

**Islamic University-Gaza
Deanship of Graduate Studies
Faculty of Science
Biological Sciences Master Program**



Prevalence of Caecal Coccidiosis among Broilers in Gaza strip

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Ph. D. Medical Parasitology

A Thesis Submitted in Partial Fulfillment of the Requirements for

The Degree of Master of Biological Sciences

2010

DEDICATION

TO EVERY SCHOLAR LOOKING FOR KNOWLEDGE,
I DEDICATE THIS SMALL DROP IN THE HUGE
OCEAN OF SCIENCE.

Acknowledgements

The researcher wishes to express his deepest gratitude and appreciation to Dr. Adnan Al-Hindi, the supervisor of this work, for his enlightening supervision, useful assistance, valuable advice and continuous support during the course of this study. Also, thanks are extended to biological science department staff and thanks and due to my family and friends.

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Prevalence of Caecal Coccidiosis among Broilers in Gaza strip

Abstract

In developing countries, animal production is being subjected to great pressure to satisfy the demand for animal protein required by the continued increase in human population, and to have surplus for international trade. Coccidiosis is a health problem resulting in significant economic losses in the world. The impacts of disease on animal agriculture include, for example, lost revenues, costs of vaccination, prevention, eradication, decontamination and restocking.

This study aims to determine the prevalence of caecal coccidiosis among broilers in Gaza strip, potential risk factors for poor bio-safety measures and the given withdrawal period of anticoccidial drugs.

This study was conducted in the Gaza strip governorates, Palestine. Randomly 390 broilers caeca were collected from poultry shops, 10 caeca from every poultry shop were sampled. Test tube flotation for caecal content and direct smear scraping of the caeca lining was done to detect *Eimeria tenella* based on the dimensions of oocysts and schizonts respectively. In addition, postmortem was done to detect the clinical coccidiosis. This study was done during September (which recorded the highest prevalence), October and November 2009.

The present study came up with the following findings: The prevalence of sub-clinical caecal coccidiosis was 54.4 %. Multi-variable associations were tested between each variable.

The bio-safety measures, farmers knowledge and protection programs against the disease did not comply with the approved standards. Accordingly, the researcher recommends that, bio-safety measures, vaccination and proper treatment must be applicative in Gaza strip.

Keywords: Prevalence, Caecal Coccidiosis, *Eimeria tenella*, Broilers, Gaza strip



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Chapter One

Introduction

1.1 Overview

Poultry are kept in backyards or commercial production systems in most areas of the world. Compared to a number of other livestock species, fewer social and religious taboos are related to the production, marketing, and consumption of poultry products. For these reasons, poultry products have become one of the most important protein sources for people throughout the world. The total number of poultry in the world has been estimated by the Food and Agriculture Organization of the United Nations (FAO) as of 14,718 million, with 1,125 million distributed throughout the African continent, 1,520 million in South America and 6,752 million in Asia, 93 million in Oceania, 3,384 million in North America and 1,844 million in Europe [1].

Diseases result when normal body functions are impaired, and the degree of impairment determines the severity of the disease. It may result from the consequences of harmful actions of infectious and parasitic agents, or it may be caused by injury or physical stress with which the bird cannot cope. Disease may also occur as the result of a deficiency of a vital nutrient or the ingestion of a toxic substance. Diseases caused by infectious and parasitic agents are frequently complex and depend upon characteristics of the host, agent, and environmental conditions on the farm. A disease resulting from parasitism depends on the number, type, and virulence of the parasite, the route of entry to the body, the defense status and capabilities of the host. The latter depends partly on the host's prior disease encounters (e.g. infectious bursal disease IBD), nutritional status, and genetic ability to organize resistance mechanisms, environmental stresses and the kind and timing of countermeasures employed (drugs or changes of environment) [2].

Parasites are creatures that live on, in or around chickens. They can cause damage directly by disturbing the chickens and affecting their growth and egg production. They can also spread certain diseases, parasites can occur inside the chicken (internal parasites e.g. worms) or on the outside of the chicken. External parasites include lice,

mites, ticks, fleas and flies; Birds are infected with these parasites when new chickens are brought onto the farm and by contact with each other [3].

Internal parasites can be classified into several types based on their body types, life cycle, and damage to their hosts. Internal parasites of poultry include roundworms or Nematodes, tapeworms or Cestodes, flukes or Trematodes, and Protozoa including coccidia, Cryptosporidia, *Histomonas* spp. (Blackhead), *Trichomonas* spp. and other blood and tissue protozoa [4].

A parasite obtains its nourishment from another organism, where it cannot live independently; these include species of coccidia that causes the poultry industry to suffer a considerable economic loss, especially in the production of broilers. Chickens are susceptible to at least 11 species of coccidia [5]. Coccidiosis remains one of the major disease problems of poultry in spite of advances made in prevention and control through chemotherapy, management and nutrition [6].

