

Benghazi University Faculty of Science Department of Zoology

INCIDENCE OF COCCIDIA (*EIMERIA* SPP.)INFECTION IN BROILER CHICKEN FARMS OF GHOT- ELSULTAN POULTRY AND DAIRY PROJECT

A thesis submitted in partial fulfillment of the requirements for

the Degree of Master of Science in Zoology

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DEDICATED

To the soul of my Father,grandmother, and my friend Fatma Al-Kotrany . To beloved mother . To my brother and sisters.

بسم الله الرحمن الرحيم



((سورة الاسراء))



جامعية بنييغازي كليية العييلوم

معدلي حدوث إلا صابة بداي الاكريات (الكوكسيديا) (Eimeria spp.) في مزايع بداري التسمين بمشروع غوط السلطان للدواجن والأبقار أطروحة مقدمة كجزء من متطلبات درجة الإجازة العليا (الماجستير) في علم الحيوان مقدمه من الطالبة : فريحه محمود لامين العمر وين

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1. Introduction

Poultary production is increasing rapidlypartly due to the low establishment cost. Its tasty meat contain high content of valuable protein low content of fat. Since1970 the meat and egg production have show increased in the size, faster than other animal food production industries in the worldwide. At present about 30 % of the world animal protein for human consumption comes from poultary production(Kinung'hi *et al*., 2004 and Windhorst, 2006).

Intestinal parasites are a major stress factor leading to malnutrition lowered performance reduced production efficiency of livestock and poultary .Anumber of these are known to be highly pathogenic causing not only heavy production losses,but also death.The coccidia are one of the most important groups of protozoa that affect many animals and avain species . Each species of coccidia is host-specific and does not infect a wide variety of animals. Infection by these protozoa parasites lead to sever intestinal disease known as coccidiosis (Yun *et al.*, 2000 and Lilic *et al* .,2009).

Coccidiosis is caused by obligate ,intercellular protozoa parasites of the phylum Apicomplexa,class sporozoa,familyEimeriidae,and genus*Eimeria*.The infection process occurred rapidly (4-7 days) whereas the organisms invade the lining of the intestine and produce tissue damage as the undergo reproduction. The results of infection lead to reduction of body weight, reduced feed efficiency,and often morbidity and mortality,in addition to increased susceptibility to other pathogens.Developmental stages of *Eimeria*

alternate between the external environment and endogenously within the single host. After an outbreak of a specific species of coccidia, the flock develops aresistance to the exposed coccidian species but remain susceptibility to other infective specie (Xiaokai *et al.*, 2009 and Shareef, 2010).

Avian coccidiosis is the major parasitic disease of the intensive poultary industry.Worldwide with economic burden estimated to cost the industry greater than \$800 million in annual losses. These estimates include the costs of prophylactic in feed medication for broilers, and losses due to mortality, morbidity and poor feed conversions of birds that survive outbreaks (Williams , 1999 and Allen and Fetter, 2002).

About1800 *Eimeria* species affect the intestinal mucosa of different animals and birds. Domestic chickens are considered susceptible to nine species of *Eimeria*. These are *E.acervulina*, *E.maxima*, *E.necatrix*, *E mivatti*, *E. brunette*, *E.tenella*, *E.praecox*, *E.mitts*, and *E.hagani*. Each of *Eimeria* species has its characteristic prevalence , specific parts of the intestinal infection , pathogenicity, and immunogenicity. Generally, three of them (*E.acervulina*, *E. tenell* and *E.maxima*) are the most pathogenic, and commonly recognized in broiler farms (Titilincu *et al*., 2007 and Haug *et al*., 2008).

Coccidia are almost universally present in poultary–raising operation, but clinical disease occur only after ingestion of relatively large number of sporulated oocysts by susceptible birds. The source of the infection are the infected birds. Whereas the disease can spread bydirect and indirect contact with the droppings of infected birds. Both clinically infected and recovered birds shed oocysts in their dropping, which contaminate feed, water, dust, litter ,people, rodent, wild birds, soil as well as equipment and insects. Coccidiosis usually occurs in growing birds and young adults. It is seldom seen in birds under three weeks or in mature birds. Birds that have coccidiosis often display a clinical characteristic include that are pale, droopy, tend to huddle, consume less feed and water, have diarrhea, and may become emaciated and dehydrated. (Chapman, 2003 and Badran and Lukesova, 2006).

In Libya ,the broiler industry has been developing rapidly in recent years. The broiler chickens are mostly reared in deep- litter system and coccidiosis has become a serious problems. Although coccidiosis is a disease known since 130 years, and in spite of continuous use of anticoccidial as food additives. It remains the most economical important parasite affecting poultary meat production. If reared under intensive production system. It remains a significant disease topoultary industry not only in Ghot EL-Sultan sector ,but also worldwide (Ziomko *et al.*, 2005).

Objectives

Theaim of thep resent study is to investigate the incidence and identification of coccidian (*Eimeria* spp.) infection in the broiler chicken farms in chosen case study area of Ghot EL-Sultan and dairy project.

2. Review of Literature

2.1 .Poultry production :

Ruff(1999) indicate that the poultry are kept in backyards or commercial production system in most areas of the world. Poultry products are one of most important protein source. They are low fat and low price for human consumption compared to a number of other livestock animals. The chicken is believed to have been domesticated nearly 500 years ago from wild birds in southeast Asia .

In the last few years the poultary industry especially the chicken meat represent 80 % of the whole production of meat that originates from birds. The total number of poultary in the world has been estimated by the Food and AgricultureOrganization(FAO)of the United Nations, to be of the order of 14.718 million, with 1.125 million distributed throughout the African continent, 1.520 million in southAmerica ,60752 million in Asia ,93 million in Oceania, 3.384 million in North America and 1.844 million in Europe (Permin and Hansen, 1998 and Lilic *et al.*, 2009).

2.2. Coccidia :

McDougald and Reid,(1997) find that coccidian is the most important parasites of poultary in distribution. Economic losses,caused by groups of the protozoan *Eimeria* species are enormous.Coccidian infection are the largest group of Apicomplexan organisms, and belong to the family of Eimeriidiae.coccidian parasites, which include the genera *Toxoplasma*, *Neospora,Hammondia,Isospora,Sarcocystis* and *Eimeria*, amongst others, share many features. All a picomplexan are obligate intercellular parasites, and theirlife cycle includes asexual invasive stages (sporozoitesand merozoites)that contain the specific group of organelles found in the anterior and give rise to name A picomplexa.They are responsible for serious human and animals diseases such as malaria, toxoplasmosis, and cryptosporidiosis .In additional defining feature of the coccidia is the oocyst. Historically the structure of the sporulated oocyst, especially the number of sporocysts and sporozoite are used as a major characteristic to differentiate genera of coccidia(Augustine,2001and Ferguson, 2002).

Coccidia are a highly successful parasites and found in most animal species worldwide .They comprise a large of obligate intracellular parasites commonlyfound in all classes of vertebrate hosts, and sort of invertebrates.The main reason for this widespread occurrence of coccidian is their reproductive ability within7-10 days of ingestion. Coccidia have a genetically fixed, self-limiting life cycle.Therefore, the severity of each coccidiosis is positively correlated with the number of infective oocysts ingested. Each oocyst may give rise to100,000 of infective oocysts in the feces. Coccidiosis become important as a disease when animals are reared under intensive rearing of chickens conditions. It induces the increase of infective oocysts in the environment (Tyzzer, 1929 ; Idris *et al.*, 1997, and Ruff, 1999).

2.3. *Eimeria* :

Most coccidia in poultary belongs to the genus *Eimeria*. They are highly host specific. They are considered as the largest genus of A picomplexan parasites that includes various species responsible for the poultry disease coccidiosis.

The genus is named for the German ZoologistTheodor Eimer.The oocyst of *Eimeria* species(*Eimeria stiedai*) is one of the first protists recognized by Antoni van Leeuwenhoek in rabit bile in 1674(Levine ,1988). *Eimeria* parasites have ahomoxenous life cycle that develops in epithelial cells of intestine , sporoblast freed in intestine become oocyst that contains four sporocysts ,each with two sporozoites . *Eimeria* species seem to be limited to specific zones within that system, specific cells within the zone,and specific locations within those cells, causing tissue damage. It results in blood loss, dehydration, malabsorpation, and increased susceptibility to other pathogens (McDougald and Reid,1991and McDougald, 2003).

Up to six species are a shown to occur simultaneously in one farm. Often clinical disease is caused by one or all nine of *Eimeria* species infecting chicken. Each species differ in their location in the gut, a characteristic degree of pathogenicity ,lesions ,and produce species- specific host immunity. *E.acervulina* and *E.mivatti* penetrate and cause lesions in the upper part of the small intestine(duodenum), *E. maxima* and *E. necatrix* that cause lesions in the mid – gut (jejunum), and *E.tenella* and *E.brunette* do cause lesions

in the lower gut(ceca and large intestine). The disease resulting in severe mucosal damage, adverse effects on the growth of infected birds and sometimes even death (Witlock and Ruff, 1977 and Shirely, 1995) (Fig.1).

Adrian *et al.*(2007) indicate that *Eimeria* are of world-wide distribution. They invade the cells of the intestine producing enteritis, diarrhoea and mortality(30- 50%) in acute forms. The bird develops a disability to absorb sugars, amino acids, vitamins, fats and minerals through the disruption of the integrity of the intestinal mucosae.



Figure (1): Diagramatic representation of the location of 8 species of poultary coccidia : a. *E.acervulina*; b. *E.brunetti*; c. *E.maxima*; d. *E.mivatti*; e. *Emitis*; f. *E.necatrix*; g. *E.praecox* and h. *E.* tenella .(Source : Adapted from Long and Reid, 1982).

2.4. Scientific Classification :

The followings is the conventional classification:

Kingdom : Protista.

Sub-kingdom: Protozoa

Phylum: Apicomplexa

Class : Sporozoea

Subclass : Coccidian

Order: Eucoccdiorida

Suborder: Eimeriina

Family : Eimeriidae

Genus: Eimeria

Species: E.tenella (Railliet and Lucet, 1891); E.acervulina; E.mittis and E.maxima (Tyzzer, 1929); E.necatrix and E.praecox (Johanson, 1930) ; E.hagani(Levine, 1938); E.brunetti (Levin e, 1942) and E.mavatti

(Edgar and Siebold, 1964).

2.5. Life Cycle of Eimeria Species :

Fantham(1910), is the first who described the life cycle of a coccidian parasite in birds. Atypical life cycle of an *Eimeria* sp. is illustrated in Figure (2).

Eimeria spp. have monoxenous life cycles, including development of the asexual and sexual stages which takes place in a single host(Fayer ,1980). The life cycles of typical *Eimeria spp*.can be divided be into three phases of development: merogony, gamegony, and sporogony (Hammond ,1973). The Eimerian life- cycle is initiated when a bird has ingested a sporulated oocyst through fecal- oral route (Fayer ,1980). Excystations occurs when the grinding action of the gizzard releases the sporocysts from the oocyst, while enzymatic action of the upper intestinal tract releases the sporozoites from the sporocysts. Sporozoites travel to a species-specific site of infection and actively penetrate enterocyste. Inside the host cell, the sporozoite transforms within 12 to 48 hours to a feeding stage called a trophozoite. The trophozoite begins to enlarge, and pass through nuclear division known as schizogony (merogony). Merogony is the asexual multiple phase of Eimeria spp. It is initiated when several mitotic nuclear divisions occur. It gets completed when elongated merozoites are released from the surface of the meront by multiple fission .



Figure (2): The Life cycle of *Eimeria spp.* in chicken .

(Source : Modified after Mehlhorn and Piekarsi, 1995).

Merozoites lyses out of the original infected intestinal epithelial cells to infect new epithelial cells completing a second cycle of merogony (Innes and Vermeulen, 2006). Some or all may go through a third schizogonous cycle, depending on the *Eimeria* species. The predetermined number of cycles ranges between two and four. Merozoites of the last cycle of merogony enter a new intestinal epithelial cells and initiate gametogony, the sexual phase of the life cycle(Current et al., 1990 and McDonald and Shirley, 2009). Initiate the sexual reproduction of the endogenous cycle (gamogony) by developing into microgamonts (males) and macrogamonts (females). Microgamonts undergo nuclear division and produces a large number of minute three active flagellate microgametes, that exite the host cell, and penetrate host cells that contain mature macrogametes. Macrogamonts have granular cytoplasm and center nucleus. They do not undergo nuclear divisions, but increase in size within the host cell allowing for the proliferation of cellular organelles include. Its wall- form bodies that are involved in the formation of an oocysts wall. The macrogamete is fertilized by the penetrating microgamete which the formation of a zygote. After the fertilization phase, the results in macrogamete mucopoteinaceous granule that is placed on the periphery of the cell, form the outer membrane of the zygote. Once the cyst wall is formed completely the oocystleaves out of the bird through feces(Hammond, 1973 and Fayer ,1980).

The life cycle of *Eimeria* will continue with sporogony of oocysts in the external environment. Sprogony is the process by which a one celled sporont (zygote)within the oocysts wall undergoes a series of divisions to form four sporocysts each contain two sporozoites. This process is known as sporulation .The time of sporulation differ from species to species depending on *Eimeria* species. Excretion of oocysts starts 4 days after infection and may last for10 days,whereas sporulation takes another two days to complete the cycle (Graat *et al.*,1994 and The merck veterinary manual , 2006). External environmental conditions such as oxygen, moisture, and optimum temperatures($21-32^{\circ}c$) (Current *et al.*, 1990). Generally coccidial infection are self-limiting , in the absent of re-infection therefore , only one cycle of development can take place.(Chapman *et al.*,2010) .

2.6 .Economic Burden :

Poultary,during coccidiosis and after therapy,have poor production results .Chicken daily growth weight is reduced and feed quantity and feed conversion rise as well as increasing concerns with prophylactic drugs use and high costs of vaccine (Magner ,1991; Chandrakesan *et a* ., 2009 ; Lilic *et al* .,2009 and Chapman *et al* .,2010) .

The poultary industry raises approximately 40 billion chickens annually and coccidiosis is the most frequently reported disease of chickens worldwide (McDonald and Shirley ,2009). Lee *et al* .(2009) suggested that this disease has the greatest impact on poultary production while Dalloul and Lillehoj (2005) determine that in-feed medication for prevention and treatment of this infection accountfor the major portion of the economic burden. However, Ruff (1999) claime that the economic loss annually exceeding \$1.5 billionUS is from a multitude of factors including decreased weight gain, decreased feed efficiency, decreased egg production in addition to the cost of treatment for the infection.

Bould *et al* .(2009)indicates that the coccidiosis has a huge economic impact in both developed and developing countries, and infections in poultary have been to decreased the growth rate of chickens by15 - 20 % during mild coccidiosis and up to as much as 30 - 40 % during severe coccidiosis .The global cost of disease is in the region of \$ 3000 million per year, and even in countries where uptake of prophylactic treatment and vaccination is high.

Williams (1999) reporte that in UK, the total of coccidial infections about 780 million broilers are estimated to be at least £ 42 million per annum, of which74% is due to sub-clinical effects on weight gain and feed conversion and 24% is the cost of prophylaxis and therapy of commercial birds . It is responsible for significant economic losses in excess of US \$3 billion annually to the worldwide poultry industry .

The annual worldwide cost estimate that about \$80 million for the American broiler industry. The U.S. broiler industry is estimated to lose between \$450 million, Out of which 17.5% of are due to the cost of prophylaxis, treatment in broilers and broiler breeders, 80% due to losses of feed concern version and weight gain ever in the presence of drug-treatment strategies (Williams ,1999a and Lee *et al.*, 2009).

Kutkat *et al.*(2009) confirme that coccidiosis is recognized as the parasite that it has the greatest economic impact on the commercial poultary industry.Current expense of preventive dedicator exceeds \$90 million in the USA and over \$3 million worldwide.

2.7 . Prevalence of Coccidiosis in Poultry Industry :

Avian coccidiosis is one of the most prevalent in the poultary industry and can be found under every possible climatic condition .

