month	Total No.	Positive	
		No.	%
October 2020	20	11	55
November	20	8	40
December	20	6	30
January 2021	20	7	35
February	20	8	40
March	20	10	50
Total	120	50	41.6
Chi-Square (χ^2)	--	--	11.638 **
** ($P \leq 0.01$)			

This partially agreed with the result of **Salam (2015)** who found that the highest infection rate in infected chickens in September and the lowest in the December and

January; while disagreed with **Al-Quraishi *et al.* (2020)** who found that the higher infection rate was in March (40%) and lower infection rate was in summer (July) reaching 21.27%. This variation might be attributed to differences in time of sampling and method of detection and sample size and geographical location of the research area. The variation in overall percentage rate may be due to the climate condition contributed in rising of infective stage. Also, in different regions, there is variation in immunity of chicken as a result for using the anthelmintic drugs that kill the parasite **(Matur *et al.*, 2010).**

4.7. Histopathology for small intestine and liver

4.7.1. Macroscopic examination

Small intestine of infected local chickens was examined macroscopically to determine the gross pathological changes. The small intestine showed the presence of yellowish and cylindrical *A. galli* parasite **(Figure 4.8A),** High burden of *A. galli* caused blockage of the intestinal lumen**(figure 4.8B**); and thickening in the wall of infected small intestines with hemorrhagic spots (**Figure 4.8C**). Samples of liver showed a significant congestion in some area (**Figure 4.9A**) and paleness in others (**Figure 4.9B**).

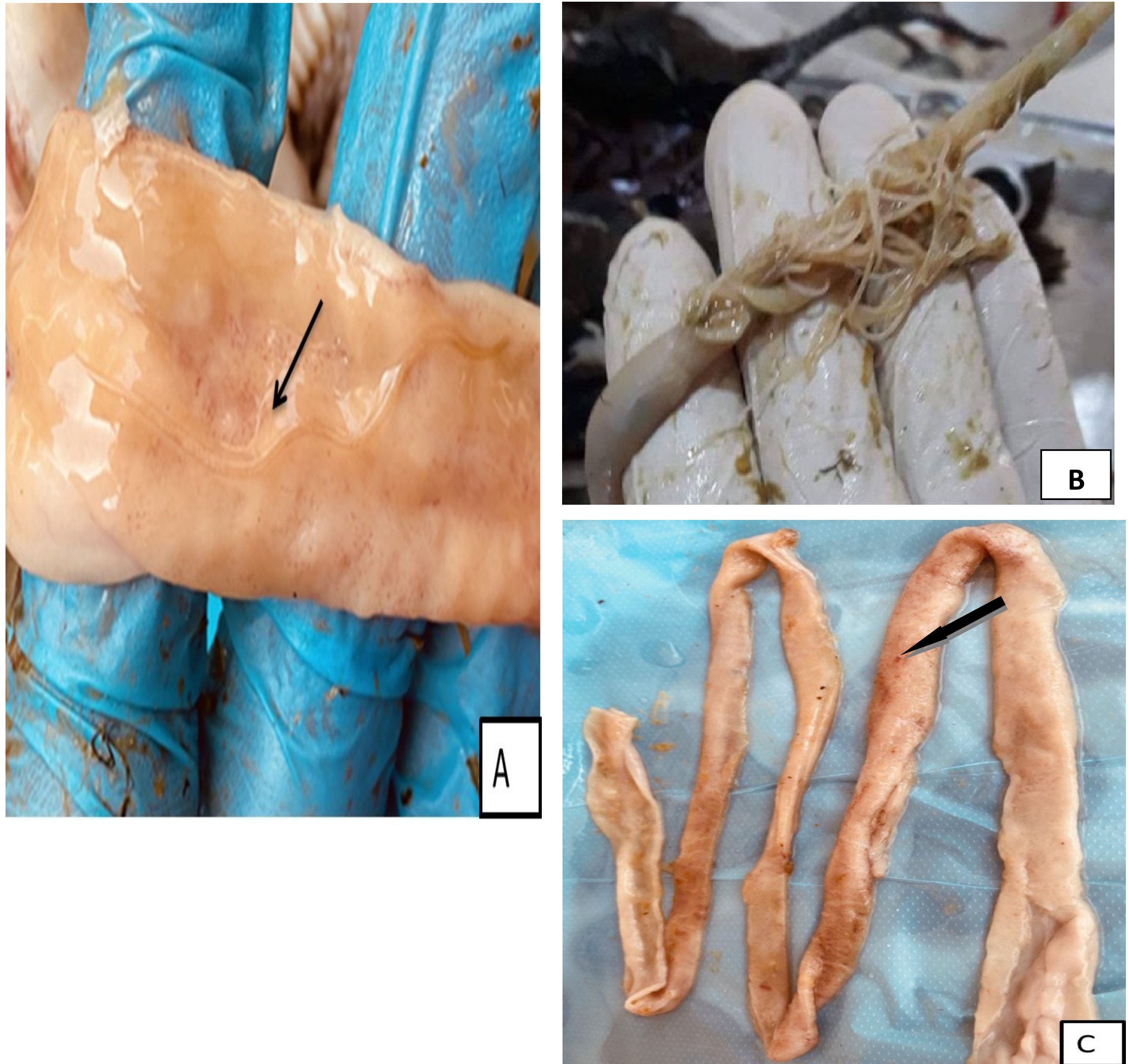


Figure (4-8): A- Small intestine with *A. galli* show yellowish worm and cylindrical in shape

B- High burden small intestine with *A. galli*

C- Thickened and haemorrghic spots in small intestines wall.

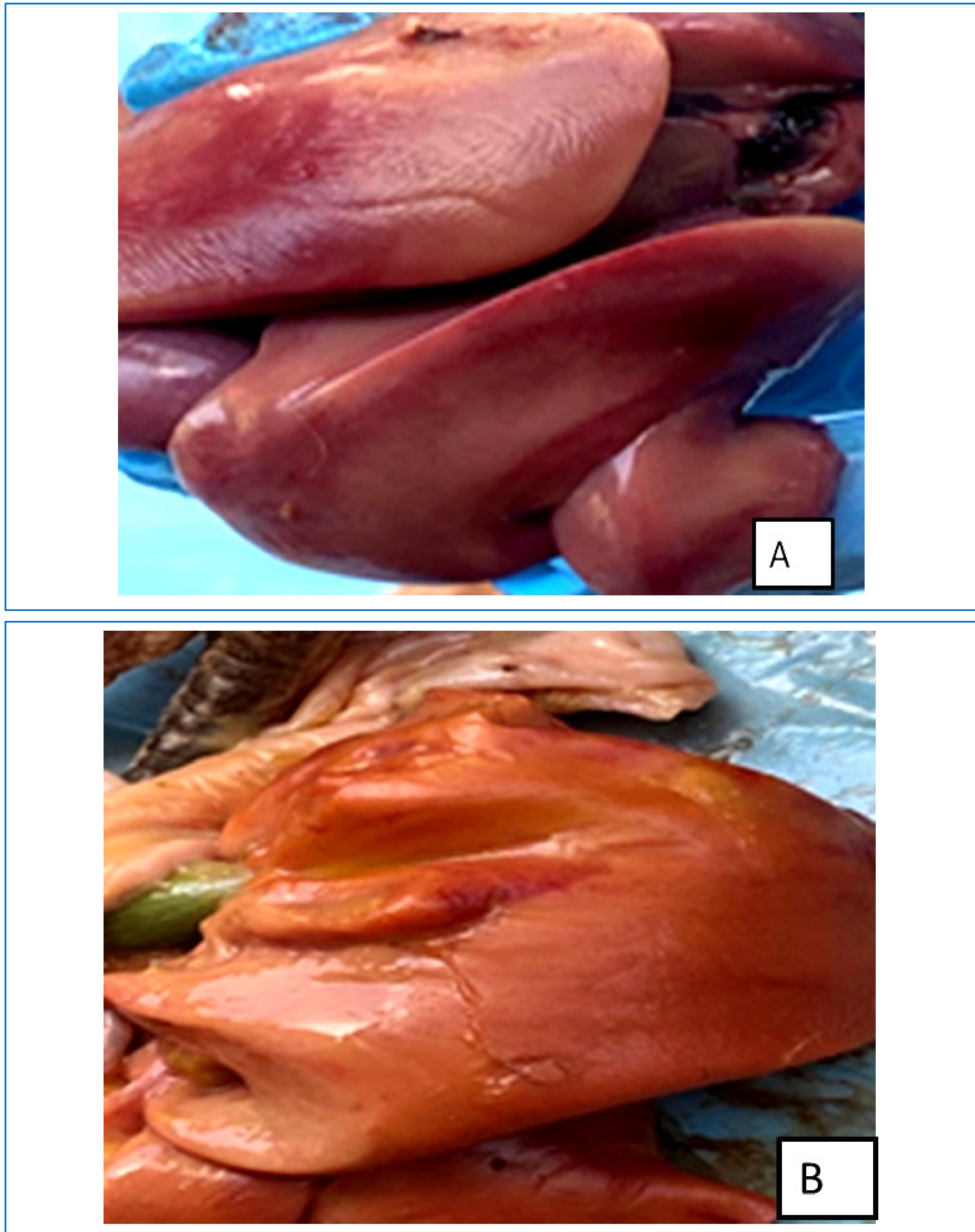


Figure (4.9): Livers of infected local chicken with *A. galli*
(A): Congestion
(B): Paleness

4.7.2. Microscopic examination

4.7.2.1. Small intestine

Histopathological examination of intestinal sections showed that there was sub-mucosal edema with mild cellular infiltration, and hyper-hyperplastic activity of sub-mucosal glands that elongated together with sub-epithelial diffuse and mononuclear cells infiltration in lamina properia (**Figure 4.10**). Microscopic examination of the intestines also revealed lymphoid depletion of mucosal lymphoid association tissue (malt) with irregular appearance of intestinal villi (**Figure 4.11**). Also, there was atrophy of villi, diffuse infiltration of mononuclear cells in lamina properia and loss of epithelia (**Figure 4.12**). In sub- mucosal layer there's focal aggregation of cellular inflammatory cell accompanied by necrotic debris of adjacent glandular tissue (**Figure 4.13**). The sub-mucosal glands showed marked proliferation with intestinal villous atrophy (**Figure 4.14**). Marked necrosis of intestinal villous tissue, sloughed epithelia and hyper plastic cryptal tissue were evident (**Figure 4.15**). Multiple foci of mineral deposition in muscular tissue with basophilic irregular mass were seen (**Figure 4.16**). The serosal tissue was infiltrated with MNCs with mild vascularity (**Figure 4.17**). Sub-mucosal gland showed hyperplasia (**Figure 4.18**) in addition to aforementioned histopathological lesions there was necrosis in some sub-mucosal glands (**Figure 4.19**)

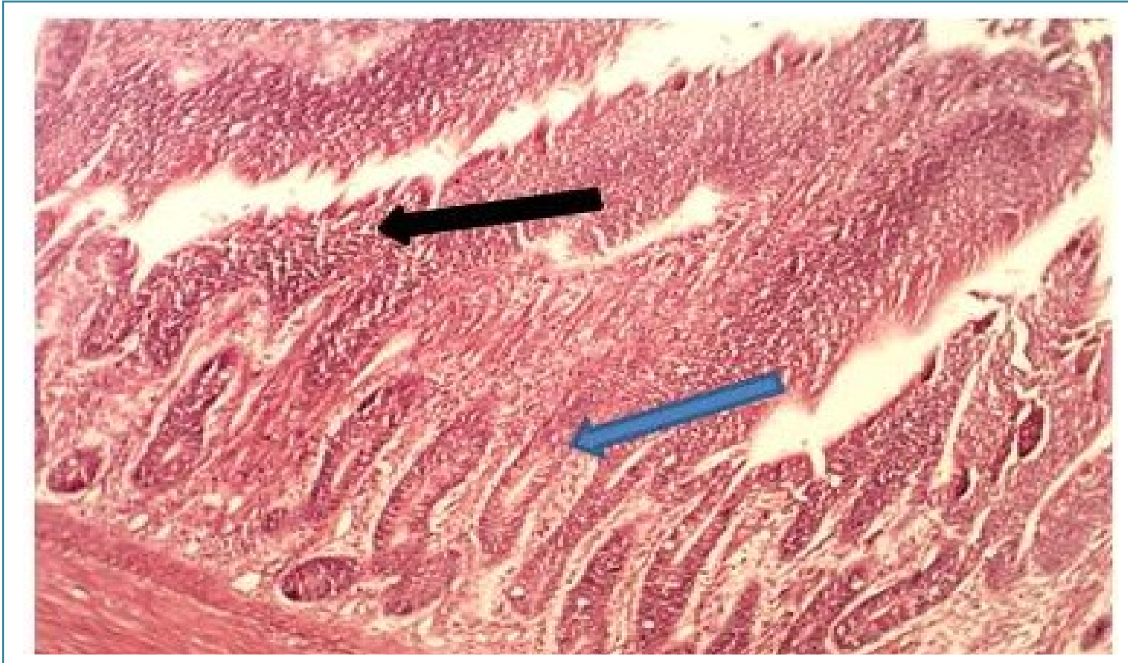


Figure (4.10): Histopathological section in small intestine shows hyperplastic activity of sub mucosal glands that show elongation to gather (Blue arrow) with sub epithelial diffuse MNCs infiltration in lamina properia (Black arrow) (H and E stain; 100×).

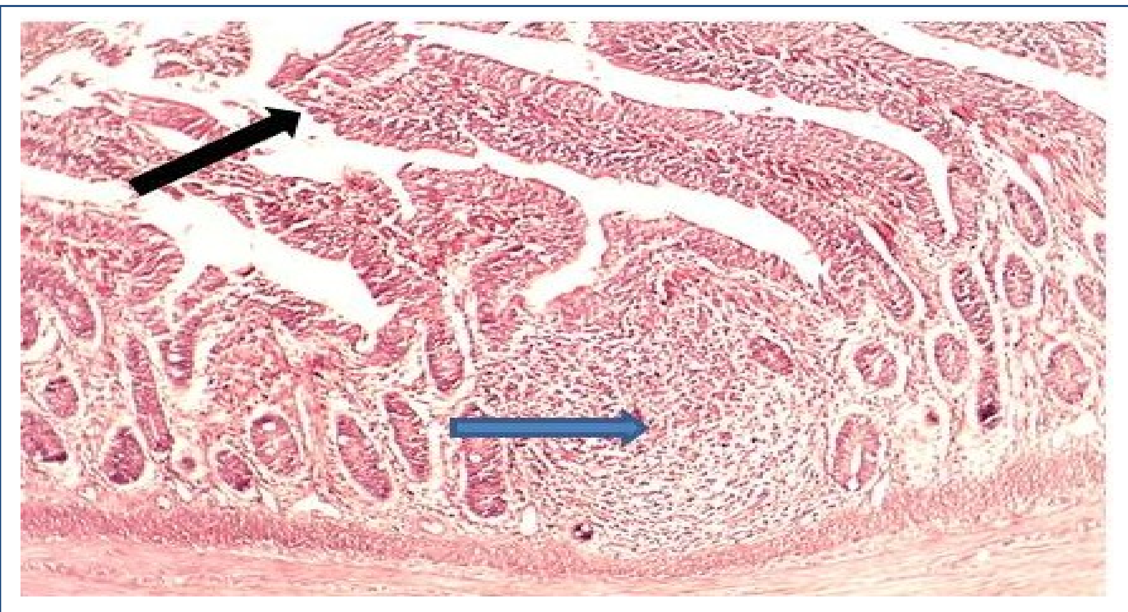


Figure **(4.11): Histopathological section in small intestine shows lymphoid depletion of mucosal lymphoid association tissue (malt) (Blue arrow) with irregular appearance of intestinal villi (Black arrow) (H and E stain; 100x).**