Coccidia of the genus *Eimeria* are predominately host-specific, each species occurs in a single host species or a group of closely related hosts, infection by coccidia should be in sufficient numbers to produce clinical manifestations of disease that is named coccidiosis. Differential identification of each species is dependent upon the following characteristics; zone of intestine parasitized, gross appearance of the lesion, oocyst morphology, minimum sporulation time, minimum prepatent time, schizont size, location of parasite in the host intestinal epithelium and Cross-immunization tests [7].

Eimeria tenella and *E. necatrix* are the most pathogenic species [8]. *E. tenella* infections are found only in the caeca and it is called caecal coccidiosis [9]. In addition it can be recognized by accumulation of blood in the caeca and by bloody droppings. Caecal cores, which are accumulations of clotted blood, tissue debris, and oocysts, may be found in birds surviving the acute stage [10, 11]. Caecal coccidiosis causes decreasing of production and economic losses, which can be significant [12].

1.2 Objectives

1.2.1 General objective

The objective of the present study is to determine the prevalence of caecal coccidiosis among broilers in Gaza strip & evaluate bio-safety procedures and their role in transmission of the disease.

1.2.2 Specific objectives

- To determine the seasonal occurrence of *E. tenella* in the infected broilers.
- To determine the prevalence differences among Gaza strip governorates.
- To determine the prevalence differences among broilers weights.
- To evaluate microscopic examinations for detection of *E. tenella*.
- To evaluate bio-safety procedures and their role in transmission of the diseases.

1.3 Significance

There is no available data on the occurrence of *E. tenella* in Gaza farms, this study will focus on the occurrence and prevalence of *E. tenella* to provide significant information due to its importance from both economic and veterinary health viewpoints.

Chapter Two

Literature Review

2.1 Demography of Gaza strip

The Gaza strip lies on the coast of the Mediterranean. It is bordered by Egypt to the south-west. Climate temperate in Gaza governorates varies from mild winters, dry and warm to hot summers, It is about 41 kilometers long, and between 6 and 12 kilometers wide, with a total area of 360 square kilometers . Internationally the area is recognized as part of the Palestinian territories (Figure 2.1) [13].

The Gaza strip was controlled by Egypt from 1948–67, and then occupied by "Israel" following the 1967 war. Pursuant to the Oslo Accords signed between "Israel" and the Palestinian Liberation Organization in 1993, the Palestinian Authority (PA) was set up as an interim administrative body to govern populated Palestinian centers, Israel unilaterally withdrew from Gaza strip in 2005. The population is estimated to be 1.5 million in July 2009. High population density, limited land access, and strict internal and external security controls have kept economic conditions in the Gaza strip, the smaller of the two areas under the Palestinian Authority even more degraded than in the West Bank. The beginning of the second Intifada in September 2000 sparked an economic downturn as result of Israeli closure policies, which were imposed to address security concerns in "Israel" and consequently this disrupted labor and trade access to and from the Gaza strip. In 2001, and even more severely in 2003, Israeli military measures in PA areas resulted in the destruction of capital, the disruption of administrative structures, and widespread business closures. The status of the crossings, which are closed to all but the most basic goods have not changed following Israel's military offensive into the Gaza strip in early 2009 [14].



Figure 2.1: Gaza strip map [15]

2.2 Poultry production

Animal production sector is one of the most important sectors of Palestinian agriculture. Its importance comes from the increasing investments in the livestock sector. The share of animal production sector of the total agricultural value had increased from 36% to 49% in the West Bank in the seventies and nineties of 20th century respectively. Similar trend occurred in the Gaza strip, as livestock sector share increase from 20% to 30% at the same periods.

The aggregate demand for various types of livestock feed as of early 1999 is estimated 25,000 tons per month (17,000 tons for the West Bank and 8,000 tons for the Gaza strip. Local feed mills currently produce about 11,000 tons per month, which accounts for only 25% of poultry feed and 55% of other livestock feed. The poultry industry in Palestine was developed from seventies until the arrival of Palestinian authority (Table 2.1) [16].

Table 2.1: The development of layers and broilers in Palestine

Year	Layers (1000)	Broilers (1000)
1970	70	3400
1973	118	4330
1975	120	3350
1978	179	2490
1981	170	3500
1984	89	4400
1987	217	16450
1990	418	16900
1993	620	18800

Now Palestinian farmers in Gaza strip breed more than 15 million broilers per year (Table 2.2) which hatched locally through importing fertilized eggs from Israel and from abroad. Palestinians consume all that in addition to different types of poultry meat such as turkey, ducks, rabbits, and imported frozen poultry products [17].