Edgar and Siebold (1964) describe that a new coccidium of chickens: *E. mivati* and they report that the incidence is as high 50 %.

Jeffers(1974) confirme that among the farms which yielded coccidian, the respective incidences of *E.acervulina*, *E.brnunetti*, *E.maxima*, *E.necatrix* and *E.tenella* are 90.6, 2.3, 86.2, 0.4, and 28.4 % respectively from 1166 (89%) of 1308 litter samples from all major broiler-producing regions of the uniated states.

Great *et al.*(1996)examine the incidence in poultary in the Netherlands and found *E.acervulina* and *E.tenella* at infection rate 63% of 4774 flocks examined.

Amoudi (1997) describes that tow new species of *Eimeria (E.jedda hensis* and *E. waeli*) in local chickens from Saudi Arabia.

Koinarski *et al.*(1997) reporte that the incidence of eimeriosis is about 20–50% of the poultry in Bulgaria and the prevalence of *E*, *acervulina* infection rate was 18.3%.

McDougald *et al.*(1997) recovere that out of 83examined samples are positive for Eimeria infection from 43 broiler and breeder farms in Argentina , *E.acervulina, E.mitis, E.praecox, E.maxima, E.tenella*, and *E.brunetti*. The detected *Eimeria* species are with prevalence rates at 93% , 67% ,56% , 42% , 14% ,and 5% respectively.

Larry (1998) reveales that some species have not been reported in all countries, recent surveys in the US, France, Argentina, Brazil, and the Czech Republic have identified all the recognized species. Thus, it is likely that the species are truly cosmoplitan and will be found wherever through surveys are conducted.

Thebo *etal* .(1998) demonstrate that the seven *Eimeria* species (*E.acervulina*, *E.preacox E.brunetti*, *E.maxima*, *E.mitis*, *Enecatrix* and *E.tenella*) of the chicken are present in Sweden .

Mattielo *et al.*(2000) indicated that from 10 poultary farms (broiler pullets, layer pullets and broilers) in the provinces of Entre Rios and Buenos Aires in Argentina are examined for presence of *Eimeria spp.*. They reporte that, *E.praecox* and *E.mittis* are found in two samples, *E.acervulina* in nine, *E.maxima* in seven, *E.necatrix* in three, *E.tenella* in seven and *E.brunette* in four of samples.

AL-Natour *et al* . (2002) study the prevalence of *Eimeria* infection among chicks in North Jordan, the result revealed that seven of *Eimeria spp*. were identified. They are *E.acervulina*, *E.brunetti*, *E.maxima*, *E.necatrix*, *E. mivati*, *E.mitis*, and *E.tenella* and 50% of the surveyed farms have six species of *Eimeria* spp., they found 23% of the farms are free of the infection .*E. tenella* is the most prevalent species 39% followed by *E.necatrix* 12%, *E. brunette* 12% and *E.maxima* 10% .

Fitz-Coy (2005) reporte that the incidence of *E.mivatti* is as high as 35 % of broiler flocks from Georgia,South and North Carolina ,Virginia , California , Texas and Arkansas.

Lobago *et al.*(2005) show that out of 465 dead birds, 370 (38.14%) are found to have clinical coccidiosis in Kombolcha poultary farms, Ethiopia. *Eimeria spp.* Identified are *E.brunetti, E.tenella .E.acervulina*, and *E.necatrix* with prevalence rates of 45.3%, 40.8%, 9.7% and 4.1%, respectively.

Bandyopadhyay *et al* .(2006) describe anewspecies, *Eimeria india* of eighty adult individuals of Gallus gallus domesticus examined from Aves , Phasianidae in India,twenty-five (31.25 %) have *E.indian* oocysts

Khan *et al* .(2006) describe that four of *Eimeria* species from 258 gut samples from broiler chicken from Rawalpinidi/Islamabad area in Pakistan , They are *E.maxima*, *E.tenella*, *E.mitis and E.necatrex* with prevalence rates at 34.10%, 30.62% ,13.95% , 7.75% respectively, and prevalence of eimeriosis is the highest in the month of september 89.74%. The lowest rates during June 89.57%.

Adhikari *et al.* (2008) indicate that out of eight dropping samples are examined of chickens have five of *Eimeria spp.* from different floor system and farming system of poultaryof Ratnangar Municipality and Chitwan District, Nepal. The prevalence rates are *E.acervulina* at 5, *E.maxima* at 5%, *E.necatrix* at 10%, *E.tenella* at 25% and *E. brunetti* at 5%.

Haug *et al.* (2008) recovere from broiler chicken in the Norway. by used PCR, five *Eimeria* spp. with the prevalence rates are *E.acervulina* at 90%, *E.tenella* at 77%, *E. maxima* at 25.5%, *E.praecox* at 10% and *E.necatrix* at 2%.

Nematollahi *et al.*(2009) reporte that the prevalence rate of *Eimeria spp.*infection is examined farms at eight dropping samples are examined of layer chicken 55.96% (out of 122/218 farms) inTabriz ,Iran .

Sun *et al.*(2009) examine fecal samples from 50 broiler farms had subclinical signs in eastern China. They reveale that the incidence rates of *E.tenella* at 90%, *E.praecox* at 88%, *E.acervulina* at 72%, *E. maxima* at 68% and *E.mitis* at 60%.

Lee *et al* .(2010) examine 356 fecal samples through microscopic examination. They are determine that 78.7% of the tested farms are positive in *Eimeria* infection. Seven *Eimeria* spp. are detecte in all positive farms by using PCR method. *E. acervulina*, *E. tenella*, *E. brunette* and *E. praecox* with prevalence rates at 87.5%, 62.5%, 59.3% and 37.5% respectively.

Each of *E maxima*, *E.mitis* and *E.necatrix* is identified in 31.3 % of the farms from different regions of Korea.

2.8 . Epidemiology :

Avian coccidiosis have been found wherever poultary are raised. In flock , disease is spreading by direct and indirect contact. An important factor in the epidemiology of coccidiosis is the survival of oocysts that are shed in the excreta of infected hosts, where normally introduces into new facilities through contaminated equipment or vehicles coming from other poultary operation, or by the movement of service personnel between older and new facilities. Once a house becomes contaminated, it is virtually impossible to totally decontaminate the environment (Muangyal, 1991and Reid *et al.*, 1994).

The highest incidence rates of coccidiosis are detected during winter and spring, especially when weather is cold and humid as compared with summer and autumn when weathr is hot and dry conditions(Maungyai *et al* ., 1990; Calnek, 1997 and Razmi and kalideri, 2000).

The species of *Eimeria* have direct life-cycles (within7–12), exposure to sporulated oocysts usually begins shortly after chicks are placed on the litter, mechanical transmission is the primary means of spread between farms and between sheds on a farm, and oocysts can be spread to broiler houses mechanically by many routes such as boots, dust, cloths, wheels, Contaminated
equipments and personnel who move between houses or farms(Long and Rowell ,1975 and Adhikari *et al* .,2008).

Rasadi *et al.*(2007) demonstrate that the primary method of spreading the *Eimeria* oocysts between poultary houses by mechanical routes such as boots,dust,cloths,wheels,litter,freeflying birds,insectes ,rodents,contaminated equipments and personal,and have a large reproduction potential ,it is very difficult to keep chickens rearing conditions .

Kiani *et al.*(2007) confirme that the sources and routes of introduction of *Eimeria* oocysts in to broiler chick' houses. The results indicate that dust around the houses, boots, wheel burrows, litter, feed ingredients and worker's hand get contaminated with *Eimeria* oocysts in 65%, 51.7%, 45%, 38.3%, 17% and 8.3% respectively from 60 houses at Suburb of Amol in Iran.

2.9 . Pathogencitaly :

Clinical disease entity depend on the number of oocysts ingested by individual birds. Lesions of the infection depend on the species and strains of coccidian causing the problems. All observed pathological effects are related to disruption of the epithelial cells lining the intestine by the release of parasite stage, and intestinal damage become visible on the 4th or 5th day postinfection. The most severe and widespread lesions occur on the 6 $^{\rm th}$ and 7 $^{\rm th}$ day post - infection . E. tenella and E. necatrix are considered to be the most common pathogenic species of *Eimeria* in domestic poultary, asexual development in the small intestine, gametogony cycle in the caecum, it causes damage by two distinct means: (1) The tips of villi are eroded and large number of degenerating epithelial cells can be seen, and (2) Isolated villi are greatly enlarged with portions of lamina propria extruding through the villus tip as a result of pressure exerted by the large developing meront in the lamina propria. *E.brunette* causes severe damage in both the ceca and large intestine, the villi of both the ileum and large intestine are completely disrupted and eroded, exposing the underlying connective tissue of the lamina propria causing extensive coagulation necrosis with accompanying sloughing of the mucosa.weight losses are often severe, although distinctive lesions may be difficult to recognize. The lesions of produced by E.acervulina and E.mivatti occure primarily in duodenal loop and the upper part of the jejunum. The morphological change in the affected area include shortened or flattened villi, decrease villous surface and get elongated crypts.

Decreased activities of digestive enzymes on the upper half of the villi, such as disaccharides, indicate a damaged brush border with a decreased digestive absorptive capacity *E.maxima* and *E.necatrix* produce their most severe lesions in the mid-intestinal area, which is readily identified by the residual yolk sac diverticulum (Stockdal and Fernando, 1975; Witlock and Ruff, 1977; Conway and McKenzie, 2007 and Lilic *et al.*, 2009).

Ali *et al* .(2002) mentione that the chicken are infected with high level of coccidian displays symptoms such as hunch up,ruffled feathers, droopy or sleep eyed appearance ,loss of appetite, decreased intake of food and water,weakness,anemia ,and decreased body weight gain or actual weight loss .The water and mucus content of fecal material is increased and blood or diarrhea may be present .

Most *Eimeria* species affect birds between 3 - 8 weeks of age, and this age group of chicken is very susceptible to coccidial infection. In broilers, peak infection of coccidiosis occur at 4 - 5weeks of age, where the pathogens causes clinical and mostaly subclinical problem in early age of the farming chick and fatal to the confined bird in rearing unite (Hofstad, 1992; Sarker, 2006 and Constantinoiu *et al*.,2007).

Williams (2003) recovere that the coccidiosis is the explicative phases which lead to damage in the intestinal tissues. Individual bird may show no clinicalsigns, suffer a mild loss of appetite, weight loss or decreased weight gain, diarrhoea (which can be bloody), dehydration and death. Resistance develops rapidly and infections can be self-limiting, but birds which consume large numbers of oocysts can be severely affected and die. Damage of the intestine caused by *Eimeria* spp. is thought to be involved in increasing the susceptibility of chickens to breaks of necrotic enteritis caused *clostridium perfringens*.

2.10. Diagnosis :

Long and Rowell(1958) indicate that after oocysts washing. They are sporulated in ashallow layer of 2% potassium dichromate at 27^{0} C.

The recovered ocysts are separated from the faeces and of the intestinal contents of infected birds by sieving, centrifugation and flotation in saturated salt solution(Joyner and Norton, 1984; Kiani *et al.*, 2007 and Al-Quraishy *et al* ., 2009)

The identification of *Eimeria* spp. is commonly accomplished through the analysis of some characteristics such as pre-patent period, morphometry of oocysts and other stages of the life cycle,site of development in the host and macroscopic lesions (Karim and Begun ,1994 and Calnek , 1997). Shirley(1975) uses a molecular biological approach for the first time to differentiate species on the basis of isoenzyme patterns of oocysts by starch block electrophoresis.

Poonsuk(1993) reportes that the diagnosis of coccidiosis in chicken is best done by postmortem examination of birds. Diagnosis on faecal examination may lead to quite erroneous results.for example the major pathology is produced before oocysts in the faeces(e.g *E.tenella*),and the presence of large number of oocysts may not necessarily indicated a serious pathogenic condition.

Shirley(1994) indicates that the recombinant DNAtechniques have been used discriminate different strains of *E.tenella*, and develop markers for precocious and drug–resistance strains .

Schnitzler *et al.*(1999) designe species - specific primers to be used in the polymerase chain reaction (PCR) to identify the seven *Eimeria species* of domestic chickens .

The diagnosis is conducted by history,location in the host, appearance of lesions and determining oocysts in faces or intestinal scrapings by microscopic examination of coccidial stage on smears taken from the lesions to deterging the species present(Larry,1998;Allen and Fetter,2002 ;Conway and McKenzie ,2007).

2.11 .Control of Chicken Coccidiosis Disease :

Permin and Hansen(1998) reveale that the protecting poultry flocks from organism contamination is an extremely important component of commercial poultry production environment. The key to controlling coccidiosis is to be on a control program that will keep the disease under control, yet allow sufficient natural immunity to develop.

2.11.1. Management and Hygiene Practices:

2.11.1.1 .Management :

Reid(1989) states that avian coccidiosis have been found wherever poultary are raised. The spread of this parasitic disease is enhanced by poor bio -security and management practices as well and by the very fact oocysts are so resistant to destruction. The coccidiosis can be controlled by good management including good ventilation, dry, clean litter, clean and decontamination of drinkers and feeders .

Ruff (1993) reportes that the infective management (such as wet litter that encourages oocyst sporulation, contaminated drinkers and high stocking density)can exacerbate the clinical signs.the coccidiosis can be controlled by good management including good ventilation, dry, clean litter, clean and decontamination of drinkers and feeders.

Williams *et al.*(1996) reporte that 95% of the coccidiosis cases observed in 22 farms in France are due to simultaneous infections regardless of type of farm management.

The management of poultary house plays a significant role in the spread of eimeriosis because coccidial oocysts are ubiquitous. They are easily disseminated in the poultary house environment, and may be a direct cause for high prevalence of coccidiosis. Management focuses on reducing the number of coccidian to keep infection at a minimum until immunity is established (Sourake, 2000; Khan *et al.*, 2006; Adhikari *et al.*, 2008 and Nematollahi *et al.*, 2009).

Chapman *et al.*(2010) states that the integrated management strategies may be designed to prevent or reduce infection, to enhance host protection, incorporating methods of maintaining gut integrity.Hygiene,anticoccidial drugs andvaccines .All play major roles.

2.11.1.2 . Hygiene :

Good hygiene, such as cleaning boots and exchanging clothes between sheds, and the eradication of rodent, assists in minimizing the transmission of oocysts. Effective farm management, such as well maintained, drip-free water lines, minimize the level of infective oocysts in the litter, as desiccation significantly reduces sporulation . Williams (1997) indicates that oocysts are resistant in the environment, both to climatic extremes and disinfectants, surviving as long as 600days in soil. However, they only last for days in litter due to heat caused by fermentation and ammonia. Only methyl bromide, carbondisulphide, ammonia or phenols can kill oocysts. The latter to can safely used under commercial conditions.

Permin and Hansen(1998) indicate that the poultry products are derived from intensive production, with control of parasitic infections through the use of veterinary medication and good sanitation.

Van-Immerseel *et al.*(2004) mentione that the enteritis in broilers may be caused by several factors including poor hygiene,management of bedding material,poor ventilation,draught,drastic changes in feed composition and low stress consistently is showing to sensitize broilers to enteritis.

The contaminated litter is the major source of infection. Isolation of *Eimeria* spp.from feed sample indicates poor management of both storing feed stock and litter disposal. Metan sodium(MS,sodium N-methyl-dithiocarbamate) if used to reduce coccidial dose cause contamination of poultry litter (Khan *et al.*, 2006 and Fetter *et al.*,2010)

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2.11.2 . Application of Anticoccidial Drugs and Vaccines :

Because most the damage caused by the nfection occurs before clinical signs become apparent, the prevention of this disease is considered to be even more important than the treatment .

Coccidiosis is the disease of greater economic transcendence, not only for the losses that it causes in the ani\mals productive performance, but also for the enomerous investments that its effective control requires(Franceschi *et al.*, 2008and Lilic *et al.*,2009).