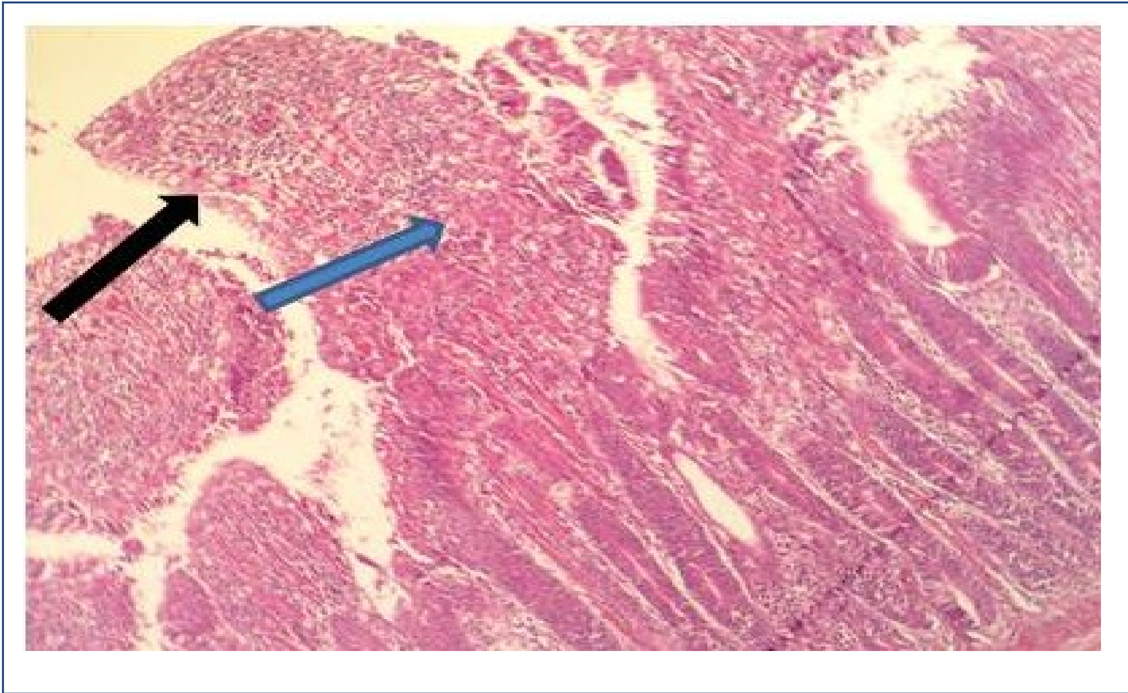


Figure (4.12): Histopathological section in small intestine shows diffuse MNCs infiltration in lamina properia (Blue arrow) with loss surface epithelia (Black arrow) (Hand E stain; 100x).

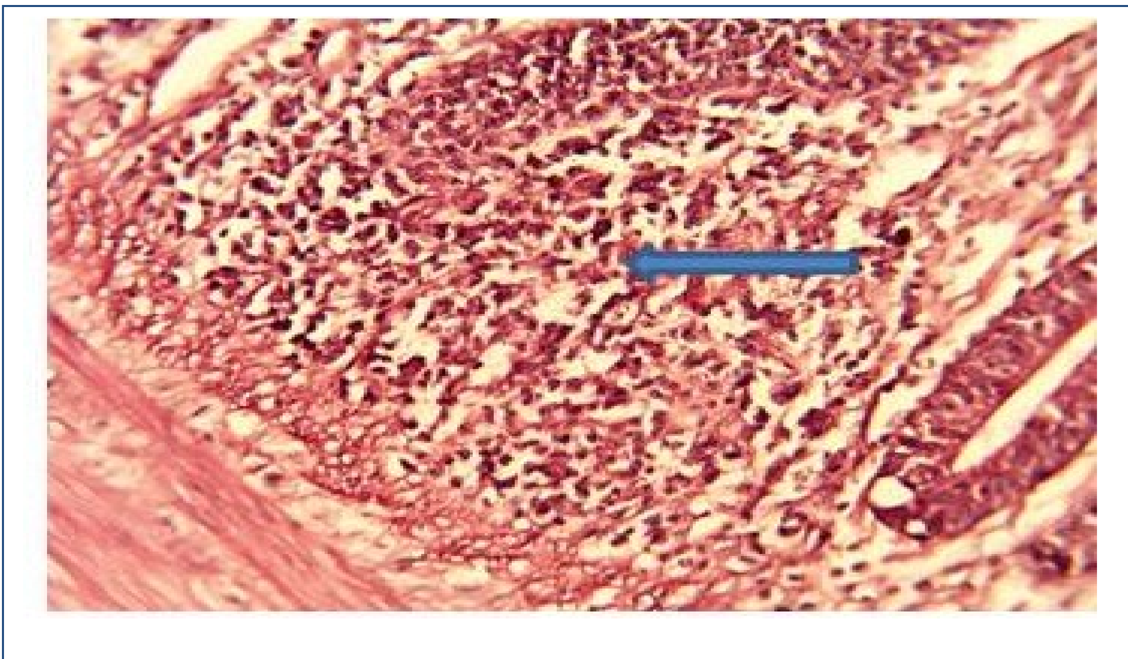


Figure (4.13): Histopathological section in small intestine shows focal cellular inflammatory cell aggregation mainly MNCs in sub mucosal layer (Blue arrow) (H and E stain; 100x)

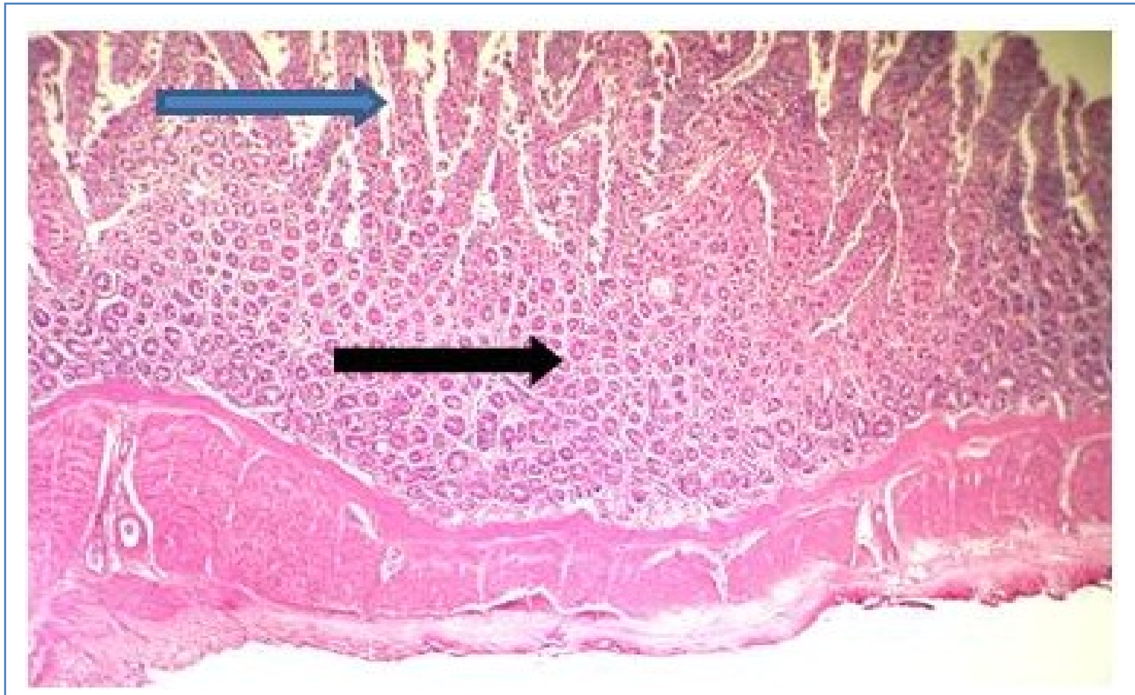


Figure (4.14): Histopathological section in intestine shows marked proliferation of sub mucosal glands (Black arrow) with atrophic villi (Blue arrow) (H and E stain; 100×)

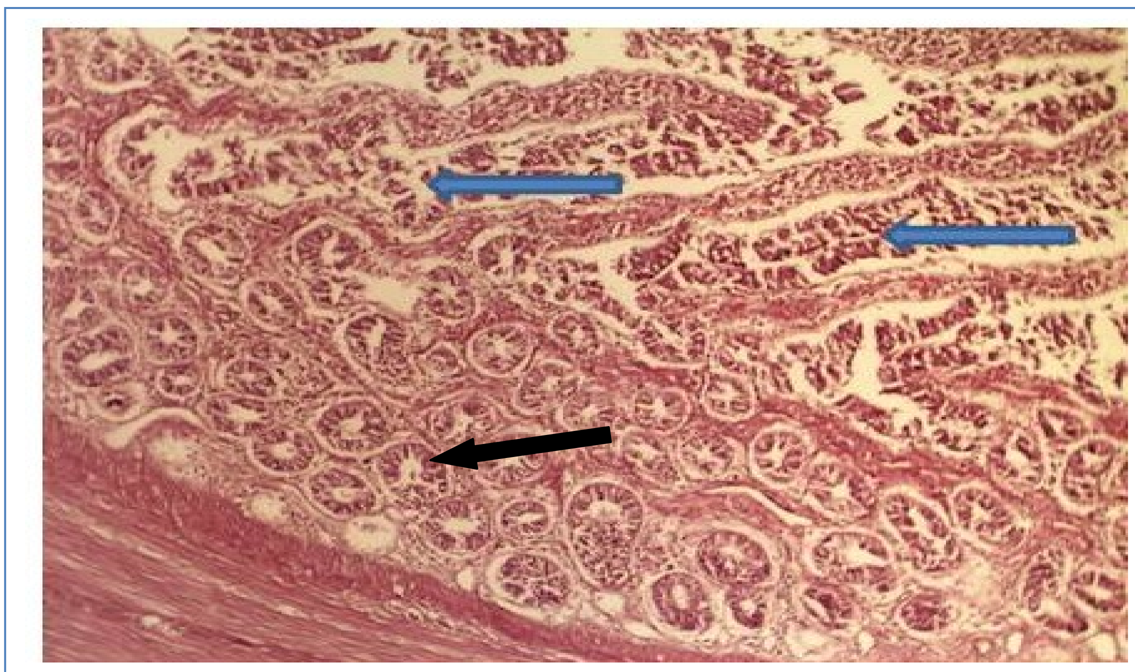


Figure (4.15): Histopathological section of small intestine shows marked necrosis of intestinal villous tissue (Blue arrow) with sloughed epithelial accompanied with hyperplastic cryptal tissue (Black arrow) (H and Estain;100x).

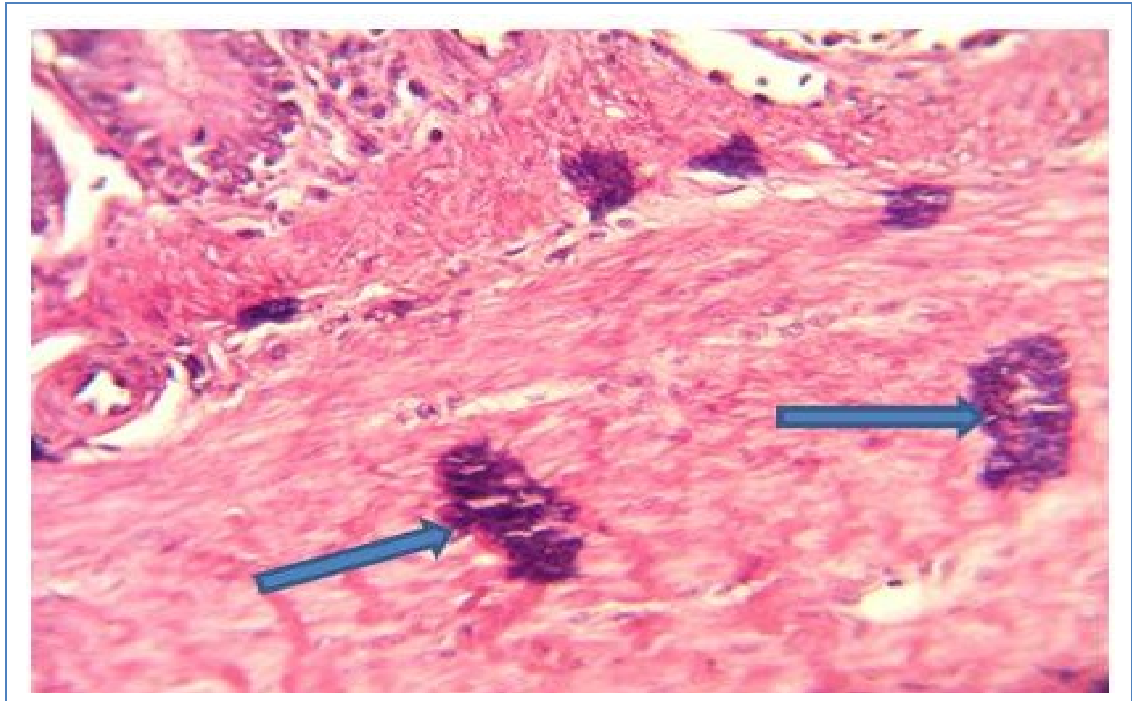


Figure (4.16): Histopathological section in small intestine shows multiple foci of irregular basophilic appearance represent mineral deposition in muscular tissue (Blue arrow) (H and E stain; 100×)

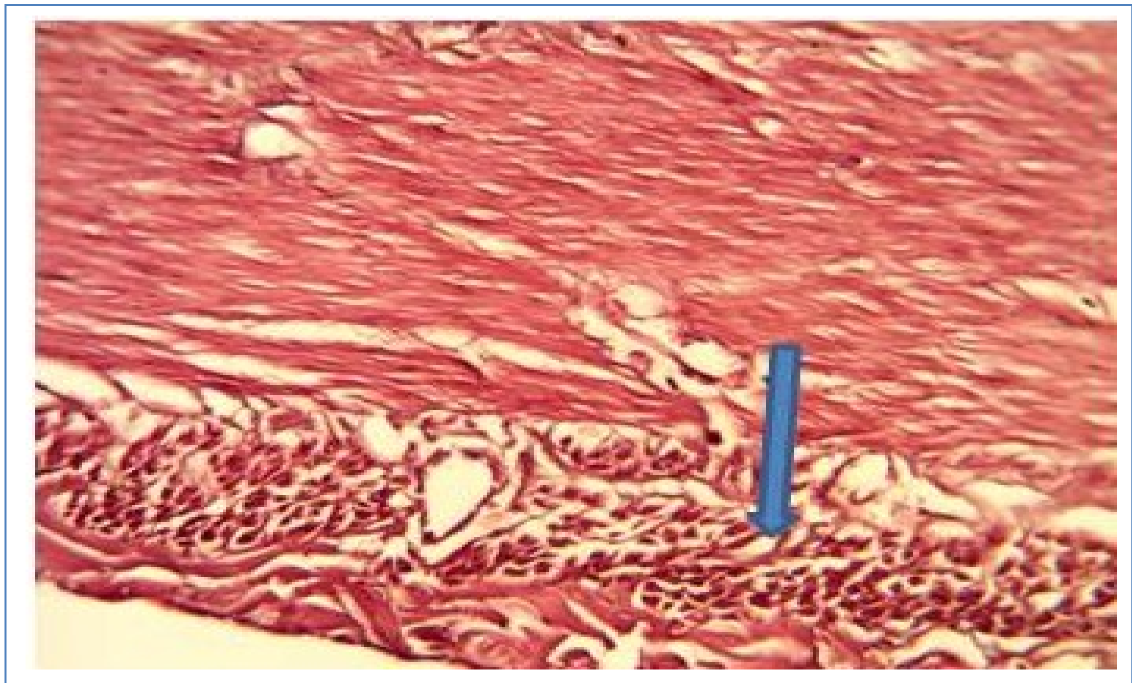


Figure (4.17): Histopathological section in small intestine shows MNCs infiltration with in serosal tissue (Blue arrow) (H and E; 100x)