2.11. 2.1. Control Using Anti- Coccidial Drugs:

Drugs and antibiotics are used to treat the symptoms of many poultry diseases. Chemotherapy are the main approach for controlling coccidiosis in most countries, because most of the damage by the infection occurs before clinical signs become apparent, the prevention of this disease is considered to be even more important than the treatment. Drugs for the prevention and treatment of coccidiosis in chickens are available since the 1940s. Various strategies, such as prophylaxis, shuttle and rotation programmes, restricted feeding programmes, drug combinations, and herapy(Chapman and Johonson ,2002 and McDougald, 2003).

Jong *et al.*(1985) indicate that the use of antibiotic in sub-therapeutic dose as additives in animal diets is used since the1950s.Anti–biotics represent a group of compounds with heterogeneous chemical structures and different physic-chemical property of antibacterial activity.

Maungyai *et al.*(1990) showe that use of coccidiostats drugs in broiler chicken in the near premarket period shall be considered carefully. The proper type of drugs should be selected . The prescribed premarketing with drawal period, normally between 3–7 days, to avoid residues of drugs in the chicken meat will be observed .

The broilers are normally fed with anti-coccidials almost throughout their lives from the first day to 5–7days before the slaughter, to avoid residues of drugs in the chicken meat shall be observed. Two types of drugs (coccidiostats and coccidiocides) are used contiiuously in the feed to prevent coccidiosis, and deferent strategies such as continuous use of a single product , the shuttle or dual and anticoccidial rotation is developed to enhance the efficacy and life time of existing products (Muangyai *et al.*, 1990 and Permin and Hansen, 1998).

In broilers, acoccidiocide is used to prevent coccidiosis. Completely inhibit the development of coccidian parasites, a results, no immunity develops in the flocks, this total lack of immunity in a broiler flock may cause a sever outbreak of coccidiosis if drug intake has fallen and the birds have ingested large numbers of oocysts.

But there are two big problems with this a approach. These are the emergence of drug resistance, and drug residues in chicken meat (McDougald ,1990and Chapman and Cherry, 1997)

McEvoy(2001) reveale that the emergence of drug resistance, and drug residues in chicken meat and to prevent that changing the anticoccidial drug and different methods of administration are used such as the 'shuttle' system . Because broilers are varying susceptibility to infection at breaks is increased with longer withdrawal.

Kitandu and Jauranova (2006) demonstrate that, for many years, prophylactic of anticoccidial drugs are the primary means of controlling chicken coccidiosis in broiler industry and it is played a major role in growth of this industry. In addition, the coccidiosis is aggravated by microflora, for example *clostridium perfringens* interacting with intestinal mucosa damage as well as the developmental stage of *Eimeria* parasites. This problem can be reduced by the use of antibacterial properties of the ionophores.

The poultary control of coccidiosis still relies heavily on adding anticoccidial drugs to feed, and there are a couple of chemicals that are marketed today, such as Amprolium, Nicarbazin, Robenidin, Diclazuril, Zoalene , Decoquinate , Halofuginone (Nacire *et al* ., 2004 and Rojs *et al* ., 2007).

2.11.2.2. Vaccination Against *Eimeria* Parasites :

Vaccines are available since1952, but have taken a long time to become accepted as an alternative to chemotherapy. Vaccines aid in preventing disease by stimulating the bird's immune system in such a way that it enhances the immune response when the bird is subsequently exposed to a pathogen .Classically, vaccines have either contained a small live dose of a weak form of the pathogen or a larger dose of a killed preparation of the disease-causing organism. However there are many different vaccines available and effectiveness depends on which vaccines are used and how they are implemented (Wong *et al* .,2004 and Ziomko *et al*., 2005).

When chickens are infected with low number of *Eimeria* parasites, protective immunity is induced after 2-3consecutive infection. Therefore, it would seem obvious that vaccines could offer excellent alternatives to drugs as controlling coccidiosis(Long and Jeffers, 1986 and Kitandu and Juranova ,2006).

Chapman (1997) examinate the vaccination of chickens against coccidiosis with live oocysts is an accepted method of disease control, especially for long-lived birds such as breeders. The appearance of drug resistance among coccidian of domestic fowl has promoted renewed interesting vaccination as a means of controlling disease in birds with shorter life spans, specifically broiler chickens. Chapman and Cherry (1997) reporte that the research has focused on ways of administering live vaccines early in the life of the bird to achieve rapid development of immunity such method include eye–spray application to newly hatched chicken .

The reluctance of broiler producers to adopt anticoccidial vaccination strategies is related to several reports on measured performance parameters associated with vaccination, weight gain and feed efficiency. Performance of vaccinated broilers has not always equaled that of medicated broilers. The reduced performance is related to mild coccidian infection associated with live oocyst vaccinatio(Chapman, 2000 and Williams, 2002 a).

Williams(1998) observes that the vaccines may be designed for rearing standard broilers for up to about 6 weeks. Attenuated, precocious lines of *Eimeria* in vaccines have low reproductive potentials, thus avoiding crowding, developing optimally, and stimulating responses with minimal tissue damage. The Live oocyst vaccines are currently the only commercially available option for the control of coccidiosis in the poultary industry other than anticoccidial feed additives. The useful lives of anticoccidial drugs may be extended by rotating them with live vaccines.

The interest is growing in controlling coccidiosis by vaccination because immunological control recognized as the only practical to anticoccidial drugs in large-scale production. The use of vaccines as part of a rotation program with in-feed anti coccidials has been proposed, and the live vaccination is Today less applied in broiler production in U.S.A., because protective immunity after natural infection takes several weeks to develop, It is not a feasible option for broilers with a short life time(Chapman *et al.*,2002 and William 2002a).

Augustine *et al* .(2001) mentione that the conventional disease control strategies have relied on prophylactic medication, because of development of drug resistance in many coccidian strains and increasing consumers demands for chemical free poultary meat, vaccination against coccidian is very attractive alternative for disease control.

Williams(2002 b) demonstrates that the resulting in increased pressure from a percentage of consumers to remove drugs from animals feeds, for these reasons here is a pressing need to move away from chemotherapeutic of coccidiosis in favor of non-medicated forms of control such as vaccination . In addition, the vaccines are also used to some extent in broilers, but compared with the breeder and layer sectors, worldwide market penetration is relatively limited. When live, anticoccidial vaccines are administered to chickens, anticoccidial drugs are not usually used concomitantly because they may kill some of the vaccinal parasites . Shirley and Smith(2007) reporte that there is a good prospect to control the disease through vaccination. Live virulent vaccines are utilized for the last50years.The live vaccines tend to givelonger immunity than killed vaccines because the live organism can colonise and survive in the host for some time and stimulate a longer and more effective immune response live attenuated vaccines are become available over the last two decades.The attenuated strains have been selected for rapid passage through the host. Consequently, they have low reproductive potential and have lost their virulence,but still have strong immunogenicity.Importantly, they cause no post-vaccinal decrease in weight gain, and they are therefore suitable for use in broiler flocks.

James *et al.*(2009) indicate that there are an increasing move towards vaccination, with growing number of producers especially in the UKand USA option to use them .But have had little penetration in regions that are most adversely affected by the disease, such as Asia, Africa, and South America primarily due to cost and availability.

2.11.3. Role of Immune Response:

Effective immunity against coccidiosis can be imparted to poultry if the birds are reared on a regular diet containing added viable sporulated coccidia oocysts at a level sufficient only to induce sub-clinical infection. Immune system is of special importance to poultary because most commercial flocks are raised under intensive rearing conditions. The primary lymphoid organs are the bursa of fabricus and the thymus and secondary lymphoid organs are lymphocytes and antigen-presenting cells are scattered throughout the body. The coccidiosis is self-limiting disease, and birds which have recovered become immune (Qurshi *et al.*, 1998 and Sharma, 2003).

Various studies are show that immunity against one strain of pathogen will not necessarily protect against a different strain of the same species; this is demonstrated with the anti-genetically variable *E.maxima* as well as *E.acervulina* (Martin *et al* .,1997and Innes andVermeulen,2006).

The immunity induced following infection with coccidiosis is highly specific and cross protection is not documented .Immunity develops rapidly following infections with *E.maxima,E.praecox*(highly immunogenic species) and probably *E.mivati* and *E.hagani*,somewhat more slowly following infections with *E.tenella* and*E.brunetti*, and is delayed following infections with *E.mitis,E.acervulina*,and *E necatrix*.Cellsfrom chickens which are immune to *E.tenella* responded well to antigens from *E.tenella* but poorly to *E.acervulina* antigens suggesting that the lack of cross-protection may be due to the absence of cross-reactiveTcells (Lee, 1993and Ilic *et al.*, 2003).

The intestinal mucosa provides both aphysiologic and immunologic barrier to pathogens.infection by *Eimeria* promotes antibody(specific/adaptive-antibod)and cell-mediated immune responses (cellular,non – specific), However,cell immunity mediated by various cell populations ,includingTcell lymphocytes,natural killer cells,mast cell and macrophages plays a major role in disease resistance but humoral immunity plays only a minor role in protection against this disease,afurthermore.There is increasing evidence that the cell-mediated immunity plays a major role in protection against this disease (Lillehoj andTrout,1996 andYun *et al.*,2000).

Lillehoj and Lillehoj,(2000) indicate that the complex life cycle of the *Eimeria* is associated with complex host immune response to the parasite, and different effectors mechanisms may be involved, depending on the species of *Eimeria*, stage of parasite development, prior host exposure, the nutritional statues of infected chickens and genetic makeup of the host .

The immunity to *Eimeria* species is acquired gradually and is not complete until the birds are 7 weeks of age, and immunization of chicken in the first few days of life is thought to be difficult because of the poor establishment of infection and the relative immaturity of the immune system .Chickens readily develop immunity from natural infection which induced after2–3life cycle of coccidian and immunity induced following infection with each of the 9 a vain *Eimeria* is species specific. Those Coccidiosis is thought to occur most frequently those birds are 3 - 6 weeks of age.

Little information is available on how rapidly immunity is acquired when 1- day old chicks are given dose of this magnitude coccidiosis thought to occur most frequently when birds are 3 - 6 weeks of age. (Wallach *et al* .,1995 and McDougald,2003).

Dalloul and Lillehoj (2005) reporte that the nutrient immune dulation, feed additives and maintenance of normal gut flora are important consideration to obtain better responses to cocci-vaccination in broilers and minimize the deleterious effects of coccidiosis.

Titilincu *et al.*(2007) demonstrate that the immune response in eimeriosis is chiefly cellular mediated and secondary humeral by means of antibodies within the cellular immune response the $CD4^+$ and $CD8^+$ cells as well as the cytokines they secrete play an important part.

2.11.4. Alternative Controls Including Natural–Product Feed Additive:

Allen *et al.*(1996) reporte that the sources of fats containing high concentration of n-3 fatty acids, such as fish oils, flaxseed oil, and whole flaxseed, when added to starter rations and fed to chicks fromone day of age. The additives don't kill *Eimeria*. Instead, they trigger a natural, biochemical response in chicks called oxidative stress. The stress results in by product compounds that doom *Eimeria* hiding in cells of the cecum, a portion of

the bird's small intestine. When mixed into a commercial diet and fed to newborn chicks or four weeks, flaxseed oil reduced by 54 % the number of cecal lesions caused by the species *E.tenella*.

Chapman (1997) reveales that the historically,the poultry industry has relied on antibiotics drugs in feed where birds reared under intensive condition on litter to prevent disease, and vaccination with live attenuated parasites- both have drawbacks.Anti-coccidial drugs are expensive and their effectiveness is hindered by widespread parasite drug resistance. The high cost of new drug development.Due to increasing concerns with prophylactic drug use and high costs of vaccines,therefore,much recent interest has been developed toward the evelopment of drug –independent control strategies against coccidiosis.

Allen *et al.*(1998) show that the feed supplementation with antioxidants such as Y-tocopherol ,found plentifully in seed oils such as wheat ,corn and soybean ,and the spice turmeric (1%),as well as its main medicinal component,curcumin(0.05%), appear effective in reducing upper – and mid–small–intestinal infections caused by *E.acervulina* and *E.maxima*.

In broiler nutrition, a alternative new feed additives are classified as probiotics, prebiotics, enzymes, organic acids, and herb extracts. Probiotic belonging to Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida, Saccharomyces, and Some other probiotics are microscopic fungi such as strains of yeasts belonging to Saccharoomyces cerevisiae species introduce desirable live microorganisms Into the gut immune tissue, improved resistance to *E.acervulina*, and increased the jejunal villus height, and decreased the villus crypt depth compared with salinomycin and control. Prebiotics promote the growth of endogenous bacteria in the gut immune tissue. The enzymes help to eliminate the anti-nutritional effects of water-soluble polysaccharides and/or change the substrates to improve proliferation of some beneficial microbial communities ,while organic acids cause the inhibition of bacterial growth. Finally, the herb extracts are very variable working mechanisms that depend on the composition (Chichlowski et al., 2007 and Eckert et al., 2010).

The *Artemisia annua* is a naturally occurring end peroxide with anti malarial properties. It has been found to be effective in reducing oocyst output from *E.acervulina* and *E.tenella* infections (OH *et al*.,1995 and Allen *et al.*,1997).

Hermans *et al* .(2006) state that there are some reports of the benefits of betaine(from sugar beets). It has a known to have beneficial effects on livestock growth and performance and in preventing enteric stress, especially as related to osmotic challenges. For broilers that exhibit flushing(mild diarrhea), betain may helpful in alleviating the problem, betaine and salinomycin significantly reduced cell invasion by *E.acervulina*.

Vitamin E, is a multifunctional nutrient essential for normal growth and development of chickens. It is a potent antioxidant,protecting a against free radical oxidative processes, and also as an immunodulator in chickens (Gore andQureshi, 1997and Boa-Amponsem *et al.*,2000).

The evidence that MOS(mannanoligosaccharide derived from the cell wall of the yeast Saccharomyces cerevisiae)suppress pathogen of the intestinal mucosa in chickens and turkeys (Spring *et al.*,2000 and Delzenne,2003).

The oregano essential oil exerted an anticoccidial effect are similar to the ionophorous antibiotic verified through the intestinal morph metric and excretion of oocysts, that after the infection with *E. tenella* and *E acervulina* the supplementation with dietary oregano oil resulted in body weight and feed conversion ratio not differing from the non-infected group, and addition resulted in improved intestinal integrity probably by reducing the impact of coccidiosis on intestinal integrity(Oviedo-Rondon *et al.*,2006 and Maria *et al*,2009).

The treatment with a wild mushromm (Ganoderma lucidum) results in a marked reduction in the number of *E.tenella* oocyst shed in the faeces, and leading toimproved weight gain, and decreased weight loss(Dalloul *et al.*,2006 and Ogbe *et al.*,2009).

Zimmermann *et al.*(2009) conclude that passive immunization of chickens with anti- coccidian IgYantibodies which expressing pea seeds provide protective immunity against coccidiosis avian coccidiosis in newly hatcheries birds.

The EMF(Electromagnetic fields) signals stimulate the production of cytokines, mediated an enhanced immune response. EMF is considered as a possible alternative to anticoccidial drugs currently used in broiler chickens infected with *Eimeria* parasites(Goodman *et al.*,1994; Simko and Mattsson , 2004 and El- Musharaf *et al.*, 2010).

3.Materials and Methods

3.1. Site of Study :

Ghot El-Sultan project is situated about 50km^{2.} South-east of Benghazi city.It is occupies an area 2500hectometer.The project considered as a one of the three largest projects poultry and dairy production complexes projects in east of Libya (Tauorga, AL-Hera, and Ghot El-Sultan sector), As well as it is agriculture and industry constructed for chicken,milk and dairy meat production.This project integrated, largely self supporting, includes grandparent farms with hatchery,parent farms,a broiler hatchery,ten broiler farms with and a processing plant with a approximately3,000 broiler chickens are slaughtered per hour (Fig. 3).

The broiler integration studied comprised of 9 farms situated about 2km from each other. Each is farm consisted of six houses(house typically have >10000chickens each). The houses are built of cement. The method of housing the broilers is an intensive deep-litter system(Fig.4 A,Band C).

The broiler-chickens are slaughtered at an average 50days of age with average live weight of 1.7–1.9 Kg,which have anticoccidial drugs (Amprolium and Nicabazin) are used on any of the broiler farms against the coccidiosis infection. The broiler-chickens are produced in different broiler parent stocks and hatcheries in Ghot El-Sultan project. The most-common breed broilers are the AL–Aseel from Tauorga project.



Figure (3):Map of Ghot EL-Sultan project, showing the collection sites of broiler chicken farms (Source Ghot EL-Sultan project)



Materials and Methods

Collection and C. Houses of broiler chickens (Source Google earth2012)

3.2 .Collection of Samples :

The present study is conducted on 900 samples of intestinal tracts. These are collected from broiler chicken farms. 100 samples are collected, 25 samples each week, and randomly from poultary processing plant (ppp) monthly, during the period from May, 2009 to April ,2010. The intestinal tracts are sampled, put in separate plastic bag, then brought to the laboratory in Zoology Department, Facuty of Science, Benghazi University for further examination. Identification of *Eimeria spp.* is bases on the location of infection, characteristics of intestinal lesions, the morphology of oocysts , and time of oocysts sporulation.

3.3 .Parasitological Technique:

Each unopened guts are examined externally for lesions and any other pathologyical characters, Then opened guts are examined forcharacteristic of lesions. Wet smears of the intestinal contents are prepared from gut scraping for the microscopic examination of the presence of *Eimeria* spp.oocyst present or absent in each sample. Oocysts isolate from intestinal content are done after concentration by flotation method by using Sheather's sugar solution. Positive samples for *Eimeria* oocysts are put in 2.5% potassium dichromat solution for sporulation .

Fifty oocysts from each intestinal part(duodenum, jejunum, ileum and caeca) are examined and measured for their morphological characteristics (Lenght,width and shape)by use an ocular micrometer,10eyepieces and X 40,X100 objectives. All measurement arein micrometers(μ m). They given as means, and followed by the shape-index (Length /Width ratio). photomicrographs are prepared using light microscope-digital camera unit (Fig.5).

3. 3.1 .Direct Wet Smear Method :

Smears are made from intestinal contents and mucosa scrapings from four different sites of each intestinal tracts. These intestinal material are diluted with distal water and mixed thoroughly. Each sample is sieved through 40–100 a tea strainer. Then by use a fine pipette to transferred . One drop is taken and placed on a microscope slide. Then the sample are microscopically examined for the presence of *Eimeria* oocysts by using normal light microscopy at X10, X4, X100 objectives and X10 eyepieces .

3.3.2. Flotation Technique :

For separate oocyst from intestinal contents applying the flotation technique by aid of Sheather Sugar flotation method (Sheather, 1923) (Appendix 1).

Intestinal contents are collected and sieved through a tea strainer to remove large particles from samples. The strained samples are poured into a centrifuge tube. The tubes are centrifuged at 2000 rpm for 5min. The supernatant fluid is decanted and sediment is mixed with Sheather' sugar solution in the centrifuge tubes .

After centrifugation, cover slides are puted at the tip of centrifuge tubes , and left for 5-10 minutes. Then placed the cover slip on a microscope slides for microscopic examination(X10, X40, and X100 magnification). Addiaton , by using the pipette the supernatant containing oocysts is removed then puted on slides for examination .

3.3.3 .Collection of Oocysts and Sporulation Technique :

After flotation, a pasture pipette is used tocollect the oocysts from the top layer of the sheater's sugar solution. From each centrifuge tube, Oocysts are washed with tap water, by centrifugation for several times . (~ 5 mm deep) of 2.5 % (w/v) a aqueous potassium dichromate solution (K₂ Cr $_2$ O₇) is placed in a50 ml flask for sporulation .

Sporulation process is performed in an incubator at $(29 - 32 \ ^{0}C)$ with a water bath trembling until oocyst sporulated. The checke is done repeatedly for determine the sporulation time of any oocysts detected (Figure 6).

Sporulted oocysys are examined microscopally concentrated by centrifugation there suspended in ashallow layer under X10, X40, andX100 magnification objective lens of light microscopic. Photomicrographs are obtained using the aid of a light microscope-digital camera (Fig. 6).

3.3.4. Histopathology Technique :

The pieces of intestinal sections are taken from upper,mid andlower parts of intestine. Each piece is opened along it's length and the luminal contents removed.All pieces flushed with saline and fixed in 10% tamponate formalin solution,embedded inparaffin wax then are sectioned and stainedwith Haematoxylin–Eosine stain (Appendix 2 and 3).Finally are examined by light microscope for coccidian.This is done as follows :-

For histopathological study,2cm from each of duodenum, jejunum, ileum and cerca are collected .Tissues are processed by standard methods and stained by Heamatoxylin and Eosin .

At the time sections preparation, the formation at 10% pieces of duodenum, mid - intestine, ileum, and ceacal that are washed with saline, then fixed in buffered formation(10%). Then, all of the fixed tissues of both fixatives are dehydrated by transferring them into ascending grades of ethyl alcohol: 70% " 80% " 90% " 100% " 100% . Duration of each transfer is one hour. The processed tissues are concentrated by centrifugation.

Then resuspended in a shallow layer cleared by three transfers, for one hour each , into absolute ethanol – xylene , xylene and xylene .

Infiltration with melted paraffin wax (melting point :58) is carried out by three transfers.One and a half an hour each .The glass containers of the paraffin wax and intestinal segments are put into an oven at 65° C. Embedding is achieved by placing the infiltrated tissues into suitable molds containing melting paraffin wax.The molds are left for 24hours at room temperature for hardening .The prepared paraffin wax blocks are then stored in a refrigerator at 4 $^{\circ}$ C until sectioning step.

Arotary microtome (Shandon ,UK) are used to prepare ribbon of seven micrometers thick sections. The sections are flattened by floating the ribbon on warm water (48° C) in a water bath (B.Braun ,Germany). Five to six histological sections are mounted on a pre cleaned glass slide whose surface are smeared with Mayer's albumin adhering mixture (Appendix 5). The slides are transferred in to an incubator oven (37° C) for 24 hours .

3.3 .5. Staining :-

Deparaffination of the mounted sections is censured by two transfers, five minutes each, in xylene. The following step of hydration is then carried out by transfers, for two minute each, through descending concentrations of ethanol : 00% " 90% " 80% " 70% " 50% . Slides are then washed with distilled water for one minute .

The hydrated section are transferred intoHarris'Hematoxylin jar for five minutes(Appendix 2).After that, the slides are kept in distilled water for one minute.Differentiation step is carried out by dipping the slides in1% acid alchol for 30 seconds, and then placed for ten minutes in jars containing tap water.The slides are then kept for two minutes in the eosin stain(Appendix3).

Later on,the stained sections are placed for two minute each through the ascending concentrations of ethyl alcohol 70% - 80% - 90% and two changes,five minutes each,in absolute alcohol.The dehydrated sections are cleared by two transfers,five minutes each,in xylene.The next step is mounting the sections with Distrene – Plasticizer – Xylene (D.P.X, Appendix 4).Glass cover slips are placed on the mounted sections and the slides are transferred for24hours into an incubation oven at 37^{0} C. Steps of preparation and staining of the histological sections are modified from Humason(1981). Photomicrograph are obtained using a light microscope-digital camera unite (Figure 5).



Figure (5): Shows the light microscope- digital camera unite.



Figure (6): Shows an incubator with a water bath trembling used for oocysts sporulation .

3.6. Statistical Analysis :

Statistical analysis are carried out to determine the incidence and significance of the data. The logistic regression used to comparison of data . The incidence is calculated as the percentage of infected broiler chickens and the number of coccidian (*Eimeria* spp.)parasites per infected intestine tract of broiler chickens .

Chi-square 2 is employed to find out the significance or non significance of the relationships between *Eimeria spp.*,month,season and single and mixed infection,and presence or absence of the parasites .

The accepted level of significance is level of 5% (P < 0.05) is considered to be significant during the test. All analysis are computed in windows environment of statistical of program (Statistical Package for Social Sientest).

4.Results

Coccidiosis is caused by obligate, intercellular protozoan parasites belong to several species of the genus *Eimeria*, It is a major problem for the poultary industry.

4.1.Incidence of Coccidian (Eimeria spp.) Infection :

The present study is carried out on Nine hundred of intestinal tract samples from nine broiler chicken farms in Ghot EL-Sultan project are examined for coccidia (*Eimeria* spp.) .The results revealed that out of the total(900) examined samples,twohundred and eighty nine (32.1%) are found infected with one or more than one of *Eimeria* sp.,whereas six hundred and eleven(67.9%) are not infected (Table 1 and Fig. 7).

There are a high significant differences in the incidence of coccidia (*Eimeria* spp.) infection(2 = 115.204, P-value = 0.000).

The results obtained from the examination of chicks intestinal mucosal scraping revealed that six species of *Eimeria* species are detected, These are *E.acervulina*, *E.necatrix*, *E.maxima*, *E.tenella*, *E.mivati* and *E.brunati*.

In the present study, the results showen that the high incidence rate of *Eimeria* spp. infection of samples examination is detected with *E.acervulina* at 26%; followed by *E. necatrix* at 14%; *E.maxima* and *E.tenella* at 13.2% each; *E.mivatti* at 11.5% and *E. brunette* at 9.4%. The high incidence rate of *Eimeria* spp. infection of positive samples is detected with *E.acervulina* at 80.97%; followed by *E. necatrix* at 43.59%; *E.maxima* and *E.tenella* at 41.17% each; *E.mivatti* at 35.98% and *E. brunette* at 29.41%.

There are a high significance differences in the incidence of coccidian (*Eimeria* spp.)infection and type of *Eimeria* spp.(2 = 104.957, P-value = 0.000). (Table 2 and Fig .8).

Table (1): Incidence of *Eimeria* spp. infection in broiler chickenfarms in Ghot EL-Sultan project (N=900);

Infected		Non-infected		Total	
Infected	(%)	Non infected	(%)	Total	(%)
289	32.1 %	611	67.9 %	900	100%

2 = 115.204 , P<0.05 , df =1 , P-value = 0.000^{***}



Figure (7): Incidence of *Eimeria* spp. infection in broiler chicken farms in Ghot EL-Sultan project (N=900).
Species of <i>Eimeria</i>	% of total examined (N=900)	% of infected (N=289)
E.acervulina	26% (234)	81%
E.necatrix	14%(126)	44%
E.maxima	13.2%(119)	41.17%
E.tenella	13.2%(119)	41.17%
E.mivatte	11.5%(104)	35.98%
E.brunette	9.4%(85)	29.41%

Table (2): In	cidence	of Eimer	<i>ria</i> spp.	infection	in examin	ed and infected
in	broiler	chicken	farms	of Ghot	El-Sultan	project :

2 = 104.957; P<0.05; df =5; P-value = 0.000^{***}



Figure (8): Incidence of *Eimeria* spp. infection in examined and infected in broiler chicken farms of Ghot El-Sultan project.

4.2 .Coccidia (Eimeria spp.):

4.2.1. Identification of *Eimeria* spp. :

The identification of *Eimeria* species is conducted through the study of some characteristics such as gross lesions, parasitic site of development in the host as well as morphologically characterisers of the oocysts by using the conent of the intestinal .

4.2.1.1 .*E.acervulina* :

Incidence :

The results obtained that out of 289 positive intestinal tracts examined , 234 (80.97 %) is found to be infected with *E. acervulina* (Table 2 and Fig. 8).

Location and Characteristic of Lesions :

The results of the present study reveale that *E. acervulina* is found limited to the upper part of small intestine (duodenum)of broiler chickens. Lesions are characterised by numerous greyshish–whit,oval or transverse patches in the upper half of the small intestine.Smears are obtained from intestinal scrapings from duodenum,contained groups of oocysts corres - ponding in size to those of *E. acervulina* (plates 1 A and B).

Description of the Oocysts :

Light microscopic examination is demonstrated that the oocysts are ovoid in shape with a bi-layered smooth wall,colourless.Polar granule is visible.Oocysts measured about 17.4 μ m (15.3 - 20.4) long with \pm SD 0.6 and about14.8 μ m(12.8 -15.3) wide with \pm SD 0.5, Index(L/W) is 1.1 μ m. (Plates 2 Aand B)

The sporoulated oocysts are ovoid in shape, the oocyst wall is smooth, colourless and double layered membrane, residuum body is visible, with four ovoid-shaped sporocysts, each containing two sporzoites.

Sporulation time : is three to foure days .(Plate 3)



Plate (1A) : Shows the lesions of duodenum caused by E. acervulina ...



Plate (1 B) : Inflamation of the duodenal mucosa caused by *E. acervulina*.
Shows the greyshish – white transvers pathes (gwp).



Plates (2A andB): Fresh non-sporulated oocysts of *E. acervulina*. Show the sporont(SPO)occupy the entire volume of the oocyst ,Outerlayer(OL),Innerlayer(IL),Polar granule (Pg) and Micropyle(M) (X100).



Plate (3): Sporulated oocysts of *E. acervulina* with four sporocysts (SPC), Oocyst wall (OW) and Oocyst residium(OR) (X100).

4.2.1.2. E. necatrix :

Incidence :

The results revealed that out of 289 infected specimens, 126 (43.59%) is infected with *E.necatrix*.(Table 2 and Fig 8).

Location and Characteristic of Lesions :

This species is recovered from mid- intestinal of broiler chickens. The gross lesions are exhibited pin-point red and white spots showed from both serosal and mucosal side. Jejenum is markedly swollen, haemorrhagic, red or brown mucus and the contents filled blood. Large schizonts are found to be in smears from the affected area. (plates 4A and B).

Description of the Oocysts :

Oocysts are collected from of smears of intestinal contents from jejunum is broadly ovoid in shape, measuring about 20.1 μ m (17.9 - 23.0) long with ±SD 0.4 by 18.8 μ m (17.9-20.4) wide with ±SD 0.6, index (L/W) is1.1 μ m.Oocyst wall smooth.micropyle not visible(Plates 5 Aand B).

Sprorulated oocyst with four sporocyst, each contain two sporozoites. double wall is visible .Oocyst residuum is presented. Polar granule not clear (Plates 6Aand B) .

Sporulation time : is from 2 - 4 days .



Plate (4 A) : Shows the inflammation in jejenum mucosa caused by *E. necatrix*. Note : Jejunum is balloon shape.



Plate (4 B) :The inflammation in jejenum mucosa caused by *E.necatrix* .Shows haemorrhagic (H) ,Pin – point red spots (PPRS) content filled with blood (CFB).



Plates (5 Aand B): Show the fresh non- sporulated oocysts of *E.necatrix* .Sporont (spo)occupy the entire volume of the oocysts ,Outer layer (OL) and Inner layer (IL) (X100).



Plates (6 Aand B): Show the sporulated oocysts of *E. necatrix* .Containing four sporocysts(SPC), Outer layer(OL), Oocyst residium (OR) and Sprozoite (S)(X 100).

4.2.1.3. *E.maxima* :

Incidence :

Eimeria maxima is recovere during the present study from the small intestinal(Jejenum) of broiler chickens . Out of 289 samples examined , One hundred and nineteen (41.17%) specimen is found to be infected with *E. maxima* .(Table 2 and Fig. 8) .

Location and Characteristic of Lesions :

The necropsy examination reveale the jejunum lesions characteristic of *E.maxima*, dilated and the wall thickened. Small red petechiae, no ballooning and the lumen gut is filled with a thick of pinkish or brown mucouid exudates (Plates7Aand B).

Description of the Oocysts :

Oocysts of *E.maxima* are obtained from smears of small intestinal scrapings. Detected oocysts in the present study are large in dimensions and ovoid in shape, measured about 25.08 μ m (25.0 – 28.16) long with ±SD 0.7 by 8.43 μ m(15.36 – 20.48) wide with ±SD 0.7, index (L/W) is1.4 μ m. Oocyst wall thickness 1.3 μ m, slightly yellow, micropyle and oocyst residuum absent. Polar granule and steida body are not clear. (plates 8A and B).