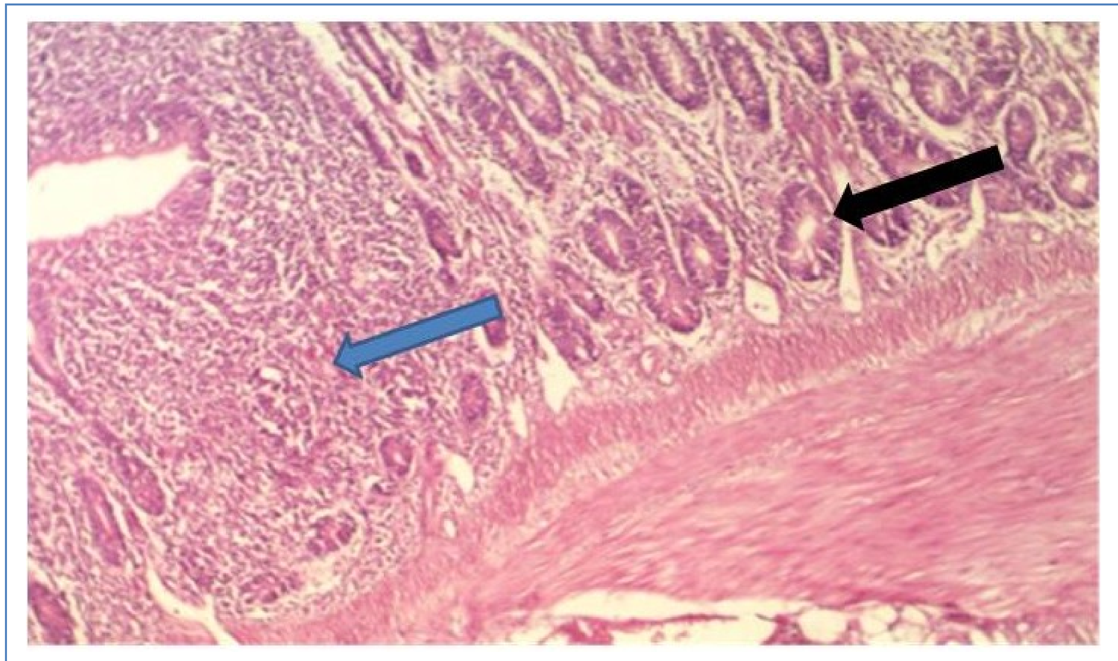


Figure (4.18): Histopathological section in small intestine shows hyperplasia of remnant sub mucosal glands (Black arrow) with prominence of lymphoid association tissue (Blue arrow) (Hand E stain; 100x)

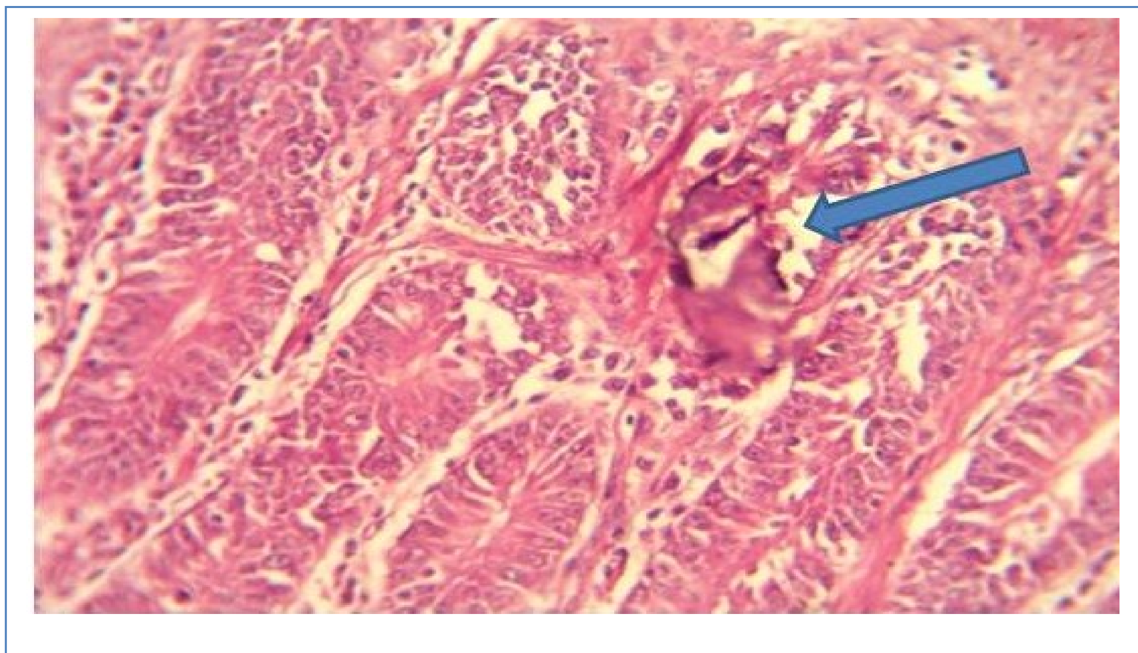


Figure (4.19): Histopathological section in small intestine shows necrosis of some sub –mucosal glands (Blue arrow) (H and E stain; 100x)

4.7.2.2. Liver

Histopathological examination of the liver section showed marked dilation and congestion of portal vein, dilation of central vein (**Figure 4.20**). Portal venous thrombus formation, mild periportal fibrosis and hyperplasia of bile duct epithelia as well as central vein thrombus (**Figure 4.21**). Dilation of sinusoid with focal heterophils aggregation with marked dilation of portal vein with varies venular cellular aggregation accompanied with necrotic finding in the bile duct. Magnification of previous figure shows diffuse necrosis of ductal tissue with vacuolation of blood vessel wall of hepatic artery wall (**Figure 4.22**). Liver parenchyma showed necrotic foci and extensive area of hemorrhage in sub capsular region (**Figure 4.23**). Small ductal proliferation with mild portal fibrosis were detected (**Figure 4.24**). Infiltration of MNCs in portal area with periductal fibrosis and necrotic debris in their lumen were observed (**Figure 4.25**). Affected hepatic tissue section showed the same lesions in the liver parenchyma, there are focal areas of hemorrhage with central lobular necrotic lesion (**Figure 4.26**). In addition to previous microscopic lesion parenchyma there's granulomatous lesion in liver mainly adjacent to the portal area (**Figure 4.27**).

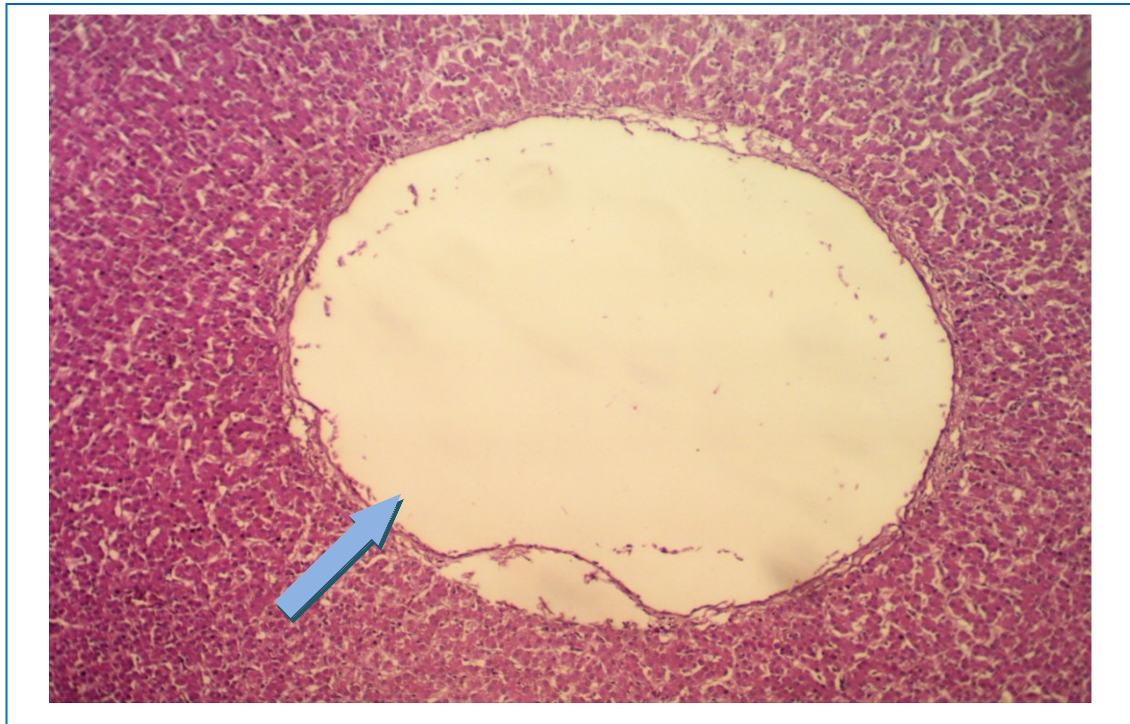


Figure (4.20): Histopathological section in liver shows dilation of central vein (Blue arrow) (H and E stain; 100×)

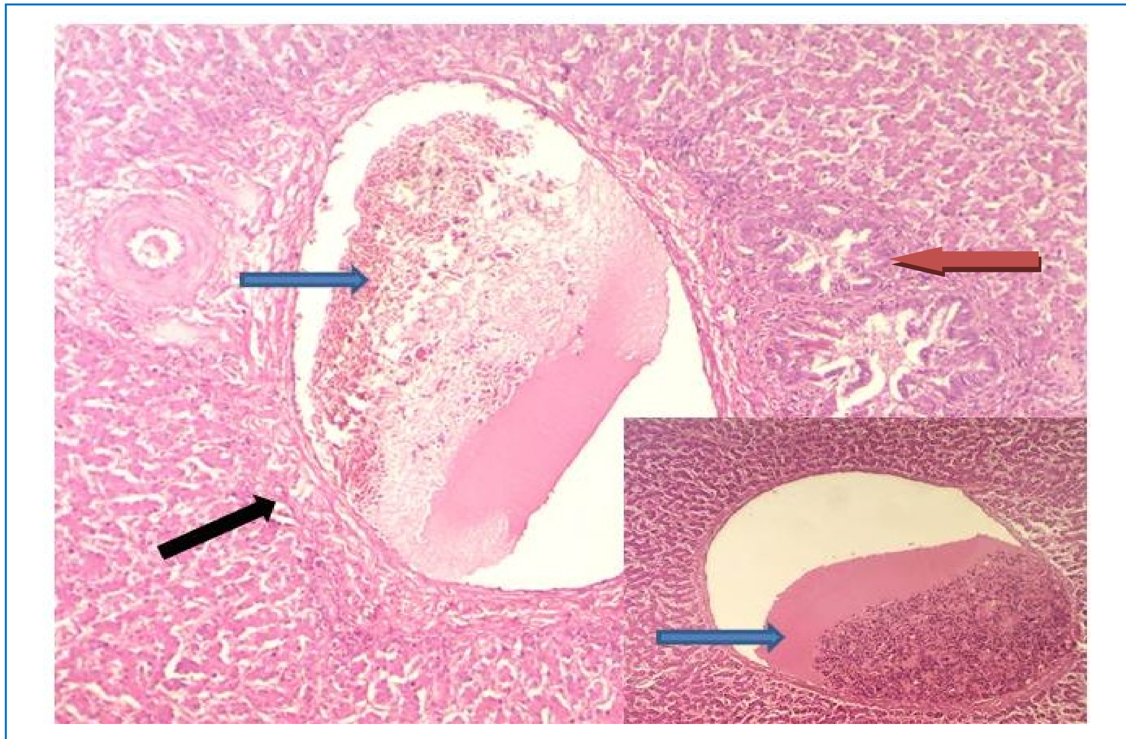


Figure (4.21): Histopathological section in liver shows portal venous thrombus formation (Blue arrow) accompanied with mild periportal fibrosis (Black arrow) and hyperplasia of bile duct epithelia (Red arrow); the inserted figure shows central vein thrombus (Blue arrow) (H and E stain; 100×).

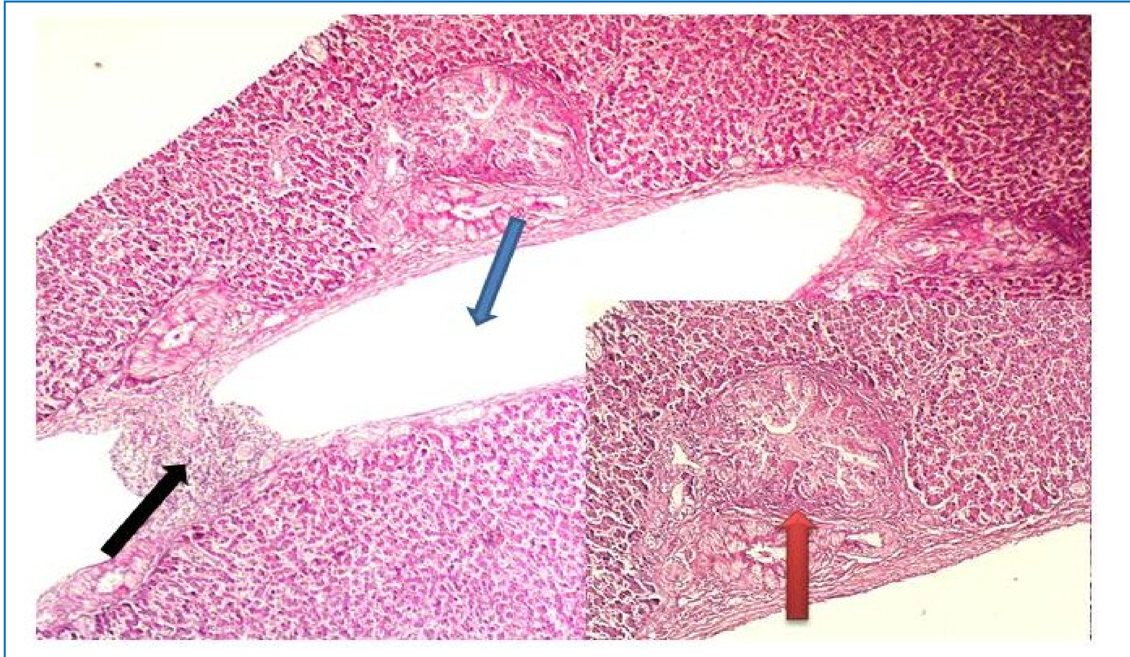


Figure (4.22): Histopathological section in liver shows marked dilation of portal vein (Blue arrow) with inflammatory cell aggregation and necrotic finding in the bile duct (Black arrow),Magnification of previous figure shows diffuse necrosis of ductal tissue (Red arrow) (H and E stain; 100×).

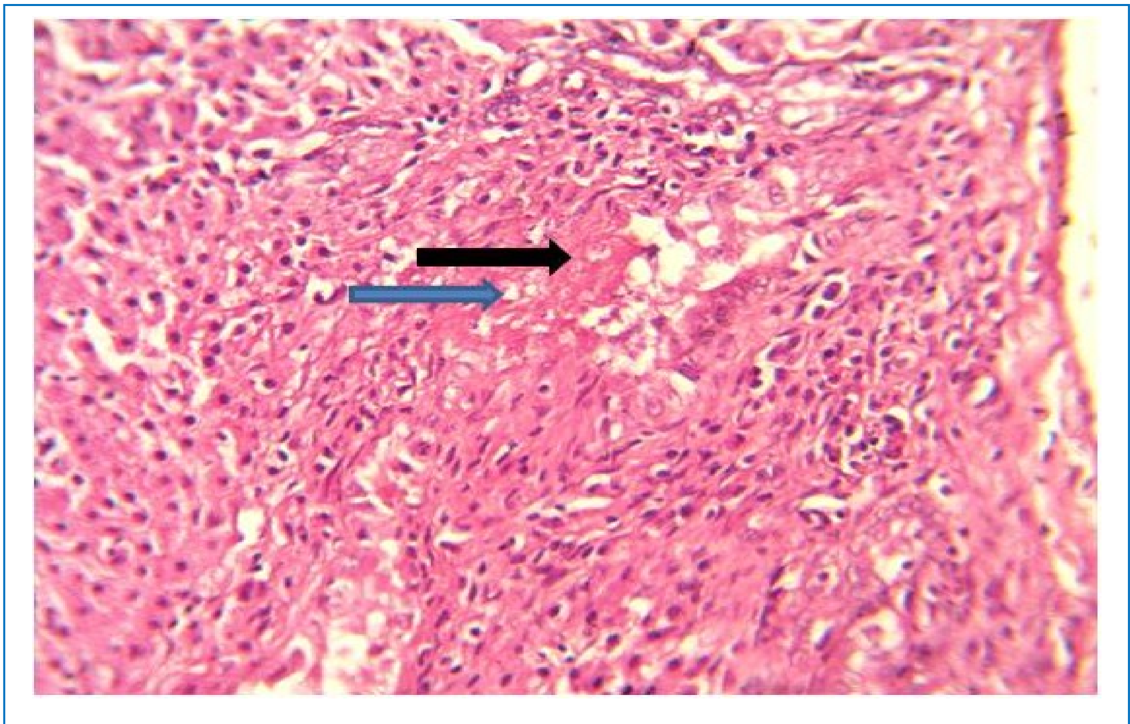


Figure (4.23): Histopathological section in liver shows necrotic foci in the liver parenchyma mainly in subcapsular region (Blue arrow) with area of hemorrhage (Back arrow) (H and E stain; 100×).

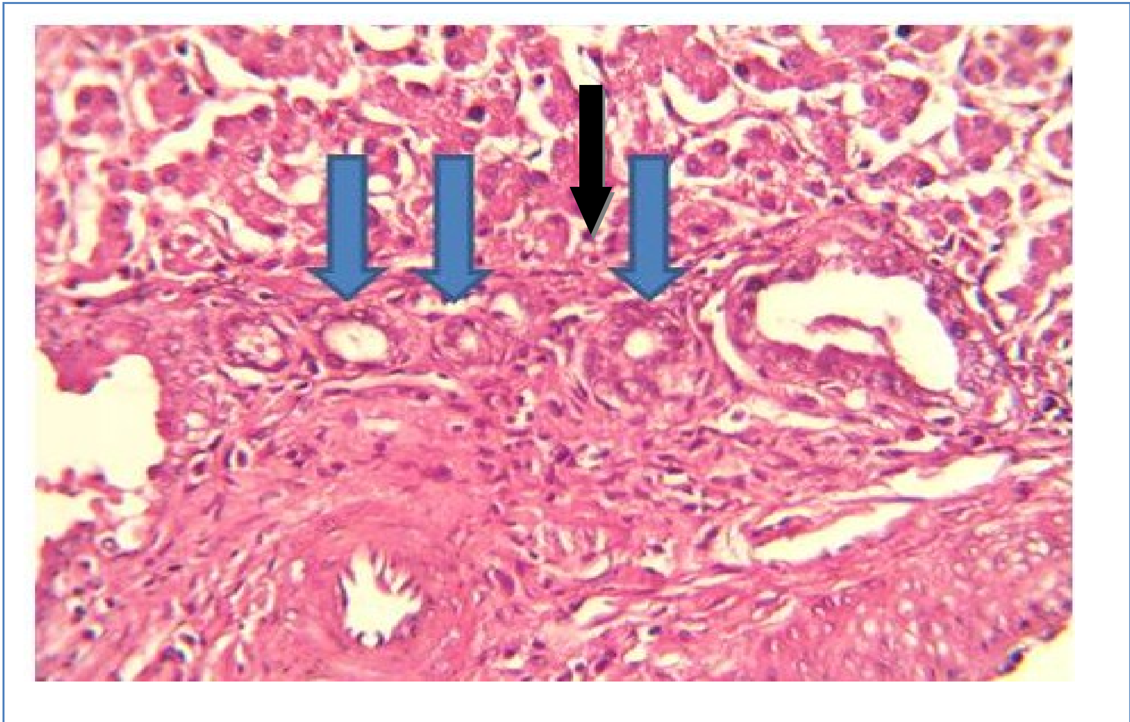


Figure (4.24): Histopathological section in liver shows small ductual proliferation (Blue arrow) with mild portal fibrosis (Black arrow) (H and E stain; 100x).

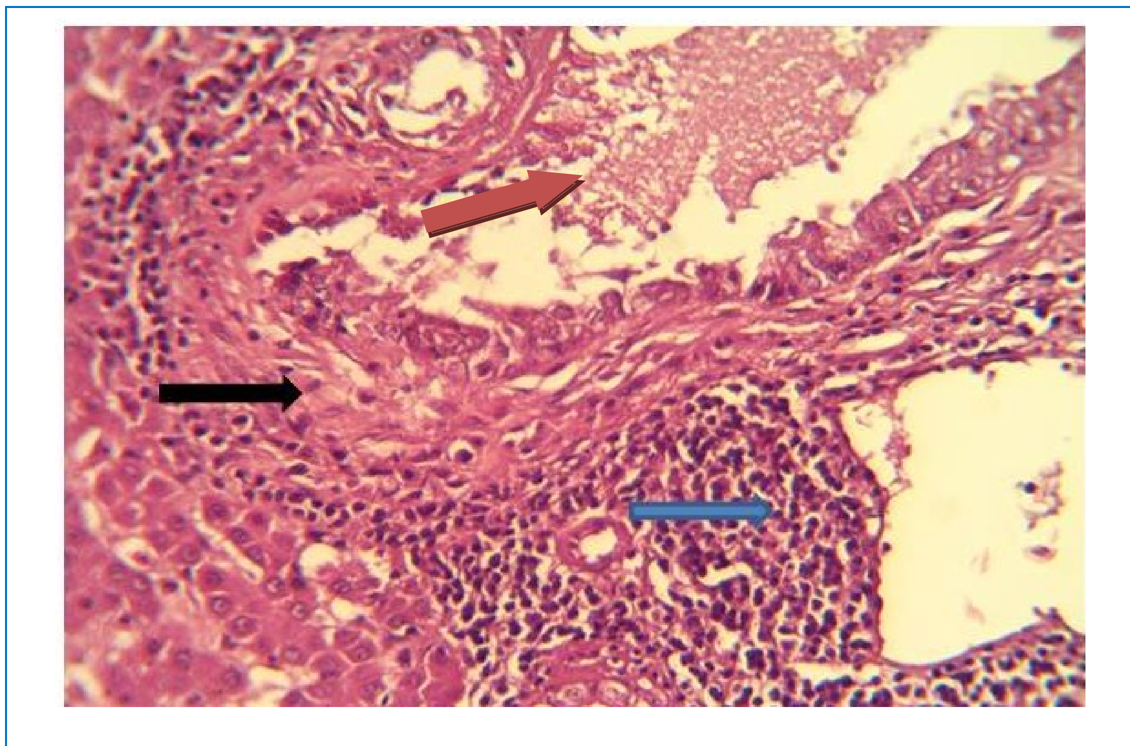


Figure (4.25): Histopathological section in liver shows marked MNCs infiltration in portal area (Blue arrow) with periductal fibrosis (Black arrow) and necrotic debris in their lumen (Red arrow) (H and E stain; 100×).

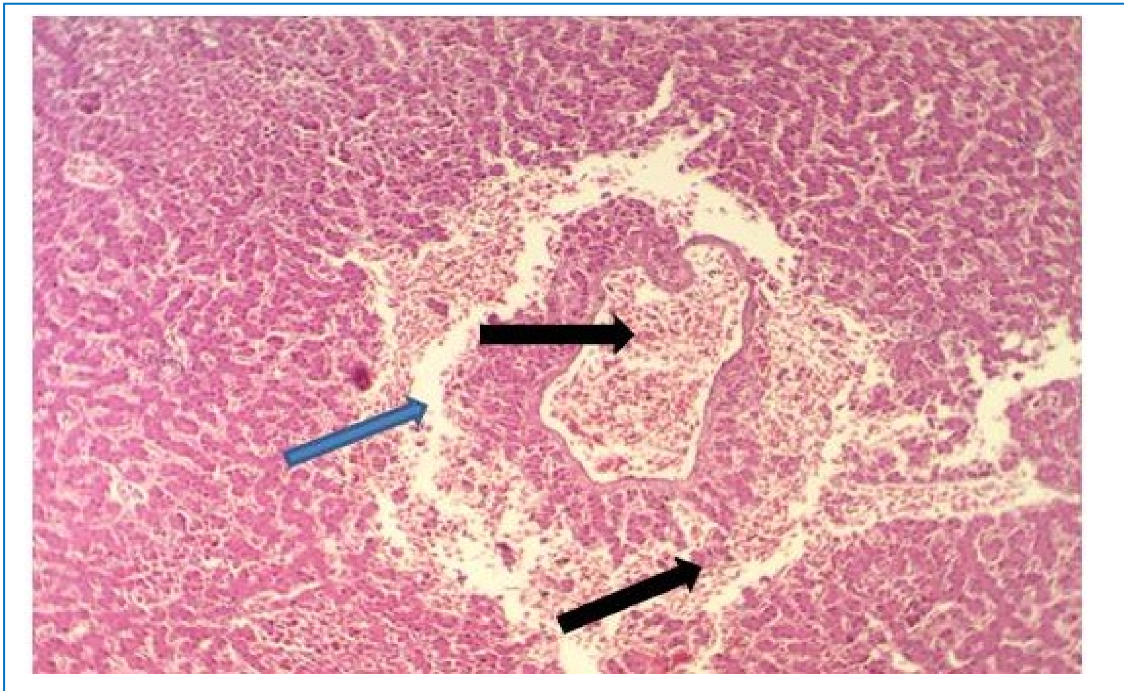


Figure (4.26): Histopathological section in liver shows focal hemorrhage (Black arrow) and central lobular necrotic lesion in liver parenchyma (Blue arrow) with hemorrhage (H and E stain; 100x).

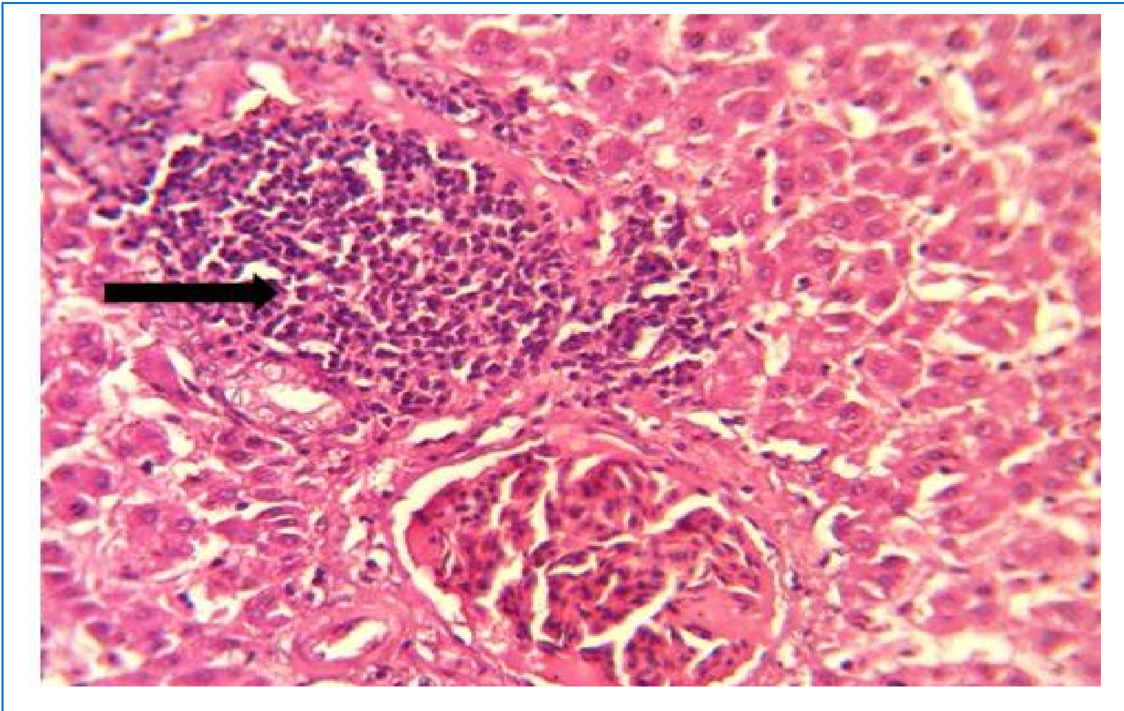


Figure (4.27): Histopathological section in liver shows granulomatous lesion in the liver parenchyma mainly adjacent to the portal area (Black arrow) (H and E stain; 100×).

In terms of histopathological changes, *A. galli* produced small intestinal obstruction, petechial bleeding in the duodenum with thickness of small intestine wall in addition to the presence of haemorrhagic spots. Liver samples showed congestion in some areas and paleness in other cases. Previous studies found that, *A. galli* mostly causes ulcerative ventriculitis **(Brar *et al,* 2016; Gopal *et al*., 2017),** intestinal wall damage and bleeding (**Skallerup *et al*., 2005),** necrotic patches of intestinal mucosa **(Rajkovic *et al*., 2019)** petechial haemorrhage in the intestinal mucosa, nodular haemorrhagic enteritis, and inflammation of the proventriculus (**Permin and Ranving, 2001; Magwisha *et al*., 2002)**. According to **Abdelqader *et al*. (2007)** and **Adang *et al*. (2010)**, if the number of parasites is large enough, some of infected chickens may survive.

According to findings of this study worms cause these severe diseases by grabbing intestinal tissues after consuming the digested meal. **Lapage (1956)** described that *A. galli* eggs hatch in the small intestine and that the early larvae live in the content of the intestine for about 9 or 10 days before they bury their head in the intestinal crypts. This hypothesis later supported in other parasitology-related references **(Soulsby, 1982; Kaufmann, 1996).** Current findings are similar to the findings of **Herd and McNaught (1975)** who recovered more larvae from the intestinal wall than in the intestinal content at 3 days post infection indicating that the larvae already at this time have moved deeper into the mucosa. According to **Tugwell and Ackert (1952)**, larvae can be in the mucosa 1 day after hatching. Even though the majority of larvae were found in the lumen as described (**Todd and Crowdus, 1952; Tugwell and Ackert, 1952)**, both of parasitological and histological results of previous study confirm that the majority of

larvae are located in the profound zones of the mucosa at day 3 post infection. **Luna-Olivares *et al.* (2012)** they reported that most larvae were observed in the intestinal lumen, but in close contact with epithelium 63% followed by with in epithelium 32% and only few in the lamina properia 5% . One reason to move into the crypts is probably to avoid being flushed out further abnormally in the intestinal tract by the peristaltic movements. The height of the villus - crypt axis compared to the size of the larvae shows that the larvae can easily locate themselves in the crypt zone. The larvae sometimes dilated the crypt lumen and exerted pressure on the surrounding crypt epithelium making almost a pocket where they are less vulnerable to be flushed out **(Luna-olivares *et al*., 2012).** Some of the larvae appeared to invade the epithelial layer. However, previous studies were unable to determine with certainty whether the individual larva anchors itself to the intestinal epithelium by breaking the intercellular bridges and thereby locating part of the body between the epithelial cells or if the larva penetrates individual epithelial cells similar to, e.g. *Trichuris* spp. (**Tilney *et al.,* 2005).**

Worms can sometimes enter the intestinal epithelium, causing necrosis and inflammation. Furthermore, it is possible that this is owing to the fact that embryonated eggs aren't fertilized. Ingesting and hatching second-stage larvae in the intestinal wall can cause macroscopic clinical lesions such as intestinal hemorrhagic enteritis, necrotic patches, and reddish spots on the intestinal wall (**Rabbi *et al.*, 2006; Adang *et al.*, 2010; Soomro *et al.,* 2010; Thomas and Reetha, 2014**). However, the Petechial haemorrhage's exact process is yet unclear. The parasite, on the other hand, is likely to penetrate deep into the mucosa. A significant number of parasites may cause petechial bleeding during penetration. In certain cases, necrotic plaques have been discovered and

demonstrated by other studies (**Permin *et al.,* 1997; Ferdushy *et al*., 2016)**, and probably, significant inflammatory response in the mucosa. Plaque can develop as a result of bacterial colonization in chicken's intestine especially in the cecum and rectum (**Nawab *et al*., 2018**).