Sporulated oocyst containing fours porocysts and sprozoites are visible . (Plate 9A).

Sporulation time : from 3 - 5 days .



Plate (7 A): Shows the lesions caused by E. maxima infection of Jejenum.



Plate (7 B): Shows thickend wall(TW), gut filled with thick pinkish mucoid exudates (ME) and blood clottes (BC).



Plates (8 Aand B): Show the fresh non- sporulated oocysts of *E.maxima* Note : Sporonts (SPO) occupy the entire volume of the oocysts ,Outer layer (OL) ,inner layer (IL) and Zygote (Z). (X100)



Plate (9): Shows the sporulated oocysts of *E.maxima*, containing four sporocysts (SPC), Outer layer(OL) and sporozoites (S) (X 100).

4.2.1. 4. *E* .tenella :

Incidence :

The results indicated that out of 289 positive infected,119(41.17%) are found infected with *E.tenella*.(Table 2 and Fig.8).

Location and Characteristic of Lesions :

Eimeria tenella is detected in the cecal specimens. Macroscopically, the appearance of gross lesions have a high degree of location specificity, especially in the caeca, and give a good indication of the *Eimeria tenella*.

The opining of the ceaca infection revealed the hemorrhagic infiltration mucosa and acuumulation of exudates in of the caecum. The examination of the mucosa scraped shows numerous mature gamonts and immature oocysts.(Plates10 A and B).

Description of the Oocysts :

E.*tenella* oocysts can be demonstrate microscopically are broadly ovoid ,with mean size of $23.0\mu m(20.4 - 25.6) \log with \pm SD 0.4$ by 19.9 $\mu m(17.92 - 23.0)$ wide with $\pm SD 0.7$, index(L/W) is 1.2 μm . Oocyst wall as smooth, yellowish color and double layered membrane of approximately 1.4 μm in thick . Micropyle is clear in the anterior end. Polar granule is present . (Plates11A and B).

The sporulated oocysts have four sporocysts which detected under the microscope. Steida body are not observed, Oocyst residuum absence. sporulated oocysts with smooth and double layer, contained four sprocyst are clear(Plate 12).

Sporulation time : is from 3 - 6 days.



Plate (10 A): Shows the lesions caused by *E. tenella* infection of caeca

Note: Caeca is enlarged and distended with blood .



Plate (10 B): Opened cecum shows clotted blood and patches of hemorrhage caused by *E. tenella*.



Plates (11 A and B): Show the fresh non- sporulated oocysts of *E.tenella* Sporonts (SPO)occupy the entire volume of the oocysts ,Outer layer(OL),Inner layer(IL),Zygote (Z) and Micropyle (M) (X100).



Plate (12): Shows sporulated oocysts of *E.tenella*, containing four Sporocysts (SPC).Outer layer (OL), Inner layer (IL), Sprozoite (S) and Polar granule (Pg) (X 100).

4.2.1.5. *E. mivatti* :

Incidence :

Atotal number of 104 out of 289 are found to be positive for *E.mivatti* infection, represente the incidence rate of 35.98 %(Table 2 and Fig .8).

Location and Characteristic of Lesions :

E.mivatti. It is primarily a parasite of the upper part of the small intestine, but infection extend from duodenum to rectum. *E.mivatti* is discovered from anterior of small intestinal .Intestines are slightly swollen , congested with scattered petechiae and whitish lesions.Lesions are numerous in anterior third of the small intestine (Plate 13).

Description of the Oocysts :

E.mivatti oocysts are recovered from smears of dudenum. The shape of oocysts are ellipsoidal to broadly ovoid .The size is15.3 μ m (14.0 – 16.6) long with ± SD 0.4 by 12.2 μ m (11.5 – 12.8) with wide ±SD 0.2, index (L/W) is1.2 μ m. Oocyst wall is colourless and smooth. Oocysts with micropyle at the front end. Steida body is not clear .(Plate14).

Sporulated oocysts contain four sporocysts each with two sprozoites . sprocyst residuum and sprozoite are visible .(Plates 15A and B).

Sporulation time : from 3 - 5 days .



Plate (13): Show the lesions caused by *E.mivatti* infection of duodenum Note :Congested with scattered petechiae and whitish lesions .



Plate (14): Shows the fresh non-sporulated oocysts of *E. mavatti*. Sporonts (SPO)occupy the entire volume of the oocysts, Outer layer (OL), Inner layer (IL), Micropyle (M) and Zygote (Z). (X100)



Plates (15A and B):Show sporulated oocysts of *E.mivatti*, conaining four sporocysts (SPC). Outer layer(OL), Inner layer (IL),Sprozoite (S) and Sporocyst residuum (SR). (X100).

4.2.1.6 . *E.brunette* :

Incidence :

In the this study *E.brunetti* is found in the contents of illum .Out of the total infected specimens (289),52 samples found to be positive for *E. brunette* infection, represente infection rate at 29.41 % .(Table 2 and Fig. 8).

Location and Characteristic of Lesions :

The lesion sites of this species is shown in the terminal ileum, caecum and rectum, a white cheese like material is found in the lumen of lower intestine and rectum, some reddening of the mucosal surface caecum are inflamed .The gut wall is thickened, bloody enteritis and lesions may extend into middle or upper small intestine .(Plate 16).

Description of the Oocysts:

Oocysts are demonstrated microscopically,that are ovoid,with mean size $24.8\mu m(23.0 - 25.6) \log with \pm SD 0.7$ by $19.9 \mu m (17.9 - 20.4) \pm SD 0.4$, index(L/W) is1.2 μm . Oocyst wall is smooth.Micropyle absent.Steida body is not observed (Plates 17A and B).

The sporulated oocysts have four sporocysts which detected under the microscope .(Plate18)

Sporulation time : is from 3-5 days.



Plate (16) : Shows the lesions caused by *E.brunette* of illume .
Note :Awhite cheese-like material in the illume ,the mucosal surface is inflamed and bloody enteritis. Gut wall is thickened .



Plates (17 A and B): Show the non-sporulated oocysts of *E. bruneeti*. Sporonts (SPO)occupy the entire volume of the oocysts. Outer layer (OL) ,Inner layer (IL) and Zygot (Z). (X100).



Plate (18): Shows sporulated oocysts of *E.brunetti*, containing four Sporocysts(SPC).Outer layer(OL) and Sporozoite (SPR)(X100).

4.3. Histopathological Findings :

The histopathological examination of Haematoxylin and Eosin stained sections of intestinal tracts of infected broiler chicken with different *Eimeria* species show that there are multiple parasitic schizonts,gametes and oocysts in the mucosa and submucosa.There are invasion of the layers of intestinal wall by different inflammatory cells mainly lymphocytes and eosinophils . Small focal areas of necrosis in underlying connective tissues are seen . There are presence of small areas of haemorrhage and necrosis separating the underlying connective tissue .The epithelium may contain sufficient parasitized cells that can produce degenerating of surrounding connective tissue .

The infected intestinal walls show inflammation over 80 % of the distal areas, the lumen is filled with blood and shedding of mucosa. Edema and necrosis are seen in muscularis mucosa and submucosal areas. There are increased numbers of inflammatory cells(eosinphils,lymphocytes, monocystes and plasma cells).

Most of the mucosa, including the muscularis layer, is destroyed. The number of oocysts attached to the mucosa is increase, but later it becomes loosened and usually expelled to the outside. The lost miscularis mucosa is not replaced. Fibrosis is see in the submucosal layer. Both as exual and sexual forms of the parasites develop beanth the nuclei of the epithelial cells.

The number of schizont when increased and the degree of inflammation is found as severe transmural inflammation affecting all layers of intestinal wall in infected birds as see in plates (19A, B, C and D).



Plates (19 A, B, C and D): Tissue section of intestine in the broiler chickens with coccidiosis.Showing intestinal wall by different inflammatory cells and various stage of *Eimeria*:Oocyst (OO), Zygote (Z), Macrogamete (MAG), Microgamete (MIG), Nucleus (N) and Parasitophorus vacuole (PV).

4.4. Incidence and Type of Infection :

The results are show that Ninty two (10.22%) out of examined samples and infective samples(31.8%) have a single infection (infected with one species of *Eimeria*) and One hundred and ninty seven(21.88%) out of examined samples and infective samples (68.2%) have mixed infection (infected with more than one species of *Eimeria*).

The results show that high significant difference is detected between single and mixed and infection with *Eimeria spp.* (2 = 38.15, P-value = 0.000).(Table 3 and Fig. 9).

4.5. Incidence and Months :

The results reveal that the infection with *Eimeria* spp.is detected during nine months of the study from May-2009 to April-2010. High incidence rate is detected of examined samples in June (6.3 %) is followed by May (6.2 %),December (5.3%), November(4.5 %), August (4.4 %), March (2.4%), April (1.4 %) and January and February (0.6 %) each .In infective samples, the high incidence rates show in June (19.72 %) is followed by May (19.38 %),December (16.61 %), November (14.19 %), August (13.84 %), March (7.61%), April (4.49 %) and January and February (2.08 %) each .

There is a high significance differences between incidence of *Eimeria* infection and months. (2 = 106.35, P-value = 0.000) (Table 4 and Fig. 10).

 Table (3): Incidence of single and mixed infection of *Eimeria* spp. in

 examined and infected in broiler chicken farms :

Type of infection					
Single	infection	Mixed	infection		
% of total examined (N=900)	%of infected (N=289)	% of total examined (N=900)	%%of infected (N=289)		
10.22% (92)	31.8 % (92)	21.88% (197)	68.2 % (197)		

^{2 = 38.15}; P<0.05; df =1; P-value = 0.000**





Month	%Overall examined (N=900)	% Of infected (N=289)		
June	6.3%(57)	19.72 %		
Мау	6.2%(56)	19.38%		
December	5.3%(48)	16.61%		
November	4.5%(41)	14.1 9%		
August	4.4%(40)	13.84%		
March	2.4%(22)	7.61%		
April	1.4%(13)	4.49%		
January	0.6%(6)	2.08 %		
February	0.6%(6)	2.08 %		
Total	32.1%(289)	100 %		

 Table (4): Incidence of *Eimeria* spp. in examined and infected and

 months in broiler chicken farms in Ghot El- Sultan project :

2 = 106.35; P<0.05; df =1; P-value =0.000****



Figure (10): Incidence of *Eimeria* spp. in examined and infected and Months in broiler farms in Ghot El- Sultan project.

4.6. Incidence of *Eimeria* species infection according to months and the six types of *Eimeri spp.* infection :

Relationship between incidence rate of *Eimeria* spp.infection and months. The results show that no significant difference exites between the type of *Eimeria spp*.and months. (*E.acervulina* p–value = 0.998, *E. necatrix* p – value = 0.416, *E.maxima* p–value = 0.981, *E. brunetti* p–value = 0.981, *E.tenella* p– value = 0.416, and *E. mivatti*, p– value = 0.437).

(Table 5 and Fig. 11).

Months	Types of <i>Eimeria sp.</i> infection (%)					
	E. acervulina	E. necatrix	E. Maxima	E. tenella	E. mivatti	E. brunette
January	2.0%(6)	0.0%(0)	2.0%(6)	0.0%(0)	0.0%(0)	0.0%(0)
February	2.0%(6)	0.0%(0)	2.0%(6)	0.0%(0)	0.0%(0)	0.0%(0)
March	7.6%(22)	0.0%(0)	3.4%(10)	0.0%(0)	5.4%(6)	0.0%(0)
April	4.4%(13)	0.0%(0)	1.7%(5)	0.0%(0)	1.3%(4)	0.0%(0)
May	12.8(37)	7.9%(23)	9.3%(27)	5.55%(16)	6.9%(20)	9.6%(28)
June	12.4%(36)	11.0%(32)	9.3%(27)	17.3%(50)	8.3%(24)	8.9%(26)
August	10.3%(30)	6.5%(19)	4.8%(14)	6.2%(18)	8.6%(25)	4.8%(14)
November	14.1%(41)	8.3%(24)	9.6%(11)	7.6%(22)	8.6%(25)	3.4%(10)
December	14.8%(43)	9.6%(28)	4.4%(13)	4.4%(13)	00.0%(0)	2.4%(7)
Total	81.0% (234)	43.65% (126)	41.2% (119)	41.2% (119)	36.05 (104)	29.4 (85)

 Table (5): Incidence of *Eimeria* spp. infection in broiler chicken according to type of *Eimeria* infection and months (N=289):

E. acervulina : 2 = 0.778 , P >0.05 , df = 7, p- value = 0.998 (Non Sig).E. necatrix : 2 = 5.000 , P >0.05 , df = 5 , p - value = 0.416 (Non Sig).E. maxima : 2 = 1.111 , P >0.05 , df = 6 , p- value = 0.981 (Non Sig).E.tenella : 2 = 5.000 , P >0.05 , df = 5 , p- value = 0.416 (Non Sig).E. mivatti : 2 = 2.333 , P >0.05 , df = 5 , p- value = 0.801 (Non Sig).E. brunetti : 2 = 5.000 , P >0.05 , df = 5 , p- value = 0.416 (Non Sig).



Figure (11):Incidence of *Eimeria* spp. infection in broiler chickens according to type of *Eimeria* infection and Months (N=289).

4.7 . Incidence and Seasons :

In the present study, the results reveale that season have effect on the incidence rate of examined samples and infected .The highest incidence rates in the examined samples are recovered during Summer at (10.77%) is followed by Spring at (10.11%) and Winter at incidence rate (6.66%) and in Autumn is found at (4.55%). In the infective samples ,the results are recovered during Summer at (33.6%) is followed by Spring at (31.5%) and Winter at incidence rate (20.8%) and in Autumn is found to be at (14.2%)

The results show that there is a high significance difference is detected between seasons and incidence rate of *Eimeria*. (2 = 28.94, P=value =0.000) (Table 6 and Fig.12).

Seasons	%of examined (N=900)	%of infected (N=289)
Summer	10.77%(97)	33.56 %
Spring	10.11(91)	31.49 %
Winter	6.66%(60)	20.76 %
Autumn	4.55(41)	14.19 %
Total	(32.1%)289	100 %

Table (6) : Seasonal incidence of *Eimeria* spp. infection in totalexamined and infected in broiler chicken farms :

2 = 28.94; P<0.05; df =3; P-value = 0.000*



Figure (12): Seasonal incidence of *Eimeria* spp. infection in total examined and infected in broiler chicken farms.

4. 8. Relationship between the incidence of six *Eimeria* spp. infection and Seasons :

In the present study, the results show that the high infection rate is detected in summer, with *E.acervulina* 28.2% (66/234); *E.necatrix*40.5% (51/126); *E.maxima* 34.5% (41/119); *E.tenella* 57.1% (68/119); *E.mivatti* 47.1% (49/104) and *E.brunetti* 47.1% (40/85).

In spring ,the incidence rates of *Eimeria* spp.are detected with *E.acervulina* 30.8% (72/234) ; *E.necatrix* 18.3% (23/126); *E.maxima* 35.3% (42/119); *E.tenella* 13.4% (16/119); *E.mavitti* 28.8% (30/104) and *E.brunetti* 32.9% (28/85).

In winter, the incidence rates of *Eimeria* spp.are detected with. *E.cervulina* 23.5% (55/234); *E.necatrix* 22.2% (28/126); *E.maxima* 21.0% (25/119); *E.tenella*10.9% (13/119); *E.mivatti* 0.0% (0/104) and *E.brunetti* 8.2% (7/85).

In an autumn,the incidence rates of *Eimeria* spp. are detected with *E.acervulina* 17.5% (41/234); *E.necatrix* 19.0% (24/126), *E.maxima* 9.2% (11/119); *E.tenella* 18.5% (22/119); *E.mivatti* 24.0% (25/104) and *E.brunetti* 11.8% (10/85) .

The results show that a high significance differences are detected between the types of *Eimeria* spp. infection and seasons(2 = 80.92, P-value = 0.000).(Table 7 and Fig. 13).

Table (7): Relationship	between the incidence	of <i>Eimeria</i>	spp. infection
and seasons	(N=289):		

suos	Infection of <i>Eimeria sp</i> . (%)					
Sea	E.	E.	E.	E.	E.	E.
	acervulina	necatrex	maxima	tenella	mivatii	brunetti
Summer	28.2 %	40.5 %	34.5%	57.1%	47.1%	47.1%
	(66)	(51)	(41)	(68)	(49)	(40)
Spring	30.8 %	18.3%	35.3%	13.4%	28.8%	32.9%
	(72)	(23)	(42)	(16)	(30)	(28)
Winter	23.5 %	22.2%	21.0%	10.9%	0.00%	8.2%
	(55)	(28)	(25)	(13)	(0)	(7)
Autumn	17.5 %	19.0%	9.2%	18.5%	24.0%	11.8%
	(41)	(24)	(11)	(22)	(25)	(10)
Total	80.98%	43.39%	41.17%	41.17%	35.98%	29.41%
	(234)	(126)	(119)	(119)	(104)	(85)

2 = 80.92 ; P < 0.05 ; d f = 15 ; P-value = 0.000^{*}



Figure (13): Relationship between the incidence of *Eimeria* spp. infection and Seasons (N=289).
4. 9. Incidence of Single and Mixed Infection and Seasons:

The highest single infection rates 41.3% (38/92) is detected in spring .Followed by summer at 31.5%, in winter at 7 % and the lowest infection rate (6.5%) is detected in autumn,while the highest mixed infection rates (34.5%) is detected in summer is followed by in spring at 26.9%, followed by winter at 20.8% It shows in winter and the lowest infection rate at 17.8% in autumn.

The results show that there is a high significant differences between the rate of infection and type of (mixed and single) infection .(2 = 9.888, P-value =0.020) (Table 8 and Fig. 14).

Infection		Total										
	Summer	Spring	Winter	Autumn								
Single N	29	38	19	6	92							
N=900	3.2%	4.2%	2.1%	0.6%	10.2%							
N=289	31.5%	6.9%2	20.8%	6.5%	31.8%							
Mixed N	68	53	41	35	197							
N=900	7.5%	5.8%	4.5%	3.8%	21.8%							
N=289	34.5%	41.3%	20.7%	17.8%	68. 1%							
Total	33.5%(397)	31.5%(391)	20.8%(260)	14.2%(41)	32.1%(289)							

Figure (14): Incidence of	a single	and	mixed	infection	of	broiler	chickens
and season :							

2 = 9.888; P<0.05; df =3; P-value =0.020⁺



Figure (14) : Incidence of a single and mixed infection of broiler chickens and season.

5. Discussion

Since 1970 the poultary production is the fast growing in the meat industry in the worldwide ,because good feed conversion in comparison to other animals species,low fat and high protein content,low price and fast production which mean a short generative time (Long and Jeffers,1986; Windhorst,2006and Lilic *et al* .,2009).

Avian coccidiosis is one of the most important and common disease caused by various species of *Eimeria* a microscopic protozoan parasites . The infection characterized by diarrhea, listlessness and variable levels a mortality in the affected birds. It is an economically important disease of the poultary industry (Braunius ,1980; Magner,1991; Williams, 1995; Kinung'hi *et al* , 2004 ; Kiani *et al* .,2007 ; Zulpo *et al*., 2007 Lee *et al*,2009andVolkers *et al*.,2010).

Coccidia of the genus *Eimeria* causes the most widespread health problems in the broiler industry and remains one of the most expensive diseses of commercial poultary production (Henken *et al* .,1994 and Yun *et al* .,2000).Birds infected with coccidial oocysts do not perform as well as non-infected birds as a result of moderate to severe damage to intestinal mucosa,birds exhibit decresed body weight gains,increased feed conversion , and in some cases,birds may appear asymptomatic,but are limited in their ability to maximize feed efficiency.According toEdgar(1992) mentione that it takes only one viable oocyst to establish the presence of coccidia in the poultary house .

The present study is the first report conducted on the presence of the chicken *Eimeria* species in Ghot EL-Sultan project in Libya up till now.On the other hand many studies are detected on commercial poultary farms in many countries throughout the world (Jeffers,1974; Long and Rowell, 1975; Macpherson,1978; Dar and Anwar,1981; Braunius,1988;Williams *et al* .,1996; Koinarski *et al*.,1997;McDougald *et a* .,1997;Thebo *et al* .,1998; Razmi and Kalideri,2000; Al-Natour *et al*.,2002; Ayaz *et al* 2003; Su *et al* ., 2003; Khan *et al*., 2006; Adhikari *et al* ., 2008; Nematollahi *et al* .,2009; Sun *et al*., 2009 and Lee *et al* .,2010).

5.1. Incidence of Coccidian (Eimeria spp.) Infection :

The present study is conducted on nine hundred of intestinal tracts obtained from nine broiler chicken farms in Ghot EL-Sultan project from June,2009 to April, 2010. The total intestinal tracts are examined to determine and idenfication the incidence rates of *Eimeria spp*. infection in the broiler farms.

The results in the present study reveale that the general incidence rate of *Eimeria* spp. is found to be 32.11%(289/900) samples are found infected, and 67.88% (611/900) samples are free of the infection. These *Eimeria* spp. parasites are natural and common intestinal infection of chicken hosts .

According toJeffers(1974)who indicate that from1308 of litter samples from all broiler- producing regions of the United States are infected with incidence at 89 % (out of 1166).

Razmi and Kalideri (2000) reporte that the prevalence of infection in Mashad, Khorasan, Iran is 38 % (out of 84 farms).

About of 50% (of 200 broiler farms) of the broiler farms in north Jordan surveyed had all six chicks infecte of *Eimeria spp.* are recovered from infection chicken in northern Jordan, and 33% of the farms are free of the infection (Al-Natour *et al.*, 2002).

On the other hand, the incidence rate of broilers infection in Islamabad, Pakistan is 71.8 % out of 359 gut samples, a dministrate by Khan *et al* .(2006) .Lobago *et al*.(2005) reporte that out of the 965 dead chickens, 370(38.34 %) are found infected with coccidiosis, that from Ethiopia.Nematollahi *et al* .(2009)reporte that the broiler farms inTabriz, Iran is infected with *Eimeria spp*. at the prevalence rate 55.96 % .

The highest incidence rate of eimeriosis in broiler chickens in the present work, may be associated with crowding factors. Intensive rearing particularly predisposes condition for coccidiosis, because large farms require more water ,feed, litter and generate greater volumes of feces may be contain a high number of oocysts in the litter. They may be represent more potential sources of infection. Hence, the moist conditions favour outbreaks of coccidiosis, and one of the factors is believe to be a more efficient for sporulation of the oocysts (Card and Nesheim, 1972 and Matter and Oester, 1989). Coccidiosis is the disease of poor management practices in the house reared chickens may be a direct cause for such a high incidence.(Sarker, 2006 ; AL-Quraishy *et al.*,2009; Nematollahi *et al.*,2009 and Chapman *et al.*,2010)

The number of oocysts eaten, strain of coccidia, environmental factors, site of development within the host, age of the bird and nutritional status of the host are acts as influencing factors for the development of the coccidiosis (Narcin *et al.*,1983and McDougald,2003).

It is reporte that the disease is more common at the farms where the poultary reared under intensive production system. In the case of extensive poultary farming the source of infection is one bird, litter is wet, chicken hygiene, mechanical routes such as boots, dust, clothes, wheels, contaminated equipment and personal and not managed properly (Hammond, 1973; Duguette, 2005; Kiani *et al.*, 2007andChapman, 2009).

Clinical is now recognized as a problem associated with growing large numbers of birds in limited areas. Confinement permits the rapid accumulation of the large numbers of oocysts required to produce clinical coccidiosis ,whereas each oocyst ingested by a host has the potential to give rise to hundreds of thousands of oocysts within the feces after seven to twelve days(Braunius,1980andRuff,1999). In the present study,the age of examined chickens are in the range between 42–49 days old. These age group may be very susceptible to coccidial infections.As similar increase in the incidence of *Eimeria* infection occurs at 4- 6 weeks of age has been showen by previous studied(Long *et al.*,1975; Braunuis,1982; Reyan *et al.*,1983; McDougald and Reid ,1991; Hofstad ,1992; McDougald *etal.*,1997; McDougald ,1998; Costa *et al.*,1999;Razmi and Kalideri,2000;Chapman,2003;Adhikari *et al.*,2008, and Bould *et al.*,2009).

Nematollahi *et al* .(2009) show that the age wise prevalence is the highest 48% in the31- 45 days age group and the least 6% in 0-15 days age groupsof chickens.Khan *et al* .(2006) reporte that the Eimeriosis disease is more common in the birds of 22 - 42 days of age at(70.75 %).

Oocyst counts in litter of commercial poultary houses are very low during the first or last weeks of broiler grow out but are high during the normal 4 - 6 weeks period. This period is very susceptible to coccidial infection, and showen the highest prevalence of infection. Also the possible reason for this broiler ages(4 - 5 weeks) may be due to the birds have not founded immunity against coccidiosis, resulting increased incidence of the disease, and high antibody levels against *Eimeria* spp. parasites are detected 2 weeks after the infection (Lillehoj and Ruff, 1987 and Constantinoiu *et al*., 2007).

5.2. Coccidia(*Eimeria spp.*) In the Present Study :

The results obtained from the present study show that the intestinal tracts are found to be infected with six species of *Eimeria*, these are *E*.*acervulin*, *E.necatri*, *E.maxima*, *E.tenella*, *E.mivatti* and *E. brunette*. These obtained is agreement with previously reporte results(Jeffers, 1974; McDougald *et al*., 1997; Williams, 1998; Al-Natour *et al*., 2002; Su *et al*., 2003; Khan *et al*., 2006; Sun *et al*., 2009 and Lee *et al*., 2010), They recorde that the same species of *Eimeria* infected domestic poultary in all over the world

The detected species in the present study are somewhat resemblance with other the records obtained from many countries, In Uniated states (Jeffers, 1974 and Gorden and Jordan , 1982), In Brazil (Franco , 1993), In Sweden (Thebo *et al.*, 1998) and In Nepal(Adhikari *et al.*, 2008). These identified *Eimeria* species except *E. necatrix* is reporter by Thakuri and Rai (1996) In the local chickens of eastern hills of Nepal . Except *E. mittis* , this results is in agreement with results reported from Swedish chickes by Thebo *et al* .(1998). Similar results are obtained by Williams *et al* .(1996) ; Al-Natour *et al* .(2002) and Nematollahi *et al* .(2009) .

Except *E. maxima* and *E.mivatti*, the present results is similar to at reporte from Ethiopia by Lobago *et al.*(2005).Except *E. mivatti*, this results is in agreement with the results are done by Su *et al* (2003) from Taiwan by using PCR methods.Except *E.praecox* and*E.mitis*, a similar to results obtained by Kutkat *et al.*(2009) from Sharkeia,Fayoum and Giza in Egypt .

Sun *et al* .(2009) regarde that seven of *Eimeria* spp.exite in most faecal samples collected from broiler chickens at50farms in Shandong ,China, and Lee *et al*.(2010) by used PCR method,They reporte that seven species of *Eimeria* .are detected in all the positive farms in different regions of Korea .

Eimeria species are identified basis on thecharacteristic of the lesions seen, shap and size of oocysts, the location of infection, and the sporulation time of oocysts will gives a good indication of the species of *Eimeria* concerned .The same is used befor for many studies(Joyner and Nortan ,1975; Joyner and Norton, 1984; Gorden and Jordan, 1982; Karim and Boegun ,1994; McDougald *et al* ., 1997; Mattielo *et al*., 2000; Adhikari *et al* .,2008 and Chapman *et al* .,2010).

The examination of oocysts morphology in the present study are examined based on certain significant factors, such as the shape and size of the oocysts ,the oocyst wall, the presence or absence of the micropyle, polar granule , retractile body, and oocyst residuum as described by Johnson and Reid(1970); Long and Reid, (1982) and Tsuji *etal.* (1997). The coccidian infection is diagnosed by determining oocysts in the faces or intestinal scrapings (Mattielo *et al.*, 2000; Allen and Fetter, 2002 and Shareef, 2010).

Identification of *Eimeria* spp.is done on the morphology of the oocysts and on the site of observed lesions(McDougald and Reid,1991; Calnek , 1997 ; Larry,1998 and Badrani andLukesova , 2006). On these basis, six species of *Eimeria* are obtained in the present studies, and the characteristic of the six species of *Eimeria spp*. are compared to those of the similar previous studies which conducted to determine the identification of coccidian (*Eimeria* spp.).

5.2.1. E. acervulina :

The present results reveale that *E.acervulina* is the commonest and with the highest infection rate (80.97 %). These results are in corresponded to many results obtained previously. These results agreement obtained by Jeffers (1974) who recorde that *E.acervulina* in broiler farms from United States at incidence rate 90.6 %. McDougald *et al*.(1997) reporte that the incidence rate of *E.acervulina* is (93 %) in broiler farms in Rios and Benos of Argentina ,Razmi and Kalideri(2000) indicate that most broiler farms from Khorsan , Iran(97 %) have *E.acervulina*, Fitz-Coy (2005) reveale that the incidence of *E.acervulina* is(97 %) in United State, Nematollahi *et al*. (2009) mentione that the incidence rate of *E.avervulina* infection is(52 %) in Tabriz, Iran .Koinarski *et al*.(1997) reporte that the incidence rate of eimeriosis is about (20 - 50 %) of the poultary populationin Bulgaria and *E.acervulina* infection rate is(18 %)

Lee *et al.*(2010)founde that the prevalence rate of *E.acervulina* is the highest(87.5%) of the tested farms are positive for *Eimeria* infection in different regions of Korea. These results may confirm that *E.acervulina* have a high biotic potential ,the prepatent (4- 6 day) and sporulation periods are short and the schizogonous cycle of *E.acervulina* is found to consist of four generations.very large numbers of oocysts are showed in theinvestigated samples in this study. The highest incidence of *E.acervulina* may be due to its the commonest *Eimeria* organisms in broiler chickens (Thebo *et al.*,1998 and Adhikari *et al.*,2008).

On the other hand, these resultes is discordant to report mentione by Lobago *et al.*(2005) they indicate that *E.acervulina* in chicken farms from Kombolcha,Ethiopia at prevalence rate(9.7%),Adhikari *et al.* (2008) obtained that prevalence rate of *E.acervulina* infection is (5%) in Nepal . Al-Natour *et al.*(2002)reveale that the incidence rate is(3%)of *E.acervulina* in northern Jordan. The present results also disagreed with the results is obtaine by Khan *et al.*(2006), They are not found *E.acervulina* infection in gut samples of broilers in Islamabad, Pakistan .

The highest incidence of *E.acervulina* may be due to it's the most common of the nine species of *Eimeria* and is prevalent throughout the world. The lesions are limited to anterior or first third of the small intestine (Williams, 1995; Allen and Fetter, 2002, and The Merck Veterinary Manual, 2008).

The predominance of ubiquitous *E.acervulina* is confirmed in broiler chickens and represents the commonest *Eimeria* spp.(Braunius ,1986 ; Jordan and Pattison ,1996 ;Williams,1999 and Razmi and Kalider. 2000). That may be due to the encystation process of *E.acervulina* is quickly which developed in the duodenum of small intestinal tract and reproduction potential from 4 to 6 days(Koinarski *et al.*, 2005). In most regions of the world , *E.acervulina* is the most commonly encountered species in broiler flocks(Conway and McKenzie ,2007).

Characteristic lesions and morphology of oocysts reporte in the present study showen that 17.4 μ m(15.3 - 20.4) long and about14.8 μ m (12.8 -15.3) wide,this consonance with results obtained by McDougald *et al.*(1997) showed that oocysts are ovoid and measured at 14 x 18 μ m and these founded is described previously byTyzzer(1929) who detected that*E*. *acervulina* oocyst is oval in shape and its measurement is17.7- 20.0 X 13.7 – 16.3 μ m, with micropyle, polar granule and without oocyst residume , also somewhat resembled obtained by(Edgar and Seibold,1964; Johonson and Reid,1970 and Long and Reid,1982).