Both gross and histological changes in the liver of infected chickens have been described by various authors. **Adang *et al*. (2010)** observed pathological changes in the chicken intestine in addition to the previously documented pathological changes in the chicken intestine. Lesions in the liver such as fatty degeneration with coagulation necrosis and changes in fatty acids were also observed. Those were similar to what have been found in the other studies (**Abdel Rahman *et al*., 2019; Sharma *et al*., 2019**). **Bsrat *et al*. (2014)** described more severe microscopical changes that demonstrated disseminated bleeding and localized necrosis. These modifications suggest that *A. galli* infection causes extensive pathogenic consequences. Not only are these changes to blame for the decline in production capacity, but they are also to blame for the increase in production costs.

4.8. Molecular study

4.8.1. DNA extraction

The genomic DNA extracted from 50 isolates of *A. galli* according to protocol of tissue DNA extraction kit (Intron Biotechnology/ South Korea). Purity and concentration were confirmed with Nanodrop spectrophotometer system. The results were that the concentrations of all 50 DNA parasite isolates were between (47,1- 69,2) ng/ µl, these concentrations were sufficient to use DNA for amplify PCR; and the DNA purity (That

was measured by reading the absorbance at the wave length 260/280 nm) of all 50 positive samples ranges from (1.83- 2).

4.8.2. PCR results

Molecular examination of extracted DNAs from all positive samples (50) revealed that the PCR amplification was successful on all isolates for the 18S rRNA gene. The amplified fragment size was approximately 724bp (**Figure 4.28)**.

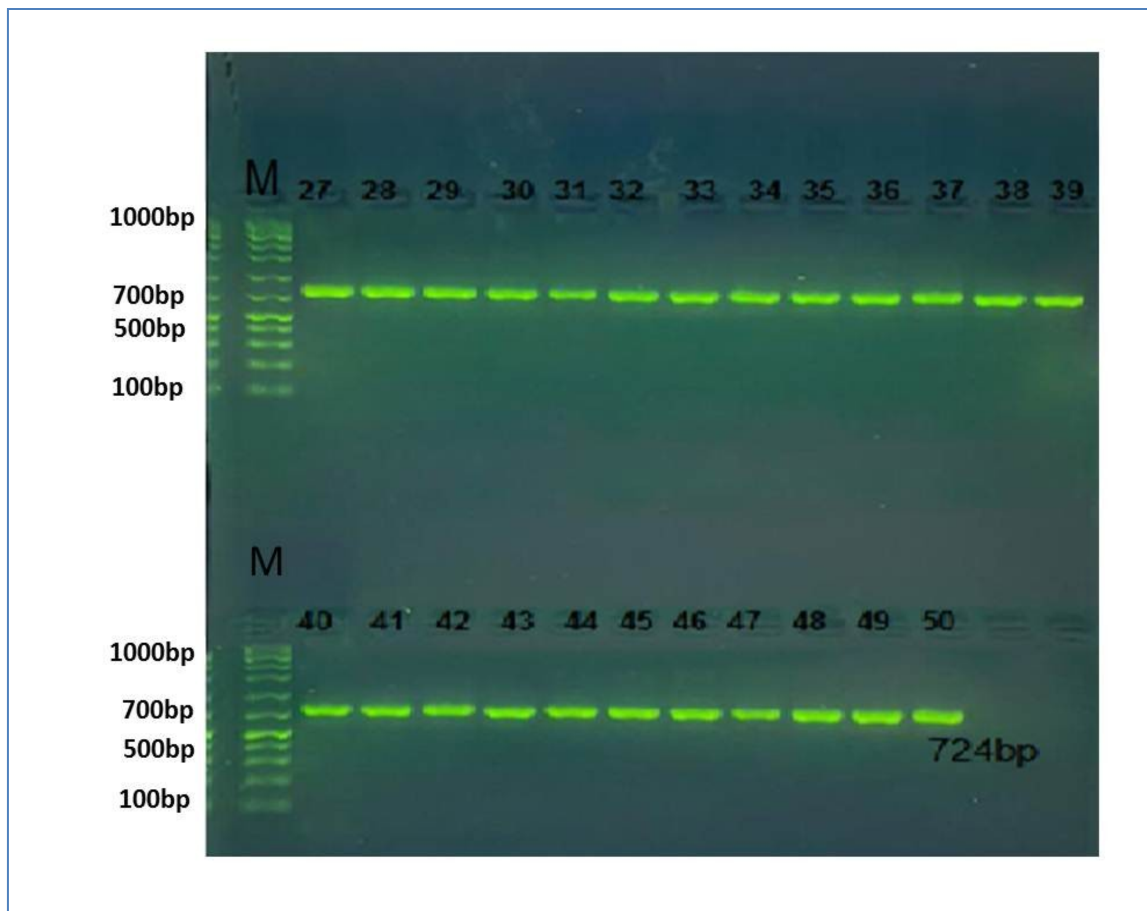


Figure (4-28): PCR products of 18sRNA 724bp. The product was run on 1.5% agarose at 70 volts, 1×TBE buffer for 1:30 hours; Lane M: Ladder marker (100); Lane (27-50): Positive PCR results for the ribosomal 18S rRNA belong to *A. galli*

4.8.3. Sequencing

4.8.3.1. Sequence alignment analysis

The 18S rRNA gene of *Ascaridia galli* were amplify by PCR method, and sent for sequencing to Macrogen company Korea. Sequence alignment analyses for 18S rRNA of *Ascaridia* isolate of Iraqi chickens were arranged by MEGA6 and NCBI. The nucleotide base alignment shows substitution modification as transversion and transition in the 18S rRNA gene as changes in the nucleotides sequence with proven isolate in database NCBI GeneBank (**Appendix 2**).

The multiple sequences analyses of 1-10 sequence with other *A. galli* credited in GenBank NCBI (**Figure 4.30 – 4.31-4-32**).

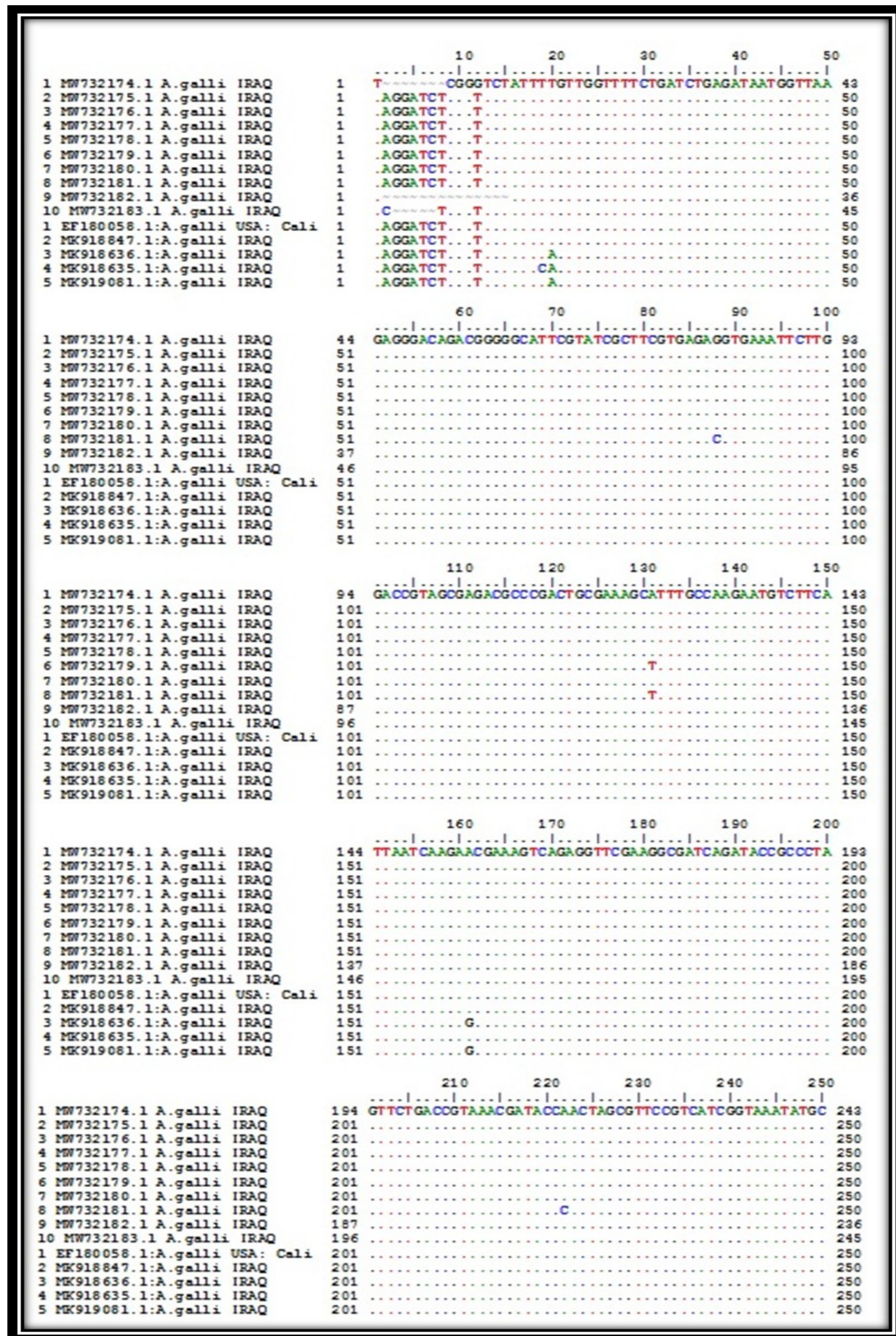


Figure (4.29): Multiple sequences of *Ascaridia galli* of 18S rRNA gene

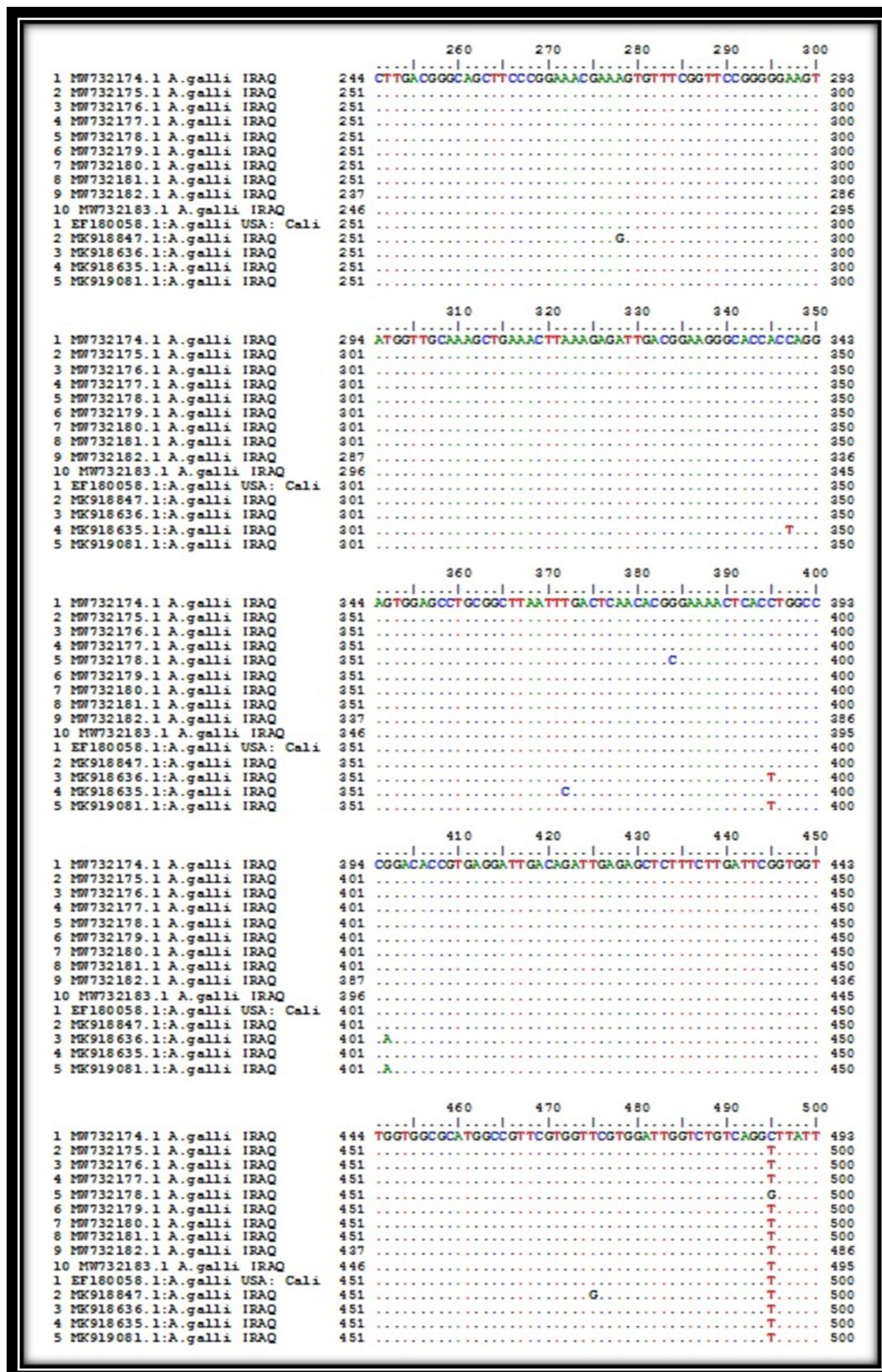


Figure (4.30): Multiple sequences of *Ascaridia galli* of 18S ribosomal RNA

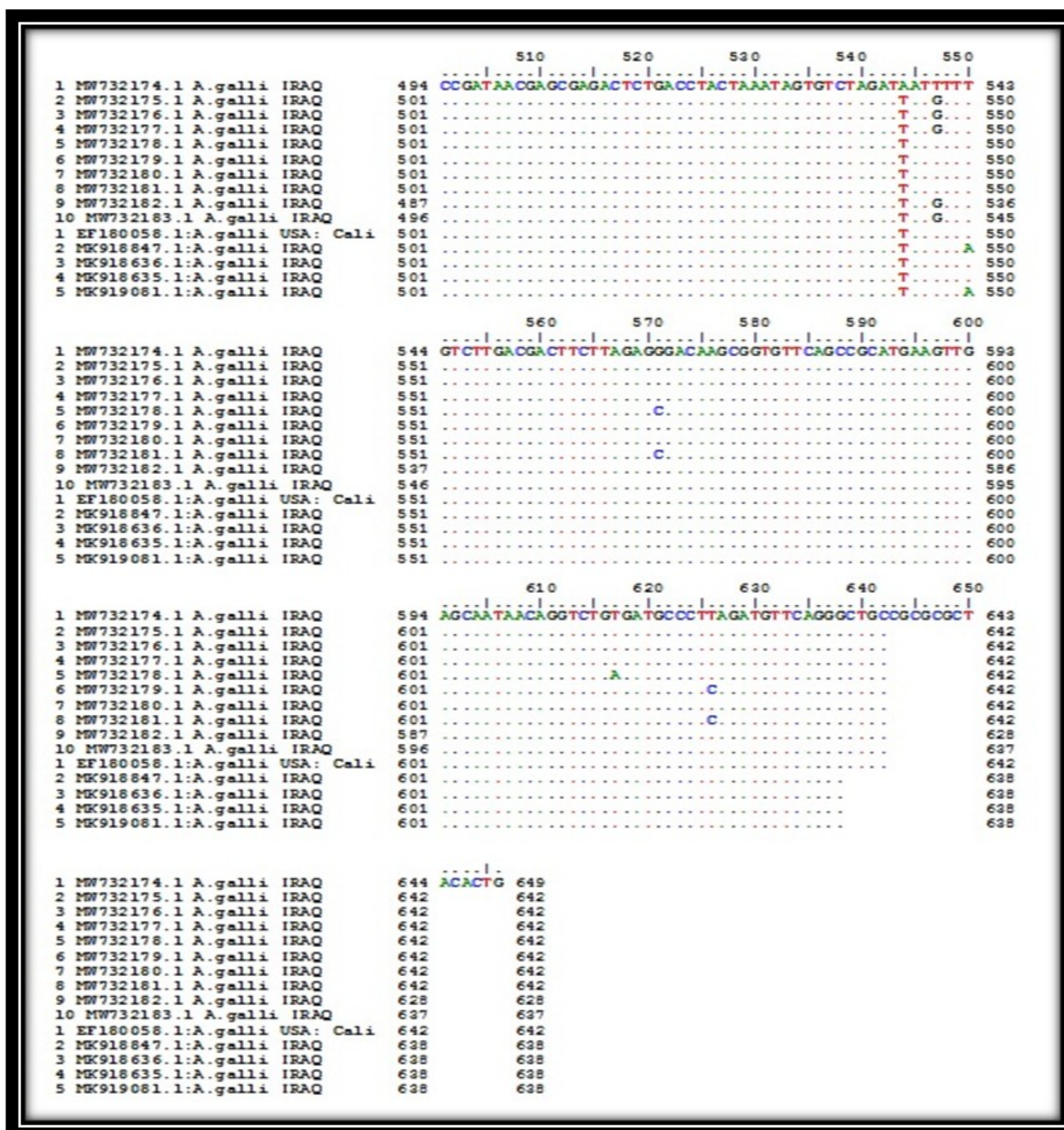


Figure (4.31): Multiple sequences of *Ascaridia galli* of 18S ribosomal RNA gene

4.8.3.2. Submission of local Iraq isolate to NCBI

Ten PCR products were chosen randomly from 50 PCR samples and sent for sequencing using the forward and reverse primers. These sequences were analyzed by BLAST-NCBI program to determine the converging sequences recorded in the GenBank Table **(4-5)**. The sequences were submitted to the NCBI Genbank database under numbers: No. 1 (MW732174.1), No. 2 (MW732175.1), No. 3 (MW732176.1), No. 4

(MW732177.1), No. 5 (MW732178.1), No. 6 (MW732179.1), No. 7 (MW732180.1), No. 8 (MW732181.1), No. 9 (MW732182.1) and No. 10 (MW732183.1) (**Appendix 3**).

Table (4.5): Type of mutation of 18S ribosomal RNA gene from *A. galli* isolates

Gene: 18S ribosomal RNA gene						
No.	**Type of substitution**	**Location**	**Nucleotide**	**Sequence ID with compare**	**Source**	**Identity (%)**
1	Transvertion	772	T\G	EF180058.1	*A. galli*	99
	Transition	1255	T\C			
	Transvertion	1304	T\A			
2	Transvertion	1307	T\G	EF180058.1	*A. galli*	99
3	Transvertion	1307	T\G	EF180058.1	*A. galli*	99
4	Transvertion	1307	T\G	EF180058.1	*A. galli*	99
5	Transvertion	1144	G\C	EF180058.1	*A. galli*	99
	Transvertion	1255	T\G			
	Transvertion	1331	G\C			
	Transvertion	1377	T\A			
6	Transvertion	891	A\T	EF180058.1	*A. galli*	99
	Transition	1386	T\C			
7	-------	-------	------	EF180058.1	*A. galli*	100
8	Transvertion	848	G\C	EF180058.1	*A. galli*	99
	Transvertion	891	A\T			
	Transvertion	982	A\C			
	Transvertion	1331	G\C			
	Transition	1386	T\C			
9	Transvertion	1307	T\G	EF180058.1	*A. galli*	99
10	Transvertion	1307	T\G	EF180058.1	*A. galli*	99

4.8.3.3. Phylogenetic Analysis

In the present study, sequences have been registered in NCBI under the following accession numbers: No. 1 (MW732174.1), No. 2 (MW732175.1), No. 3 (MW732176.1), No. 4 (MW732177.1), No. 5 (MW732178.1), No. 6 (MW732179.1), No. 7 (MW732180.1), No. 8 (MW732181.1), No. 9 (MW732182.1) and No. 10 (MW732183.1) and compared with the NCBI- GenBank *Ascaridia galli* isolates (**Table 4.6, Figure 4.33**). (EF180058.1) USA isolate closely related to local isolates with high identity 99%-100% and *A. galli* (MK918847.1, MK918636.1, MK918635.1, MK919081.1) Iraqi isolates showed 99% identity to our isolates.

Table (4.6): NCBI-BLAST Homology Sequence identity (%) between local *Ascaridia galli* local isolates and NCBI-BLAST submitted *A. galli*

No.	Accession No.	Country	Host	Source	Identity (%)
1	**EF180058.1**	USA: California, UC Riverside	Gallus gallus (Zuk lab strain)	*A. galli*	99
2	**MK918847.1**	Iraq	Columba livia	*A. galli*	99
3	**MK918636.1**	Iraq	Columba livia	*A. galli*	99
4	**MK918635.1**	Iraq	Columba livia	*A. galli*	99
5	**MK919081.1**	Iraq	Columba livia	*A. galli*	99

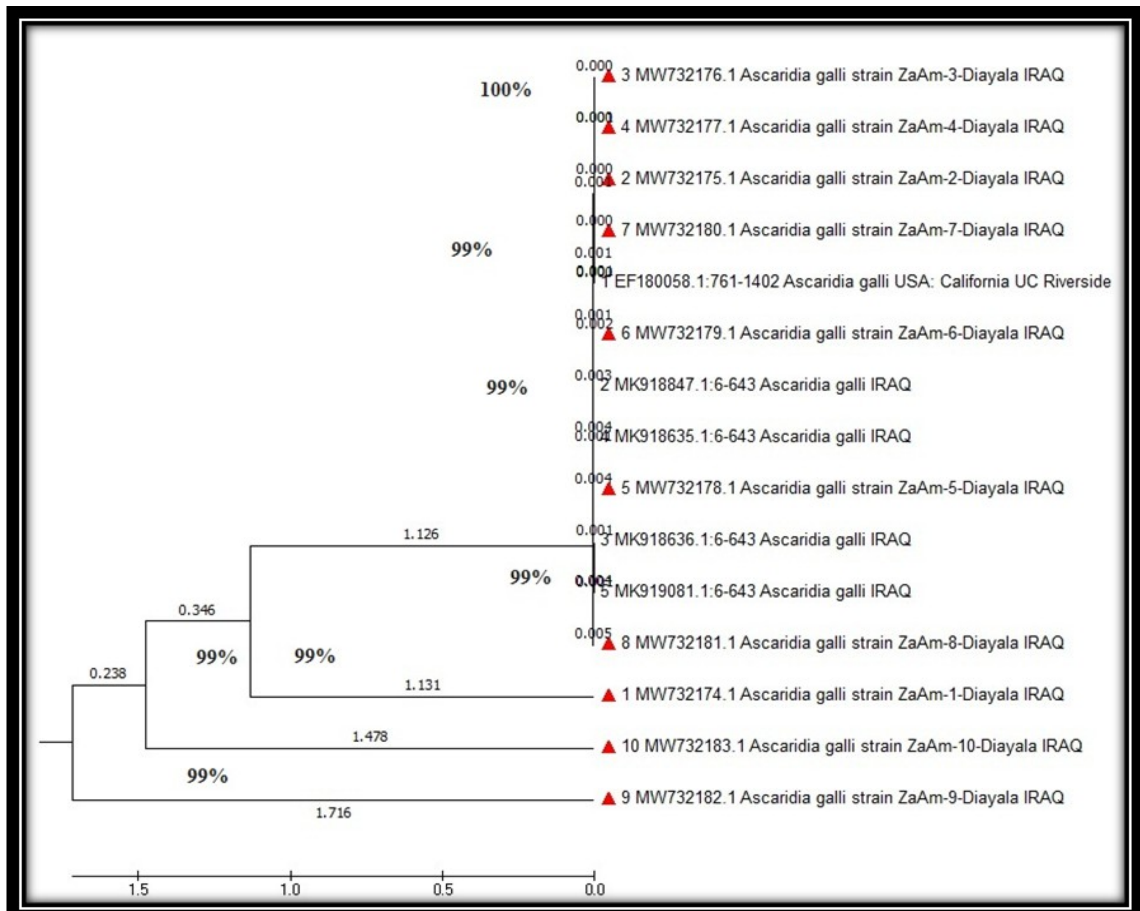


Figure (4.32): Neighbor-joining tree *A. galli* of 18S rRNA gene with genetic variation

Chapter Five

Conclusions and Recomindations

5. Conclusions and recommendations

5.1. Conclusions

1. Field study showed high infection rate with *Ascaridia galli* in slaughtered local chicken in Diayala province.
2. There was significant impact of the sex and months on rate of infection.
3. *A. galli* infection rates in adults chicken were more than young's.
4. Result of histopathology concluded that the more infected small intestine lesions involved loss of epithelia, villi atrophy and necrosis of sub-mucosal gland as well as infected liver showed sever lesions in some cases furthermore gross lesions also recorded.
5. This is first study for using PCR technique with phylogenetic tree of *A. galli* in local chicken in Diyala Province-Iraq. The result of sequences analysis for 10 isolates recorded at National Center for Biotechnology Information (NCBI). Sequence recorded in GenBank under the following accession numbers: (MW732174.1), (MW732175.1), (MW732176.1), (MW732177.1), (MW732178.1), (MW732179.1), (MW732180.1), (MW732181.1), (MW732182.1) and (MW732183.1) belong to *Ascardia* galli.

5.2. Recommendations

1. Maintain strict hygiene and regular deworming with anthelmintic treatment in local chickens to minimize the ascridiosis infection.
2. There is a need for epidemiological study among other gastro-intestinal helminthes in local chickens with aiding of molecular technique to determine the genetic similarity and their phylogenetic tree in the world.
3. Additional studies are essential for conducting ascaridiosis prevalence in further Iraqi province.
4. Additional morphological studies for all intestinal nematode in local chickens are necessary.
5. Focus on larvae and their diagnosis using tools beside the PCR

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Appendices

Appendices

Appendix no.1: Measurement the lengths of adult females and males *Ascaridia galli* (mm)

No.	Female (mm)	Male (mm)
1	60	48
2	68	55
3	75	55
4	70	52
5	75	45
6	70	40
7	70	40
8	82	45
9	75	48
10	75	38
11	75	38
12	70	51
13	60	44
14	70	36
15	75	36
16	67	37
17	40	38
18	70	42
19	60	41
20	73	39

Appendix 2: Sequencing ID in GenBank, Score, Expect, Identity of DNA sequence for 18Sr RNA gene in *Ascaridia galli*

1

Ascaridia galli **18Ssmall subunit ribosomal RNA gene, partial sequence ID: EF180058.1 Length: 1718 Number of Matches: 1 Range 1:768 to 1416**

GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1158 bits(1283)	0.0	646/649(99%)	0/649(0%)	Plus/Plus

```
Query  1     TCGGGTCTATTTTGTTGGTTTTCTGATCTGAGATAATGGTTAAGAGGGACAGACGGGGGC  60
Sbjct  768   ....T.......................................................  827
Query  61    ATTCGTATCGCTTCGTGAGAGGTGAAATTCTTGGACCGTAGCGAGACGCCCGACTGCGAA  120
Sbjct  828   ............................................................  887
Query  121   AGCATTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGGCGATC  180
Sbjct  888   ............................................................  947
Query  181   AGATACCGCCCTAGTTCTGACCGTAAACGATACCAACTAGCGTTCCGTCATCGGTAAATA  240
Sbjct  948   ............................................................  1007
Query  41   TGCCTTGACGGGCAGCTTCCCGGAAACGAAAGTGTTTCGGTTCCGGGGGAAGTATGGTTG  300
Sbjct  1008  ............................................................  1067
Query  301   CAAAGCTGAAACTTAAAGAGATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTT  360
Sbjct  1068  ............................................................  1127
Query  361   AATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGACAGATTGA  420
Sbjct  1128  ............................................................  1187
Query  421   GAGCTCTTTCTTGATTCGGTGGTTGGTGGCGCATGGCCGTTCGTGGTTCGTGGATTGGTC  480
Sbjct  1188  ............................................................  1247
Query  481   TGTCAGGCTTATTCCGATAACGAGCGAGACTCTGACCTACTAAATAGTGTCTAGATAATT  540
Sbjct  1248  .......T................................................T...  1307
Query  541   TTTGTCTTGACGACTTCTTAGAGGGACAAGCGGTGTTCAGCCGCATGAAGTTGAGCAATA  600
Sbjct  1308  ............................................................  1367
Query  601   ACAGGTCTGTGATGCCCTTAGATGTTCAGGGCTGCCGCGCGCTACACTG  649
Sbjct  1368  .................................................  1416
```

2

Ascaridia galli **18Ssmall subunit ribosomal RNA gene, partial sequence ID: EF180058.1 Length: 1718 Number of Matches: 1 Range 1:761to 1402**

GenBank **Graphics**

Score	Expect	Identities	Gaps	Strand
1154 bits(1279)	0.0	641/642(99%)	0/642(0%)	Plus/Plus

```
Query  1     TAGGATCTCGGTTCTATTTTGTTGGTTTTCTGATCTGAGATAATGGTTAAGAGGGACAGA  60
Sbjct  761   ............................................................  820
Query  61    CGGGGGCATTCGTATCGCTTCGTGAGAGGTGAAATTCTTGGACCGTAGCGAGACGCCCGA  120
Sbjct  821   ............................................................  880
Query  121   CTGCGAAAGCATTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAA  180
Sbjct  881   ............................................................  940
Query  181   GGCGATCAGATACCGCCCTAGTTCTGACCGTAAACGATACCAACTAGCGTTCCGTCATCG  240
Sbjct  941   ............................................................  1000
Query  241   GTAAATATGCCTTGACGGGCAGCTTCCCGGAAACGAAAGTGTTTCGGTTCCGGGGGAAGT  300
Sbjct  1001  ............................................................  1060
Query  301   ATGGTTGCAAAGCTGAAACTTAAAGAGATTGACGGAAGGGCACCACCAGGAGTGGAGCCT  360
Sbjct  1061  ............................................................  1120
Query  361   GCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGAC  420
Sbjct  1121  ............................................................  1180
Query  421   AGATTGAGAGCTCTTTCTTGATTCGGTGGTTGGTGGCGCATGGCCGTTCGTGGTTCGTGG  480
Sbjct  1181  ............................................................  1240
Query  481   ATTGGTCTGTCAGGTTTATTCCGATAACGAGCGAGACTCTGACCTACTAAATAGTGTCTA  540
Sbjct  1241  ............................................................  1300
Query  541   GATTATGTTTGTCTTGACGACTTCTTAGAGGGACAAGCGGTGTTCAGCCGCATGAAGTTG  600
Sbjct  1301  ......T.....................................................  1360
Query  601   AGCAATAACAGGTCTGTGATGCCCTTAGATGTTCAGGGCTGC  642
Sbjct  1361  ..........................................  1402
```

3

Ascaridia galli **18Ssmall subunit ribosomal RNA gene, partial sequence ID: EF180058.1 Length: 1718 Number of Matches: 1 Range 1:761 to 1402**

GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1154 bits(1279)	0.0	641/642(99%)	0/642(0%)	Plus/Plus

```
Query  1     TAGGATCTCGGTTCTATTTTGTTGGTTTTCTGATCTGAGATAATGGTTAAGAGGGACAGA  60
Sbjct  761   ............................................................  820
Query  61    CGGGGGCATTCGTATCGCTTCGTGAGAGGTGAAATTCTTGGACCGTAGCGAGACGCCCGA  120
Sbjct  821   ............................................................  880
Query  121   CTGCGAAAGCATTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAA  180
Sbjct  881   ............................................................  940
Query  181   GGCGATCAGATACCGCCCTAGTTCTGACCGTAAACGATACCAACTAGCGTTCCGTCATCG  240
Sbjct  941   ............................................................  1000
Query  241   GTAAATATGCCTTGACGGGCAGCTTCCCGGAAACGAAAGTGTTTCGGTTCCGGGGGAAGT  300
Sbjct  1001  ............................................................  1060
Query  301   ATGGTTGCAAAGCTGAAACTTAAAGAGATTGACGGAAGGGCACCACCAGGAGTGGAGCCT  360
Sbjct  1061  ............................................................  1120
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Sbjct  1121  ............................................................  1180
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Sbjct  1181  ............................................................  1240
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Sbjct  1241  ............................................................  1300
Query  541   GATTATGTTTGTCTTGACGACTTCTTAGAGGGACAAGCGGTGTTCAGCCGCATGAAGTTG  600
Sbjct  1301  ......T.....................................................  1360
Query  601   AGCAATAACAGGTCTGTGATGCCCTTAGATGTTCAGGGCTGC  642
Sbjct  1361  ..........................................  1402
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4

Ascaridia galli 18Ssmall subunit ribosomal RNA gene, partial sequence ID: EF180058.1 Length: 1718 Number of Matches: 1 Range 1:761 to 1402

GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1154 bits(1279)	0.0	641/642(99%)	0/642(0%)	Plus/Plus

```
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Sbjct  821   ............................................................  880
Query  121   CTGCGAAAGCATTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAA  180
Sbjct  881   ............................................................  940
Query  181   GGCGATCAGATACCGCCCTAGTTCTGACCGTAAACGATACCAACTAGCGTTCCGTCATCG  240
Sbjct  941   ............................................................  1000
Query  241   GTAAATATGCCTTGACGGGCAGCTTCCCGGAAACGAAAGTGTTTCGGTTCCGGGGGAAGT  300
Sbjct  1001  ............................................................  1060
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Sbjct  1061  ............................................................  1120
Query  361   GCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGAC  420
Sbjct  1121  ............................................................  1180
Query  421   AGATTGAGAGCTCTTTCTTGATTCGGTGGTTGGTGGCGCATGGCCGTTCGTGGTTCGTGG  480
Sbjct  1181  ............................................................  1240
Query  481   ATTGGTCTGTCAGGTTTATTCCGATAACGAGCGAGACTCTGACCTACTAAATAGTGTCTA  540
Sbjct  1241  ............................................................  1300
Query  541   GATTATGTTTGTCTTGACGACTTCTTAGAGGGACAAGCGGTGTTCAGCCGCATGAAGTTG  600
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Query  601   AGCAATAACAGGTCTGTGATGCCCTTAGATGTTCAGGGCTGC  642
Sbjct  1361  ..........................................  1402
```

Ascaridia galli 18Ssmall subunit ribosomal RNA gene, partial sequence ID: EF180058.1 Length: 1718 Number of Matches: 1 Range 1:761to 1402

GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1141 bits(1264)	0.0	638/642(99%)	0/642(0%)	Plus/Plus

```
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Sbjct  761   ............................................................  820
Query  61    CGGGGGCATTCGTATCGCTTCGTGAGAGGTGAAATTCTTGGACCGTAGCGAGACGCCCGA  120
Sbjct  821   ............................................................  880
Query  121   CTGCGAAAGCATTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAA  180
Sbjct  881   ............................................................  940
Query  181   GGCGATCAGATACCGCCCTAGTTCTGACCGTAAACGATACCAACTAGCGTTCCGTCATCG  240
Sbjct  941   ............................................................  1000
Query  241   GTAAATATGCCTTGACGGGCAGCTTCCCGGAAACGAAAGTGTTTCGGTTCCGGGGGAAGT  300
Sbjct  1001  ............................................................  1060
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Sbjct  1061  ............................................................  1120
Query  361   GCGGCTTAATTTGACTCAACACGCGAAAACTCACCTGGCCCGGACACCGTGAGGATTGAC  420
Sbjct  1121  .......................G....................................  1180
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Sbjct  1181  ............................................................  1240
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Sbjct  1301  ..............................G.............................  1360
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Sbjct  1361  ................T.........................  1402
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6

***Ascaridia galli* 18Ssmall subunit ribosomal RNA gene, partial sequence ID: EF180058.1 Length: 1718 Number of Matches: 1 Range 1:761 to 1402**

GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1150 bits(1274)	0.0	640/642(99%)	0/642(0%)	Plus/Plus

```
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Sbjct  761   ............................................................  820
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Sbjct  821   ............................................................  880
Query  121   CTGCGAAAGCTTTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAA  180
Sbjct  881   ..........A.................................................  940
Query  181   GGCGATCAGATACCGCCCTAGTTCTGACCGTAAACGATACCAACTAGCGTTCCGTCATCG  240
Sbjct  941   ............................................................  1000
Query  241   GTAAATATGCCTTGACGGGCAGCTTCCCGGAAACGAAAGTGTTTCGGTTCCGGGGGAAGT  300
Sbjct  1001  ............................................................  1060
Query  301   ATGGTTGCAAAGCTGAAACTTAAAGAGATTGACGGAAGGGCACCACCAGGAGTGGAGCCT  360
Sbjct  1061  ............................................................  1120
Query  361   GCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGAC  420
Sbjct  1121  ............................................................  1180
Query  421   AGATTGAGAGCTCTTTCTTGATTCGGTGGTTGGTGGCGCATGGCCGTTCGTGGTTCGTGG  480
Sbjct  1181  ............................................................  1240
Query  481   ATTGGTCTGTCAGGTTTATTCCGATAACGAGCGAGACTCTGACCTACTAAATAGTGTCTA  540
Sbjct  1241  ............................................................  1300
Query  541   GATTATTTTTGTCTTGACGACTTCTTAGAGGGACAAGCGGTGTTCAGCCGCATGAAGTTG  600
Sbjct  1301  ............................................................  1360
Query  601   AGCAATAACAGGTCTGTGATGCCCTCAGATGTTCAGGGCTGC  642
Sbjct  1361  ........................T.................  1402
```

7

Ascaridia galli **18Ssmall subunit ribosomal RNA gene, partial sequence ID: EF180058.1 Length: 1718 Number of Matches: 1 Range 1:761 to 1402**

GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1159 bits(1284)	0.0	642/642(100%)	0/642(0%)	Plus/Plus

```
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Sbjct  761   ............................................................  820
Query  61    CGGGGGCATTCGTATCGCTTCGTGAGAGGTGAAATTCTTGGACCGTAGCGAGACGCCCGA  120
Sbjct  821   ............................................................  880
Query  121   CTGCGAAAGCATTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAA  180
Sbjct  881   ............................................................  940
Query  181   GGCGATCAGATACCGCCCTAGTTCTGACCGTAAACGATACCAACTAGCGTTCCGTCATCG  240
Sbjct  941   ............................................................  1000
Query  241   GTAAATATGCCTTGACGGGCAGCTTCCCGGAAACGAAAGTGTTTCGGTTCCGGGGGAAGT  300
Sbjct  1001  ............................................................  1060
Query  301   ATGGTTGCAAAGCTGAAACTTAAAGAGATTGACGGAAGGGCACCACCAGGAGTGGAGCCT  360
Sbjct  1061  ............................................................  1120
Query  361   GCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGAC  420
Sbjct  1121  ............................................................  1180
Query  421   AGATTGAGAGCTCTTTCTTGATTCGGTGGTTGGTGGCGCATGGCCGTTCGTGGTTCGTGG  480
Sbjct  1181  ............................................................  1240
Query  481   ATTGGTCTGTCAGGTTTATTCCGATAACGAGCGAGACTCTGACCTACTAAATAGTGTCTA  540
Sbjct  1241  ............................................................  1300
Query  541   GATTATTTTTGTCTTGACGACTTCTTAGAGGGACAAGCGGTGTTCAGCCGCATGAAGTTG  600
Sbjct  1301  ............................................................  1360
Query  601   AGCAATAACAGGTCTGTGATGCCCTTAGATGTTCAGGGCTGC  642
Sbjct  1361  ..........................................  1402
```

8

Ascaridia galli 18Ssmall subunit ribosomal RNA gene, partial sequence ID: EF180058.1 Length: 1718 Number of Matches: 1 Range 1:761 to 1402

GenBank **Graphics**

Score	Expect	Identities	Gaps	Strand
1136 bits(1259)	0.0	637/642 (99%)	0/642(0%)	Plus/Plus

```
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Sbjct  821   ..........................G.................................  880
Query  121   CTGCGAAAGCTTTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAA  180
Sbjct  881   ..........A.................................................  940
Query  181   GGCGATCAGATACCGCCCTAGTTCTGACCGTAAACGATACCCACTAGCGTTCCGTCATCG  240
Sbjct  941   ........................................A...................  1000
Query  241   GTAAATATGCCTTGACGGGCAGCTTCCCGGAAACGAAAGTGTTTCGGTTCCGGGGGAAGT  300
Sbjct  1001  ............................................................  1060
Query  301   ATGGTTGCAAAGCTGAAACTTAAAGAGATTGACGGAAGGGCACCACCAGGAGTGGAGCCT  360
Sbjct  1061  ............................................................  1120
Query  361   GCGGCTTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGAC  420
Sbjct  1121  ............................................................  1180
Query  421   AGATTGAGAGCTCTTTCTTGATTCGGTGGTTGGTGGCGCATGGCCGTTCGTGGTTCGTGG  480
Sbjct  1181  ............................................................  1240
Query  481   ATTGGTCTGTCAGGTTTATTCCGATAACGAGCGAGACTCTGACCTACTAAATAGTGTCTA  540
Sbjct  1241  ............................................................  1300
Query  541   GATTATTTTTGTCTTGACGACTTCTTAGAGCGACAAGCGGTGTTCAGCCGCATGAAGTTG  600
Sbjct  1301  .............................G..............................  1360
Query  601   AGCAATAACAGGTCTGTGATGCCCTCAGATGTTCAGGGCTGC  642
Sbjct  1361  .........................T................  1402
```

9

Ascaridia galli 18Ssmall subunit ribosomal RNA gene, partial sequence ID: EF180058.1 Length: 1718 Number of Matches: 1 Range 1:775 to 1402

GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1129 bits(1251)	0.0	627/628(99%)	0/628(0%)	Plus/Plus

```
Query  4     TATTTTGTTGGTTTTCTGATCTGAGATAATGGTTAAGAGGGACAGACGGGGGCATTCGTA  63
Sbjct  775   ............................................................  834
Query  64    TCGCTTCGTGAGAGGTGAAATTCTTGGACCGTAGCGAGACGCCCGACTGCGAAAGCATTT  123
Sbjct  835   ............................................................  894
Query  124   GCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGGCGATCAGATACC  183
Sbjct  895   ............................................................  954
Query  184   GCCCTAGTTCTGACCGTAAACGATACCAACTAGCGTTCCGTCATCGGTAAATATGCCTTG  243
Sbjct  955   ............................................................  1014
Query  244   ACGGGCAGCTTCCCGGAAACGAAAGTGTTTCGGTTCCGGGGGAAGTATGGTTGCAAAGCT  303
Sbjct  1015  ............................................................  1074
Query  304   GAAACTTAAAGAGATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGA  363
Sbjct  1075  ............................................................  1134
Query  364   CTCAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGACAGATTGAGAGCTCT  423
Sbjct  1135  ............................................................  1194
Query  424   TTCTTGATTCGGTGGTTGGTGGCGCATGGCCGTTCGTGGTTCGTGGATTGGTCTGTCAGG  483
Sbjct  1195  ............................................................  1254
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Sbjct  1255  ....................................................T.......  1314
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Sbjct  1315  ............................................................  1374
Query  604   TGTGATGCCCTTAGATGTTCAGGGCTGC  631
Sbjct  1375  ............................  1402
```

10

Ascaridia galli 18Ssmall subunit ribosomal RNA gene, partial sequence ID: EF180058.1 Length: 1718 Number of Matches: 1 Range 1:766 to 1402

GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1145 bits(1269)	0.0	636/637(99%)	0/637(0%)	Plus/Plus

```
Query  1     TCTCGGTTCTATTTTGTTGGTTTTCTGATCTGAGATAATGGTTAAGAGGGACAGACGGGG  60
Sbjct  766   ............................................................  825
Query  61    GCATTCGTATCGCTTCGTGAGAGGTGAAATTCTTGGACCGTAGCGAGACGCCCGACTGCG  120
Sbjct  826   ............................................................  885
Query  121   AAAGCATTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGGCGA  180
Sbjct  886   ............................................................  945
Query  181   TCAGATACCGCCCTAGTTCTGACCGTAAACGATACCAACTAGCGTTCCGTCATCGGTAAA  240
Sbjct  946   ............................................................  1005
Query  241   TATGCCTTGACGGGCAGCTTCCCGGAAACGAAAGTGTTTCGGTTCCGGGGGAAGTATGGT  300
Sbjct  1006  ............................................................  1065
Query  301   TGCAAAGCTGAAACTTAAAGAGATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGC  360
Sbjct  1066  ............................................................  1125
Query  361   TTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACCGTGAGGATTGACAGATT  420
Sbjct  1126  ............................................................  1185
Query  421   GAGAGCTCTTTCTTGATTCGGTGGTTGGTGGCGCATGGCCGTTCGTGGTTCGTGGATTGG  480
Sbjct  1186  ............................................................  1245
Query  481   TCTGTCAGGTTTATTCCGATAACGAGCGAGACTCTGACCTACTAAATAGTGTCTAGATTA  540
Sbjct  1246  ............................................................  1305
Query  541   TGTTTGTCTTGACGACTTCTTAGAGGGACAAGCGGTGTTCAGCCGCATGAAGTTGAGCAA  600
Sbjct  1306  .T..........................................................  1365
Query  601   TAACAGGTCTGTGATGCCCTTAGATGTTCAGGGCTGC  637
Sbjct  1366  .....................................  1402
```

Appendix 3:

1

Ascaridia galli strain ZaAm-1-Diayala small sub unit ribosomal RNA gene, partial sequence, Gen Bank: MW732174.1

FASTA Graphics

Go to:

```
LOCUS       MW732174                 649 bp    DNA     linear   INV 17-MAR-2021
DEFINITION  Ascaridia galli strain ZaAm-1-Diayala small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION   MW732174
VERSION     MW732174.1
KEYWORDS    .
SOURCE      Ascaridia galli
  ORGANISM  Ascaridia galli
            Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Rhabditida;
            Spirurina; Ascaridomorpha; Heterakoidea; Ascaridiidae; Ascaridia.
REFERENCE   1  (bases 1 to 649)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Ascaridia galli 18S small subunit ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 649)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-MAR-2021) Department of Parasitology, Diayala
            university, veterinary medicine;Baghdad university, veterinary
            medicine, iraq, Diayala 00964, Iraq
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            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /host="domestic chickens"
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                     /clone="ZaAm-1"
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      121 agcatttgcc aagaatgtct tcattaatca agaacgaaag tcagaggttc gaaggcgatc
      181 agataccgcc ctagttctga ccgtaaacga taccaactag cgttccgtca tcggtaaata
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      421 gagctctttc ttgattcggt ggttggtggc gcatggccgt tcgtggttcg tggattggtc
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      541 tttgtcttga cgacttctta gagggacaag cggtgttcag ccgcatgaag ttgagcaata
      601 acaggtctgt gatgccctta gatgttcagg gctgccgcgc gctacactg
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2

Ascaridia galli strain ZaAm-1-Diayala small sub unit ribosomal RNA gene, partial sequence, Gen Bank: MW732175.1

FASTA Graphics

Go to:

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LOCUS       MW732175                 642 bp    DNA     linear   INV 17-MAR-2021
DEFINITION  Ascaridia galli strain ZaAm-2-Diayala small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION   MW732175
VERSION     MW732175.1
KEYWORDS    .
SOURCE      Ascaridia galli
  ORGANISM  Ascaridia galli
            Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Rhabditida;
            Spirurina; Ascaridomorpha; Heterakoidea; Ascaridiidae; Ascaridia.
REFERENCE   1  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Ascaridia galli 18S small subunit ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-MAR-2021) Department of Parasitology, Diayala
            university, veterinary medicine;Baghdad university, veterinary
            medicine, iraq, Diayala 00964, Iraq
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      541 gattatgttt gtcttgacga cttcttagag ggacaagcgg tgttcagccg catgaagttg
      601 agcaataaca ggtctgtgat gcccttagat gttcagggct gc
//
```