E.acervulina. is detected in the present study is recovere from the appearance of characteristic lesions and their limited site to anterior of the small intestine(duodenum). These results may indicate that this *E acervulina* has a higher degree of site specificity of intestinal tracts. Lesions observed are with numerous greyish – white, pin-point or transverse patches, these are with visible from the surface of the duodenum. Similar lesions for *E.acervulina* is observen in previous studies(Jeffers, 1974; Witlock and Ruff , 1977; Ruff and Wilkins, 1980; McDougald *et al* ., 1997; Razmi and Kalideri , 2000 and Conway and Mckenzie, 2007).

Nemmatollahi *et al.*(2009) confirme that the entire villus tip is removed exposing the lamina propria core and oocysts are found within the damaged epithelial cells surrounding the lamina propria core which causes nutrient malabsorption, and reduced of weight gain .Koinarski *et al.*(2005) demonstrated that *E.acervulina* as early as the5 th post infection day caused significant damage to the intestinal tract.

Assis *et al.*(2010) show that intestinal villus measurements and absorptive area are directly affected by *E.acervulina* and that there is direct and positive correlation between the macro and microscopic findings observed in intestinal coccidiosis. *E.acervulina* causes shortening of villi and reduction in the intestinal absorptive area, affecting broiler growth.

E.acervulina infections ,one of the milder and most common species of the coccidian ,and causing extensive economic losses in poultary industry throughout the world and this is confirmed with (Oikawa and Kawaguchi , 1971 ;Weber ,1997 and Conway and McKenzie , 2007). In some regions, the infection rates with *E.acervulina* are higher than provoked by *E. tenella* (Jeffers, 1974 ; Kucera, 1990 ; Williams *et al.*, 1996).

5.2.2. *E.necatrix* :

E.necatrix is recovere from jejunum samples at incidence rate (43.59 %) of positive examined broiler guts . Al-Natour *et al.*(2002) recovere that the prevalence of *E. necatrix* is(12 %)from (50 %) of the broiler farms in Jordan infected. On the other hand,Khan *et al.*(2006)showen that the incidence rate of *E.necatrix* is (7.75 %) in Islamabad ,Pakistan . Adhikari *et al.* (2008) reporte that out of 125 samples are found tobe positive for *E. necatrix* with prevalence rate (10 %) of chicken .Sun *et al.*(2009) They mentione that the incidence infection in the Shandong , China is (26 %).

Lee *et al* .(2010) mentione that the prevalence rate of *E.necatrix* is found at (62.5 %) from positive fecal samples examined from 356 chicken farms are collected randomly from different regions of Korea.

Concerning other reports, Lobago *et al.* (2005) detecte that in dead chickens low infection rates, at Ethiopia with prevalence is (4.1%) found infected with *E. necatrix*, and Jeffers (1974) confirme that out of 1166 from litter samples in broiler producing regions of United States with incidence rate at (0.4%), may due to climate, crowding ,and management factors .

The gross lesions are exhibit in this study are pin- point red and white spots observed from both serosal and mucosal side in the mid gut of the intestinal samples. It is markedly swollen, haemorrhagic, red or brown mucus , and the contents filled with blood. The same results is obtained by Johnson and Reid(1970); Long and Reid(1982); McDougald *et al*.(1997) and Thebo *et al*.(1998).

The ocysts are obtained from jujenum, described that broadly ovoid in shape . Size measured, about 20.1 μ m (17.9 - 23.0) long by 18.8 μ m(17.9 - 20.4) width and with index (L/W)1.1 μ m.Oocyst wall is smooth, micropyle and oocyst residuum are absent, but polar granule present, this observation are in greement with previous studies (Johnson, 1930 and Thebo *et al.*, 1998).

E.necatrix is one from two *Eimeria* spp. parasitize the jejunum of the bird. It causes a more chronic disease, and impair the bird's ability to absorption of nutrients and physiological change in the jejenum. It is considered as the most pathogenic species of *Eimeria* in domestic poultary. Infection of this species has particular feature compare with the nine other species in chicken, asexual stage development in the small intestine and its sexual stage development in the ceaca . *E.necatrix* causes of ruptured villar epithelium resulting in exposure of the lamina propria, which allow leakage of blood components into the lumen causing blood streak intestinal contents. Birds heavily infected with *E.necatrix* may die before any marked infect is noticed in weight or before blood is found in the feces(Stockdale and Fernando ,1975; Witlock and Ruff, 1977 and Conway and McKenzie , 2007).

5.2.3. *E. maxima* :

E.maxima is diagnosed in 119 gut specimens, with incidence rate at (41.17 %) out of 289 positive guts examined. This findings of the present study are correspond to those results founde by Razmi and Kalideri (2000) They record that the *E.maxima* is reporte from broiler chickens from Mashhad, Iran with prevalence rate at (41%). In broilers *E.maxima* infection shows the highest prevalence (34.1%), is reported by Khan *et al*.,(2006) in Pakistan.

McDougald *et al*. (1997) conducted a survey on *E.maxima* infection in 43 broiler farms in the Entre Rios and Buenos. Aires districts of Argentina .The infection rate is at 42 % of the examined samples for coccidia. Lee *et al*.(2010) detecte that *E.maxima* is found to be at 31.3% of faecal samples from 356 chicken farms in Korea.

Jeffers (1974) conferrs that the incidence rate of *E.maxima* is (86 %) out of 1166 positive samples in United States . The results show that the prevalence of *E.maxima* is found to be at (68 %),out of 50 small-scale farms in China (Sun *et al* .,2009).

Fitz-Coy (2005) reveale that the incidence of *E.maxima* (64%) in United State. In most regions of the world, *E.acervulina* and *E.maxima* are the most commonly encountered species in broiler flocks (Conway and McKenzie 2007).

Titilincu *et al.*(2007) indicate that *E.tenella*; *E.acervulina*; *E.maxima*; *E.mitis*; *E.necatrix* and *E.brunette* are the most frequent of the eimerian species that parasitize in hen ,three of them that are *E.acervulina*; *E.tenell* and *E.maxima* are frequently found in broiler farms.

On the other hand, the present results are not corresponded to the results conducted on the prevalence rate of *E.maxima* from 218 broiler farms in Tabriz, Iran is at (12 %) (Nematollahi *et al.*, 2009). Al-Natour *et al*. (2002) obtaine that the prevalence rate of *E.maxima* among broiler chicks is (10 %), out of 200 broiler farms in northern Jordan. Adhikari *et al.*(2008) founde that (5 %) infection of *E.maxima*, out of 125 samples are positive in Ratnangar Municipality and Chitwan District, Nepal.

The appearance of lesions characteristic, their site in the small intestine, large oocysts are confirmed that *E.maxima*. There is production thickened mucosa, the mucosal surface is inflamed and the intestinal contents consist of a pinkish mucoid exudates. The content samples collected is found to contain oocysts seen in the mucoid exudates characteristic of *E. maxima*. Similiar observation obtained by Witlock and Ruff (1977); Long and Reid (1982) and Thebo *et al*. (1998).

The detected oocysts in the present study are ovoid in shape .They are measured is about 25.08 μ m by 18.43 μ m. Most closely resemble as the same is observed previously (Witlock and Ruff , 1977 and Long and Reid , 1982) who indicate that *E.maxima* oocysts are large,brownish ,ovoid oocysts of which were longer than 30 μ m .Tyzzer (1929) demonstrate that the shape of oocyst is ovoid, with polar granule and oocyst residuum absent, oocysts is measured is 27.0 – 34.4 X16.0 – 28.0 μ m.

On the other hand ,Norton and Helen (1976) reporte that the oocyst of the weybridge and houghton strains of *Eimeria* and afresh field isolate are similar .The are mreasured on average 30.9 X 22.4 μ m .McDougald *et al* .(1997) is mentioned. that the oocysts are measured with 20 X30 μ m .

E.maxima, infects the chick jejuna mucosa, causes reduced weight gain and nutrient malabsorption due to sloughing and villous atrophy. Intestine loses tine and becomes flatten and dilated (Ruff and Wilkins ,1980). In most rgions of the world, *E.maxima* is the most commonly encountered species in broiler flocks (Conway and Mckenzie ,2007).

5.2.4 . E. tenella :

E.tenella is detected in 119(41.17%) out of 289 positive samples are examined. The results in the present work are relatively similar to results founded in Kombolcha poultary Ethiopia, incidence rate(40%)out of 370(Lobago *et al*., 2005). As well as the found by Al-Natour *et al*.(2002) report that the incidence of infection in 200 broiler farms in Jordan with prevalence rates at (39 %).

On the other hand ,the incidence rate of infection in 50 broiler farms of China is of the order of 90 % (Sun *et al*., 2009).Fitz-Coy (2005) reveale that the incidence of *E.tenella* is 64% in United State .Lee *et al*.(2010) mentione that *E.tenella* is at 62.5 % out of 356 fecal samples from chicken farms in Korea.

Jeffers (1974) found that the incidence rate of *E.tenella* infection in United States is of the order of 28.4 %. Great *et al.* (1996) investigate the incidence rate of infestation in poultary from the Netherlands are found the *E.acervulina* and *E.tenella* infection found at 63%. McDougald *et al.* (1997) reveale that the *E.tenella* is suspected in 14 % of 43 samples ,in Argentina.

The appearance of special lesions in the ceaca pouch, their site are indicated of *E.tenella* in the ceca pouches (localized to the ceca only). A similar lesions characterstic are observed in previous studies by(McDougald *et al.*, 1997and Cornelissen *et al.*, 2009).

Lesions is obtained in the ceca samples is characterized by accumulation of clotted blood in the lumen, due to its extensive destruction of mucosa with histological lesions (Baba *et al.*, 1987 and Olimpia and Duma , 2009).

E.tenella is a one of the most common and pathogenic coccidian observation. It is considered to be comparable to the one in the present study . It may indicate that *E.tenella* have a higher degree of lesions and location specificity of chickens .The highly pathological changes which are discussed previously by Calnek(1997); Yadav and Gupta(2001); Zulpo *et al* .(2007);Olimpia and Duma(2009)are mainly due to the second generation schizonts .

The resulte show the measurement of oocysts (23.0 μ m in length by 19.9 μ m in width.In the present study is somewhat in agreement with the founded by Al-Quarishy *et al* .(2009).It detects oocysts measurements as 21.6 μ m X 19 μ m .

On the other hand, the reports reveal by Railliet and Lucet (1891) and Edgar(1955) found that oocyst is ovoide with micropyle, polar granule and without oocyst residuum. Measurement is $19.2 - 26.0 \times 16.0 - 22.0 \mu m$. thickness of oocyst wall $1.5 \mu m$. 24.4 X 18.2 μm long.

5.2.5. E. mivatti :

The results obtained from the present study is examined show that 104 out of 289 positive examined samples is infected with *E.mivatti*. It representes an incidence rate at 35.98 % .This result is agreement with the results reported by Fitz-Coy (2005) who reveale that the incidence of *E. mivatti* is as high as 35% of broiler flocks from Georgia, Southand North Carolina, Virginia, California, Texas and Arkansas. On the other hand, Edgar and Seibold (1964) report that during a persistent coccidiosis outbreak on poultary farms in Florida, the incidence rate of *E. mivatti* is 50 % .

These result show disagree with the results reported by AL-Natour *et al* .(2002). They find that the prevalence rate of *E.mivatti* is 2%. Some species of coccidia in broiler flocks with a somewhat lower incidence of *E. mivatti* infections is less likely to be observed in broiler flocks. Possibly because of the shorter growing time of broiler birds(Conway and McKenzie, 2007).

In this study the collected *E.mivatti* oocysts are ellipsoidal to broadly ovoid in the shape and oocyst measurements is $14.0 - 16.6 \times 11.5 - 12.8 \mu m$. The same measurments is obtained from Florida, Canada by Edgar and Seibold (1964) describe that the oocysts are ellipsoidal or broadly oval ,with micropyle and polar granule . the measurement are $13.7 - 17.0 \times 10.1 - 15.3 \mu m$. The same characteristic for oocysts of *E.mivatti* are reported by Witlock and Ruff (1977) and Thebo *et al*.(1998).

E.mivatti is the most recently described species of chicken coccidian. It is developed in the anterior third of small intestine, but infection extends from duodenum to rectum. Intestines are slightly swollen ,oedematous ,congested with scattered petechiae and contents. They are white orcreamy. These observations are in agreement with results described by (Edgar and Seilbold ,1964; Witlock and Ruff, 1977 and Conway and Mckenzre, 2007).

5.2.6. *E. brunette* :

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The exanimation of 289 infected samples show that *E.brunette* is detected in 85 samples with an incidence rate at 29.41 % .The high incidence rates of *E.brunette* at 59.3% of fecal is sampled from 356 of chicken farms in Korea (Lee *et al* .,2010). Lobago *et al*.(2005) mentions that *E. brunetti* is reported during the firest time in Ethiopia with prevalence rates at 45.3 % .

The result findings show disagreement with that obtained by Jeefers (1974). It is reported that the incidence of *E.brunetti* infection found to be at 2.3 %, out of 1166 litter samples from all major broiler producing regions of United States .McDougald *et al* .(1997) mention that out of 43 poultary farms examined found to be at 5% are typical of *E.brunette* in Argentina .Similar result are obtained by Adhikari *et al* . (2008) in Nepal

Sun *et al.*(2009) indicate the prevalence of *E.brunette* is of the order of 8% out of 50 farms. The prevalence rates of *E.brunette* is 12% of 200 briolerfarms examined in Northern Jordan(Al-Natour *et al.*,2002). Mattielo *et al.*(2000) found that *E.brunette* is found in 4 samples from 10 litter samples. These are examined for presence of ther *Eimeriaspp.*in Argentina. *E.brunette* is encou- ntered more rarely than other species (Kucera, 1990 and Williams *et al.*,1996). Some species of *Eimeria* in broiler chicken farms, such as *E.brunette* is reported at low rates , possibly because of the shorter growing time of broiler birds (Conway and McKenzie, 2007).

The present study shows that the appearance of lesions is in the lower intestine extending down into the large intestine and rectum .This site indicate the *E.brunette*. Long (1964)reveale that *E.brunette* infection detected from the characteristic lesions between the ceca.

In the present study the middle and lower small intestine and rectum, show a white cheese like material ,some reddening of the mucosal surface caecum and colaca are inflamed. In some infection the gut wall is thickened . Asimilar observations is reporte by Long and Reid (1982) and McDougald and Reid (1997).

E.brunette oocyst is ovoided in shape and with mean size 24.8 X 19.9 μ m. These results do represent somewhat correspond to those describe byLevine (1942). It reveals that *E.brunette* oocysts are oval in shape. Its size is 24.0-30.0 X 20.0 – 23.0 μ m and with polar granule.

According to Adhikari *et al.*(2008) show that the oocyst measurement are 23.75 X 19.52 μ m. The same descripe is showed by Boles and Becker (1954); Long and Reid(1982) and Thebo *et al.*(1998)

5.3. Types of Infections (Single and Mixed) :

The present study reveals that the incidence rate is 31.83 % of infected with a single type of infection ,while 68.16 % samples are found is infected with mixed type. A similar results obtained by Williams (1998) ; Aryal (2001) ; Ali *et al.* (2006); Cornelissen *et al.*(2009) and Sun *et al.*(2009). Mixed of intestinal protozoan parasites appear to be a characteristic of parasitic infections. There is a seasonal effect up on incidence rates of *Eimeria* spp. infection .

The results in the present study, coincide with the one found by Adhikari *et al* .(2008). Is reporte the incidence rates of mixed infection is(64%). This is report as mentioned in Nepal . Nematollahi *et al*.(2009) showe that 122 brioler farms out of 218 farms have mixed infection inTabriz, Iran.