3

Ascaridia galli strain ZaAm-1-Diayala small sub unit ribosomal RNA gene, partial sequence, Gen Bank: MW732176.1

FASTA Graphics

Go to:

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DEFINITION  Ascaridia galli strain ZaAm-3-Diayala small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION   MW732176
VERSION     MW732176.1
KEYWORDS    .
SOURCE      Ascaridia galli
  ORGANISM  Ascaridia galli
            Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Rhabditida;
            Spirurina; Ascaridomorpha; Heterakoidea; Ascaridiidae; Ascaridia.
REFERENCE   1  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Ascaridia galli 18S small subunit ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-MAR-2021) Department of Parasitology, Diayala
            university, veterinary medicine;Baghdad university, veterinary
            medicine, iraq, Diayala 00964, Iraq
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            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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      481 attggtctgt caggtttatt ccgataacga gcgagactct gacctactaa atagtgtcta
      541 gattatgttt gtcttgacga cttcttagag ggacaagcgg tgttcagccg catgaagttg
      601 agcaataaca ggtctgtgat gcccttagat gttcagggct gc
//
```

.

4

Ascaridia galli strain ZaAm-1-Diayala small sub unit ribosomal RNA gene, partial sequence, Gen Bank: MW732177.1

FASTA **Graphics**

Go to:

```
LOCUS       MW732177                 642 bp    DNA     linear   INV 17-MAR-2021
DEFINITION  Ascaridia galli strain ZaAm-4-Diayala small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION   MW732177
VERSION     MW732177.1
KEYWORDS    .
SOURCE      Ascaridia galli
  ORGANISM  Ascaridia galli
            Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Rhabditida;
            Spirurina; Ascaridomorpha; Heterakoidea; Ascaridiidae; Ascaridia.
REFERENCE   1  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Ascaridia galli 18S small subunit ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-MAR-2021) Department of Parasitology, Diayala
            university, veterinary medicine;Baghdad university, veterinary
            medicine, iraq, Diayala 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /clone="ZaAm-4"
                     /country="Iraq"
                     /collection_date="2020"
                     /collected_by="Zainab Fadhil Rahman, Amer murhum AL-amery"
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                     /product="small subunit ribosomal RNA"
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      121 ctgcgaaagc atttgccaag aatgtcttca ttaatcaaga acgaaagtca gaggttcgaa
      181 ggcgatcaga taccgcccta gttctgaccg taaacgatac caactagcgt tccgtcatcg
      241 gtaaatatgc cttgacgggc agcttcccgg aaacgaaagt gtttcggttc cgggggaagt
      301 atggttgcaa agctgaaact taaagagatt gacggaaggg caccaccagg agtggagcct
      361 gcggcttaat ttgactcaac acgggaaaac tcacctggcc cggacaccgt gaggattgac
      421 agattgagag ctctttcttg attcggtggt tggtggcgca tggccgttcg tggttcgtgg
      481 attggtctgt caggtttatt ccgataacga gcgagactct gacctactaa atagtgtcta
      541 gattatgttt gtcttgacga cttcttagag ggacaagcgg tgttcagccg catgaagttg
      601 agcaataaca ggtctgtgat gcccttagat gttcagggct gc
//
```

5

Ascaridia galli strain ZaAm-1-Diayala small sub unit ribosomal RNA gene, partial sequence, Gen Bank: MW732178.1

FASTA **Graphics**

Go to:

```
LOCUS       MW732178                 642 bp    DNA     linear   INV 17-MAR-2021
DEFINITION  Ascaridia galli strain ZaAm-5-Diayala small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION   MW732178
VERSION     MW732178.1
KEYWORDS    .
SOURCE      Ascaridia galli
  ORGANISM  Ascaridia galli
            Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Rhabditida;
            Spirurina; Ascaridomorpha; Heterakoidea; Ascaridiidae; Ascaridia.
REFERENCE   1  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Ascaridia galli 18S small subunit ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-MAR-2021) Department of Parasitology, Diayala
            university, veterinary medicine;Baghdad university, veterinary
            medicine, iraq, Diayala 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /country="Iraq"
                     /collection_date="2020"
                     /collected_by="Zainab Fadhil Rahman, Amer murhum AL-amery"
     rRNA            <1..>642
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      121 ctgcgaaagc atttgccaag aatgtcttca ttaatcaaga acgaaagtca gaggttcgaa
      181 ggcgatcaga taccgcccta gttctgaccg taaacgatac caactagcgt tccgtcatcg
      241 gtaaatatgc cttgacgggc agcttcccgg aaacgaaagt gtttcggttc cgggggaagt
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      541 gattattttt gtcttgacga cttcttagag cgacaagcgg tgttcagccg catgaagttg
      601 agcaataaca ggtctgagat gcccttagat gttcagggct gc
//
```

6

Ascaridia galli strain ZaAm-1-Diayala small sub unit ribosomal RNA gene, partial sequence, Gen Bank: MW732179.1

FASTA Graphics

Go to:

```
LOCUS       MW732179                 642 bp    DNA     linear   INV 17-MAR-2021
DEFINITION  Ascaridia galli strain ZaAm-6-Diayala small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION   MW732179
VERSION     MW732179.1
KEYWORDS    .
SOURCE      Ascaridia galli
  ORGANISM  Ascaridia galli
            Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Rhabditida;
            Spirurina; Ascaridomorpha; Heterakoidea; Ascaridiidae; Ascaridia.
REFERENCE   1  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Ascaridia galli 18S small subunit ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-MAR-2021) Department of Parasitology, Diayala
            university, veterinary medicine;Baghdad university, veterinary
            medicine, iraq, Diayala 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /country="Iraq"
                     /collection_date="2020"
                     /collected_by="Zainab Fadhil Rahman, Amer murhum AL-amery"
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                     /product="small subunit ribosomal RNA"
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      121 ctgcgaaagc ttttgccaag aatgtcttca ttaatcaaga acgaaagtca gaggttcgaa
      181 ggcgatcaga taccgcccta gttctgaccg taaacgatac caactagcgt tccgtcatcg
      241 gtaaatatgc cttgacgggc agcttcccgg aaacgaaagt gtttcggttc cgggggaagt
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      421 agattgagag ctctttcttg attcggtggt tggtggcgca tggccgttcg tggttcgtgg
      481 attggtctgt caggtttatt ccgataacga gcgagactct gacctactaa atagtgtcta
      541 gattattttt gtcttgacga cttcttagag ggacaagcgg tgttcagccg catgaagttg
      601 agcaataaca ggtctgtgat gccctcagat gttcagggct gc
//
```

7

Ascaridia galli strain ZaAm-1-Diayala small sub unit ribosomal RNA gene, partial sequence, Gen Bank: MW732180.1

FASTA Graphics

Go to:

```
LOCUS       MW732180                 642 bp    DNA     linear   INV 17-MAR-2021
DEFINITION  Ascaridia galli strain ZaAm-7-Diayala small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION   MW732180
VERSION     MW732180.1
KEYWORDS    .
SOURCE      Ascaridia galli
  ORGANISM  Ascaridia galli
            Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Rhabditida;
            Spirurina; Ascaridomorpha; Heterakoidea; Ascarididae; Ascaridia.
REFERENCE   1  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Ascaridia galli 18S small subunit ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-MAR-2021) Department of Parasitology, Diayala
            university, veterinary medicine;Baghdad university, veterinary
            medicine, iraq, Diayala 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /clone="ZaAm-7"
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                     /collection_date="2020"
                     /collected_by="Zainab Fadhil Rahman, Amer murhum AL-amery"
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      481 attggtctgt caggtttatt ccgataacga gcgagactct gacctactaa atagtgtcta
      541 gattattttt gtcttgacga cttcttagag ggacaagcgg tgttcagccg catgaagttg
      601 agcaataaca ggtctgtgat gcccttagat gttcagggct gc
//
```

8

Ascaridia galli strain ZaAm-1-Diayala small sub unit ribosomal RNA gene, partial sequence, Gen Bank: MW732181.1

FASTA Graphics

```
LOCUS       MW732181                 642 bp    DNA     linear   INV 17-MAR-2021
DEFINITION  Ascaridia galli strain ZaAm-8-Diayala small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION   MW732181
VERSION     MW732181.1
KEYWORDS    .
SOURCE      Ascaridia galli
  ORGANISM  Ascaridia galli
            Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Rhabditida;
            Spirurina; Ascaridomorpha; Heterakoidea; Ascaridiidae; Ascaridia.
REFERENCE   1  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Ascaridia galli 18S small subunit ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 642)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-MAR-2021) Department of Parasitology, Diayala
            university, veterinary medicine;Baghdad university, veterinary
            medicine, iraq, Diayala 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /clone="ZaAm-8"
                     /country="Iraq"
                     /collection_date="2020"
                     /collected_by="Zainab Fadhil Rahman, Amer murhum AL-amery"
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      121 ctgcgaaagc ttttgccaag aatgtcttca ttaatcaaga acgaaagtca gaggttcgaa
      181 ggcgatcaga taccgcccta gttctgaccg taaacgatac ccactagcgt tccgtcatcg
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      301 atggttgcaa agctgaaact taaagagatt gacggaaggg caccaccagg agtggagcct
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      481 attggtctgt caggtttatt ccgataacga gcgagactct gacctactaa atagtgtcta
      541 gattattttt gtcttgacga cttcttagag cgacaagcgg tgttcagccg catgaagttg
      601 agcaataaca ggtctgtgat gccctcagat gttcagggct gc
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```

9

***Ascaridia galli* strain ZaAm-1-Diayala small sub unit ribosomal RNA gene, partial sequence, Gen Bank: MW732182.1**

FASTA Graphics

Go to:

```
LOCUS       MW732182                 628 bp    DNA     linear   INV 17-MAR-2021
DEFINITION  Ascaridia galli strain ZaAm-9-Diayala small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION   MW732182
VERSION     MW732182.1
KEYWORDS    .
SOURCE      Ascaridia galli
  ORGANISM  Ascaridia galli
            Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Rhabditida;
            Spirurina; Ascaridomorpha; Heterakoidea; Ascaridiidae; Ascaridia.
REFERENCE   1  (bases 1 to 628)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Ascaridia galli 18S small subunit ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 628)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-MAR-2021) Department of Parasitology, Diayala
            university, veterinary medicine;Baghdad university, veterinary
            medicine, iraq, Diayala 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /clone="ZaAm-9"
                     /country="Iraq"
                     /collection_date="2020"
                     /collected_by="Zainab Fadhil Rahman, Amer murhum AL-amery"
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                     /product="small subunit ribosomal RNA"
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//
```

10

Ascaridia galli strain ZaAm-1-Diayala small sub unit ribosomal RNA gene, partial sequence, Gen Bank: MW732183.1

FASTA Graphics

Go to:

```
LOCUS       MW732183                 637 bp    DNA     linear   INV 17-MAR-2021
DEFINITION  Ascaridia galli strain ZaAm-10-Diayala small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION   MW732183
VERSION     MW732183.1
KEYWORDS    .
SOURCE      Ascaridia galli
  ORGANISM  Ascaridia galli
            Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Rhabditida;
            Spirurina; Ascaridomorpha; Heterakoidea; Ascarididae; Ascaridia.
REFERENCE   1  (bases 1 to 637)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Ascaridia galli 18S small subunit ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 637)
  AUTHORS   Zainab,F.R. and AL-Amery,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-MAR-2021) Department of Parasitology, Diayala
            university, veterinary medicine;Baghdad university, veterinary
            medicine, iraq, Diayala 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /isolation_source="small intestine"
                     /host="domestic chickens"
                     /db_xref="taxon:46685"
                     /clone="ZaAm-10"
                     /country="Iraq"
                     /collection_date="2020"
                     /collected_by="Zainab Fadhil Rahman, Amer murhum AL-amery"
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//
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الخلاصة

اجريت الدراسة خلال الفترة من الاول من تشرين الاول 2020 ولغاية الاول من شهر اذار 2021 ، حيث تم فحص 120 من الدجاج المحلي في محافظة ديالى للكشف عن مدى أنشار الطفيلي الاسكاريديا , الفحص الجزيئي و دراسة الآفات في الدجاج المصاب, وكانت نسبة الاصابة 41,66%.

اظهرت الدراسة وجود فروق معنوية بنسبة الاصابة وبمستوى ($P \leq 0.05$) بين الذكور والاناث ، حيث اظهرت الاناث نسبة الاصابة اعلى 50% (33/66) مقارنة مع الذكور31,48 % (17/54) ، واظهرت نتائج الدراسة عدم وجود فروق معنوية بين الاعمار الكبيره بنسبة الاصابة 50% (24/48) والاعمار الصغيرة بنسبة الاصابة 36.11% (26/72) . فروق معنوية عالية في الدجاج المذبوح بين الاشهر وبمستوى ($P \leq 0.01$) حيث ,سجل شهرتشرين الاول اعلى نسبة اصابة (55%),في حين كان اقل نسبة اصابة (30%) في شهركانون الاول.

واظهرت الدراسة المرضية النسجية لامعاء الدقيقة والكبد بواسطة الميكروتوم التقليدي فقدان الظهارة ,ضمور الزغابات ,تنخر في غدد المخاطية ,تفاعلات الخلوية بالاخص خلايا وحيدات النواة وخلايا الالتهابية واظهرت الكبد ورم الحبيبي ,مناطق نزفية في نسيجه وكذلك تنخر في انسجة قناة الصفراء , وخثرة في الوريد البابي الكبدي , مع تسجيل الافات العيانية والتي شملت وجود الطفيلي داخل تجويف الامعاء, وجود بقع نزفية , تثخن جدار الامعاء ,اضافة الى شحوب واحتقان الكبد.

هدفت الدراسة تحديد نوع اسكارس على المستوى الجزيئي والتحري عن وجود جين 18S rRNA حيث تم استخلاص الحامض النووي DNA من الديدان البالغة المعزولة من الامعاء الدقيقة للدجاج المحلي المصاب طبيعيا باستعمال عدة باستخلاص DNA. تم تضخيم الجين باستخدام بادئات خاصة للجين بعملية بلمرة.

اعطى التفاعل البلمره نتائج ايجابية على جين DNA عند 720bp,حيث تم ارسال عشر عينات موجبة لغرض تسلسل القواعد النتروجينية ورسم الشجرة الوراثية , تم تسجيل التسلسل بالبنك الجيني في المركز الوطني للمعلومات التقنية الحيوية تحت رقم انضمام (MW732174.1), (MW732175.1), (MW732176.1), (MW732177.1), (MW732178.1), (MW732179.1), (MW732180.1), (MW732181.1), (MW732182.1) و (MW732183.1) حيث اظهرت تطابق بنسبة (100% -99%) مع العزلة الامريكية

الخـــلاصـــة **ب**

USA والمسجلة تحت الرقم انضمام (EF180058.1) ومع عزلات العراقية تحت رقم انضمام (MK918847.1

MK918636.1, MK918635.1, MK919081.1,) اعطت تطابق (99%).

جمهورية الـعـراق

وزارة التعليم العـالي والبـحث العلمي

جـــامـعـة بـغـــداد

كليـــة الـطـب الـبـيـطـري

فـــرع الـــطفيليـــات

الكشف الطفيلي والجزيئي لأنواع الاسكارديا في الدجاج المحلي في محافظة ديالى ، العراق

رســالــة

مُقـــدمة إلى مجلـس كـــليـــــة الـــطب البـيـطري – جامعة بـغداد

وهـي جـزء مـــن متـطـلبـــــات نيل درجـــة الـــمـاجستـيــر

في علوم الطب البيطري / الــطـفـيـلـيـــــات

من قبل

زيـنـب فـــاضـل رحـمـن

بإشـــــراف

الاستـــاذ الـــدكـتـــور

عامــــر مــــرحم الـــعـــامـــري

٢٠٢٢ م　　　　١٤٤٣ هـ