Infection with a single species of *Eimeria* is rare in natural conditions, and mixed infections being the rule and common. Mixed infection of intestinal protozoa parasites appear to be a characteristic of parasitic infections and common in coccidia infection. This may be due to obligator nature of the species of coccidia (Williams, 1998; Ali *et al.*, 2006; Kutkat *et al.*, 2009 and Nematollahi *et al.*, 2009).

The highest incidence rate of mixed infection may be due to opportunistic nature of the mild pathogenic species of *Eimeria*.i e.*E.maxima* and*E. acervulina* which starts infection in the bird under sufficient stress due to initial infection with pathogenic species.(Williams, 1999 and Adhikari *et al* .,2008).

According to Williams (1999) demonstrats that the pathogenic species have a cosmopolitan distribution and can cause infection simultaneously. Thus, d isease caused by *Eimeria* species better represents a disease complex.

5.4. Incidence of Infection and Months :

The infection with *Eimeria* is observed during all the months of the study. However, the incidence rate of examined samples is a higher during the months of Juneat 6.3%, followed by May 6.2%, December 5.3%, November 4.5%, August 4.4%, March 2.4% and April 1.4%. The lowest rate of infection is detected during the months January and February at 0.6 % each The incidence rates of positiv samples is a higher in the months of June with 19.72%, followed by May 19.38%, December 16.61 %, November 14.19 %, August 13.84 % . March 7.61% and April 4.49 % . the lowest rate of infection is detected during the months January and February at 2.08 % each. The high incidence rates of *Eimeria* infection may be due to effect of favorable environment for sporulation and survival of the oocysts in litter with the poor management practices in broilers farmers of Ghot El-Sultan project .This may be due to a direct cause .Also this may be due to the high level of humidity and heat during these months of the years. In addition under optimal conditions with adequate moisture and oxygen, the oocysts are infective.

Astudy in Pakistan shows that the prevalence of eimeriosis in broiler chickens in the months of September is 89.7%,October 84.6%, and November 82.9% is conducted by Khan *et al.*(2006). On the other hand .The highest prevalence rate of eimeriosis at 50 % is showed during the March and the least at10% during April and September in Nepal poultary farms by Adhikari *et al.*(2008). This result may be due to the high level of humidity during these months of the year in Nepal .

5.5. Incidence of Infection and Seasons :

The results of the present study show that the seasonal effect on the incidence rate of infection. The highest incidence rate of examined samples observed during the summer samples is found at 10.77 %, followed by the Spring at 10.11% . Whileas the lowest infection rate is reporte during the winter is obtaine at 6.66%, is followed by an autumn is at 4.55 %. The highest incidence rate of positive examined samples is observed during summer at 33.56 %, followed by the Spring at 31.49%. The low infection rate is reporte during the winter samples is found to be at 20.76 %, is followed by an autumn is at 14.19 % .These presented results are inagreement with the results are obtained by Adhikari et al. (2008) reveale that the incidence rate of infection is the highest during summer and spring seasons with rate 33 %. The incidence rate during winter is at 23 % and the least during autumn with rate 14 %. The incidence rate of infection is a higher during summer and spring seasons, This may be due to the heat and humid climate where litter is wet. The high humidity favours sporulation and survival of the oocysts in litter. It increases the spread of oocysts in chicken farms (Jordan and Pattison, 1996).

The obtaine result in the present investigation is disagree with the results obtained byBraunius,(1988); Jordan andPattison,(1996) and Khan *et al*. (2006)They recorde that the highest prevalence rates of *Eimeria* infection are during winter and an autumn. Razmi andKalideri(2000)reporte that infection rate during winter and spring are a higher than summer and autumn In Iran, these results may due to the rain fall in these seasons (Maungyai *et al.*, 1990 and Calnek, 1997).

6. Summary

1. Since 1970 poultry production increasing in the siz faster than other food production animal industries, because low fat and high protein content only, low price and fast production which mean a short generative time.

2. Coccidiosis is aperament health problem in poultary industry especially in intensive production system. These disese caused by protozoa parasite belong to the various of *Eimeria* spp. .It is economically very important to reduce growth of the poultry worldwide not only in Ghot EL-Sultan project .

3. According to the information available on the chicken *Eimeria species* reported in Ghot EL-Sultan project .This study is the first record to determine the incidence rate and to identified the coccidia (*Eimeria* spp.) infection in broiler farms in Ghot EL-Sultan project up till now.

4. Nine hundred of intestinal tract of broiler chicken farms are collected randomly from poultary processing plant (PPP) of Ghot EL- Sultan project . From May in 2009 to April in 2010. These samples are examined for the incidence rate of *Eimeria* spp .infection.

5. The results show that, two hundred and eighty nine (32.1 %) of examined samples are found to be infected with different species of *Eimeria* and six hundred and twelve (67.8%) are non infected.

6. There are a high significant differences in the incidence rate of coccidian (*Eimeria* spp.) infection and non infection between the broiler chicken in Ghot EL-Sultan project ($^2 = 115.204$, P-value =0.000).

7. The result reveale that six species of *Eimeria* are recoverd during the examination of the site and samples . Identification of the different spp. is done bassis on the site of infection, characteristics of intestinal lesions , morphology of oocysts , and sporulation time of oocyst. The detecte species are *E. acervulins*, *E.necatrex*, *E. maxima*, *E. bruneti*, *E. tenella*, and *E. mivatte*.

8. The highest infection rates of the examined samples is found to be with *E.acervulina* at incidence rates 26% (234/900), is followed by *E.necatrix* 14% (126/900), *E.tenella* and *E.maxima*13.2% (119/900), *E.mivatte* 11.5%, (104/289) and *E.brunette* 9.4% (85/900). The highest infection rates of the infected examined samples is found with *E.acervulina* at incidence rates 80.97 %, followed by *E.necatrix* 43.59 % (126/289), *E.tenella* and *E.maxima*41.17 % (119/289), *E.mivatte* 35.98%, (104/289) and *E.brunette* 29.41% (85/289).

9. Ahigh Significant difference is exits between the incidence and types of *Eimeria* spp. ($^2 = 104.957$, P -value =0.000).

10. The present results reveale that one hundred and ninty seven (10.22 %) of examined samples have mixed infection (Inection with more than one of species of *Eimeria*) and ninty two(21.88%) have a single infection(Inection with a single species of *Eimeria*). The incidence rate of infected examined samples is found to be at 68.2 % have mixed infection and 31.8 % have a single infection.

11. There is a high significant difference is observed between single and mixed infections ($^2 = 38.15$, P- value=0.000).

12. The results showe that the highest incidence rates of examined samples during the months are 6.3% in June, is followed by 6.2 % in May, 5.3% in December, 4.5% in November, 4.4% in August, 2.4% in March , 1.4% in April , and 0.6% in January and February . The incidence rates of infected samples are19.72% in June,followed by19.38% in May, 16.61% in December ,14.19% in November ,13.84% in August, 7.61% in March , 4.49% in April , and 2.08% in January and February .

13.There are a high significant differences between the incidence rates and months ($^2 = 106.35$, P= 0.000).

14. The results reveale that no significant differences are exist between the types of *Eimeria species* infection and months, *E. acervulina* p– value = 0.998 ; *E.necatrix* p – value = 0.416 ; *E.maxima* p– value = 0.981 ; *E.brunette* p– value = 0.981; *E.tenella* p–value = 0.416 ; and *E.mivatte* p– value = 0.801 .

15. High incidence rates with *Eimeria* infection of examined samples are show during the summer 10.77 %, is followed by spring 10.11 % and low infection rates are during winter 6.66 % and Autume 4.55 %. The incidence rates of infected samples are show during the summer 33 .56 %, is followed by spring 31.49 % and low infection rates are in winter 20.76 % and Autume 14.19 %.

16. The results reveale that there is a high significant differences observed between the incidence of coccidia (*Eimeria* spp.) infection and the seasons ($^2 = 28.94$, P-value = 0.000).

17. The results show that there is a significant differences between seasons and mixed and single infection ($^2 = 9.888$, P-value = 0.02).

18. The results reveale that there are a high significant differences is detected between in types of infection and seasons ($^2 = 80.92$, P-value = 0.000).

7. Conclusion

The poultary production is increasing rapidly growing livestock sector in the developing countries ,due to low establishment cost ,and its tasty meat contain high content of valuable protein and low content of fat.

Coccidia is one of the most important of protozoa that affect avian species, causing sever intestinal disease known as coccidiosis. It has the greatest economic important on the poultary industry worldwide .

Controlling coccidiosis are required the first line of defence is the application of hygien standard in poultary farms to reduse the number of oocyst in the environment. Follwing point should be considered to maintain good hygene:

1)Put water and feeders at aheight level with the backs of the birds .

2)Avoid moisture and humidity in litters to reduce the oocust spotulation.

3)Avoid over growing in the house.

4)Control of coccidiosis bychemotherapy.Anticoccidial medication is commonly added to poultary feed as apreventaive against the disease . In poultary industry use adrug rotation or shuttle programe to reduce resistance .Most anticoccidial are with drawn aweek for prevent residue in the meat .

5) Prevention and control of coccidiosis by vaccination programs.

6)Other bio-control measures such as requiring attendents to change boots, and cloths between houses .Dust, wheels, Contaminated equipments and personnel who move between houses or farms .

6) Future studies should be done to idengification of coccidian (*Eimeria spp.*) in grand parent and parent chicken farms .

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A ppendixes:

(Appendix -1)

SHEATHER'S SUGAR SOLUTION (Flotation Solution)

Sucrose		500 g
Tap wate	r	350 ml
Phenol		5 ml
	(Sheater, 1923).	

(Appendix – 2)

HARRIS HAEMATOXYLIN STAIN SOLUTION

Haematoxylin	001.0 g
Absolute ethyl alcohol	010.0 ml
Potassium(or Ammonium)alum	020.0g
Distilled water	200.0 ml
Mercuric oxide	000.5g

Dissolve potassium alum in distilled water and boil. Dissolve haematoxylin in ethyl alcohol, then add the solution to potassium alum solution and continue boiling for half a minute .Add mercuric oxide ,mix and cool rapidly in cold water bath. Add a few drops of acetic acid toprevent mentallic luster and brighten nuclear structure .

(Humason ,1981).

(Appendix – 3)

EOSIN STAIN SOLUTION

Eosin Y(C.I.45380)	000.5 g
Ethyl alcohol (70%)	100.0 ml
Glacial acetic acid	. one drop

To prepare the working solution ,dilution with equal volume of 70% ethyl alcohol and add 2-3 drops of glacial acetic acid .

(Haumason ,1981).

(Appendix – 4)

DPX: Distrene ,Plasticizer ,Xylene

A mixture of Distrene-80, Dibutyl or Tricresy Phthalate as Plasticizer and Xylene as solvent. The proportion of each of the three components is variable depending on the manufacturer. It is a recommended replacement for the Canada Balsam mounting medium

(Appendix – 5)

Mayer's Albumen Adhering Mixture

Egg White	 50 ml
Glycerine	 50 ml
Formalin	 10 ml

(Humason ,1981)

8.الـخــلاصـة

 ازدادت صناعة منتجات الدواجن منذ عام 1970 أسرع من المنتجات الحيوانية الأخرى ، ذلك لأنها تعتبر من أهم مصادر البروتين وقلة محتواها الدهنى و انخفاض الأسعار إذا ما قورنت باللحوم الحيوانية الأخرى .

2) مرض الكوكسيديوز واحد من أهم المشاكل الصحية الدائمة فى صناعة الدواجن المكثفة للدواجن . يسببها طفيل أولى يتبع لأنواع مختلفة من جنس الايميريا ،ويعتبر هذا المرض واحد من أهم الأمراض المؤثرة اقتصادياً فى العالم ليس فقط فى مشروع غوط السلطان .

3) تعتبر هذه ا لدراسة والتى أجريت فى مشروع غوط السلطان ،وبناءً على المعلومات المتوفرة هى أول دراسة فى المشروع لتحديد معدل حدوث الإصابة ومعرفة أنواع الايميريا الموجودة فى مزارع بداري التسمين.

4) تم تجميع تسعمائة عينة من امعاء بدارى التسمين من المجزر الآلي بالمشروع من تسع مزارع لبدارى التسمين ،خلال الفترة من شهر مايو (2009) الى شهر ابريل (2010) .

5) أأهرت النتائج أن مائتان وتسعه وثمانون من العينات المفحوصة وبنسبة (32.1%)كانت مصابة بأنواع مختلفة من طفيل الايميريا ، وستمائة وإحدى عشر بنسبة (67.9 %) لم تكن مصابة .

6) لوحظ وجود فروق معنوية كبيرة بين معدل الإصابة و عدم الإصابة (111.204 = ² ، P= 0.000).

7) أظهرت النتائج وجود ستة أنواع من طفيل الايميريا ، وذلك بفحص الامعاء لتحديد مكان وصفات الاصابة و بناءً على صفات وشكل وقياس الأكياس البيضيه ،ووقت التجرثم والانواع هي

. E.brunetti z E.mivatti , E.tenella, E.maxima, E.necatrix, E acervulina

8) أظهرت النتائج أن طفيل E. acervulina قد سجل أعلى معدل حدوث إصابة في الحالات المفحوصة بـ 26%) ويليها E. necatrix بمعدل إصابة (14%) و كان معدل الإصابة بـ المفحوصة بـ 26%) ويليها E. brunetix بمعدل إصابة (11.5) و E. brunetta بمعدل إصابة (9.4%). ومعدل حدوث الإصابة بأنواع من طفيل الايميريا في الحالات الموجبة كانت كالتالى :

بنسبة E.brunetti – E.mivatti – E.tenella – E.maxima – E.necatrix – Eacervulina بنسبة (1.5% ه. 80.97) و 80.97% على التوالي]

10) مائة وسبعه وتسعون (21.88 %) من الأمعاء المصابة أظهرت أن إصابة مختلطة (الإصابة بأكثر من نوع من الايميريا) ، و اثنان وتسعون (22.10 %) كانت إصابة مفردة (الإصابة بنوع واحد من الايميريا) في الحالات المفحوصة. وكانت نسبة معدل حدوث الاصابة المختلطة في الحالات الموجبة بـ (86.2%) وكان معدل حدوث الإصابة المفرده بنسبة (31.8%).

(11) لوحظ من خلال النتائج وجود فروق معنوية كبيرة بين معدل حدوث الإصابة ونوع الإصابة (11) لوحظ من خلال النتائج وجود أو معنوية كبيرة بين معدل حدوث الإصابة (11) لوحظ من خلال النتائج وجود أو معنوية كبيرة بين معدل حدوث الإصابة ونوع الإصابة (11) لوحظ من خلال النتائج وجود أو معنوية كبيرة بين معدل حدوث الإصابة ونوع الإصابة (11) لوحظ من خلال النتائج وجود أو معنوية معنوية كبيرة بين معدل حدوث الإصابة ونوع الإصابة و

14) أعلى معدل حدوث إصابة لطغيل الاميريا سجلت في فصل الصيف بنسبة (33.56%) ، يليه فصل الربيع (31.49%) ، وأقل نسبة سجلت في فصل الشتاء (20.76%) ، وفصل الخريف (14.19%) و في الحالات المفحوصة أظهرت النتائج أعلى معدل للإصابة في فصل الصيف بنسبة (10.77%) يليه فصل الربيع بمعدل (10.11%) ، وأقل معدل سجل في فصل الشتاء (6.66%) ، ويليه فصل الخريف بنسبة (4.55%) .

P-V = 0.000 لوحظ وجود فروق معنوية كبيرة بين معدل حدوث الإصابة وفصول السنة (15) P-V = 0.000 . (²=106.35,

(16) النتائج أظهرت وجود فروق معنوية مقارنة بمعدل الإصابة أثناء فصول السنة ونوع الإصابة (16) المفردة والمختلطة) (16V = 0.000 (المفردة والمختلطة)

17) لوحظ وجود فروق معنوية عند مقارنة معدل الإصابة أثناء فصول السنة ونوع الايميريا .

.($^{2} = 80.92$, P-V = 0.000)