Table 2.2: Poultry capacity in Gaza strip

Governorate	No. of hatcheries	Capacity/ eggs million	Hatched eggs/million	No. of Broilers farms	No. of Broilers /million	No. of Layers farms	No. of Layers
Gaza north	2	9.4	2.87	108	2.13	26	128050
Gaza	1	19.2	9.4	132	2.24	63	426800
Gaza med	3	18.4	3	230	3.54	26	122560
Khan younis	3	15.6	5.8	353	4.54	21	76000
Rafah	1	7.2	0.4	254	3	6	74500
Balady	-	-	-	-	-	-	70000
Total	10	70	21.6	1077	15.45	142	897910

2. 3 Chicken's caeca

The small intestine of poultry is relatively simple and short but highly efficient nevertheless, it is easily divided into 3 parts; duodenum, proximal small intestine (jejunum) and distal small intestine (ileum). The proximal and distal small intestines do not show distinguishing histological differences that define the jejunum and ileum of other vertebrates. The ileocaecal junction is found at the base of the distal small intestine and top of the large intestine. This junction is the site where twin caecal pouches join the linear portion of the intestine and is the location of the largest element of the gut immune tissue in the caecal tonsils. The caeca are thin-walled pouches that contain the anaerobic microflora responsible for fermentation in the bird. Finally, the large intestine, short and simple in poultry, joins the small intestine with the cloaca, the common receptacle for urinary, fecal, and reproductive products (Figure 2.2) [18].

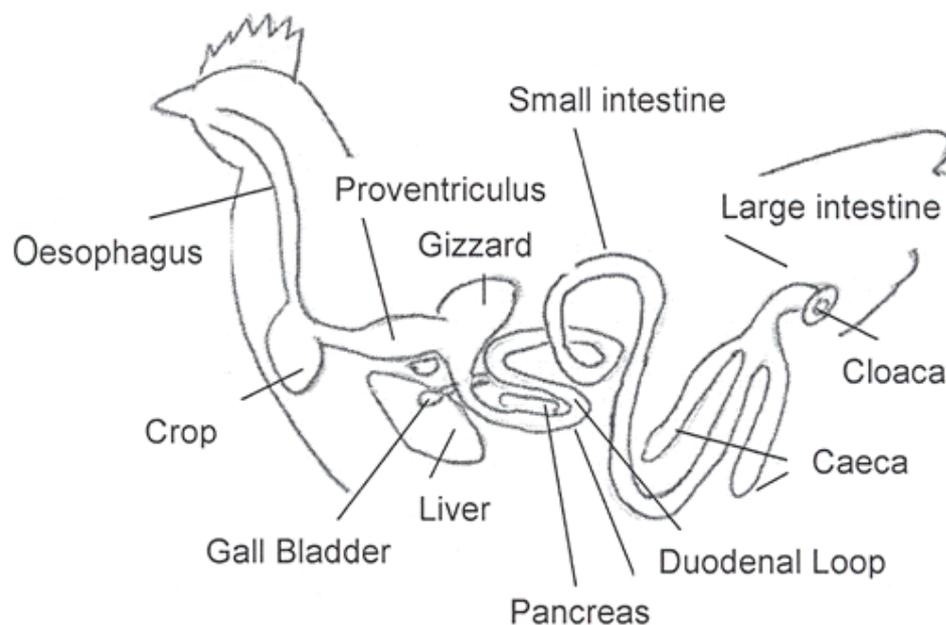


Figure 2.2: Chicken digestive system [19]

2.4 Etiology of *E. tenella*

Chicken are susceptible to at least 11 species of coccidia [5]. *E. tenella* and *E. necatrix* are the most pathogenic species [20, 8]. *E. tenella* causes caecal coccidiosis are almost universally present in poultry, raising operations, but clinical disease occurs only after ingestion of relatively large numbers of sporulated oocysts by susceptible birds. Both clinically infected and recovered birds shed oocysts in their droppings, which contaminate feed, dust, water, litter, and soil. Mechanical carriers (e.g. equipment, clothing, insects and other animals) may transmit oocysts. Fresh oocysts are not infective until they sporulate under optimal conditions (21-32°C) with adequate moisture and oxygen; this requires 1-2 days. The prepatent period is 4-7 days. Sporulated oocysts may survive for long periods, depending on environmental factors. Oocysts are resistant to some disinfectants commonly used around livestock but are killed by freezing or high environmental temperatures [21].

2.4.1 Taxonomy of *E. tenella*

Classification	
Kingdom:	Protista
Phylum:	Apicomplexa
Class:	Conoidasida
Order:	Eucoccidiorida
Family:	Eimeriidae
Genus:	Eimeria
Species	<i>Eimeria tenella</i>
Binomial name:	<i>Eimeria</i>
Mnemonic:	EIMTE
Taxon identifier:	5802
Common name:	Coccidian parasite
<i>E. tenella</i> and related strains	
NO	Strain name
1	<i>E. tenella</i> Beijing
2	<i>E. tenella</i> H
3	<i>E. tenella</i> Houghton
4	<i>E. tenella</i> LS18
5	<i>E. tenella</i> PAPt38
6	<i>E. tenella</i> Penn State
7	<i>E. tenella</i> Weybridge
8	<i>E. tenella</i> Wisconsin, Wis [22].

2.6 Life cycle

Fantham in 1910 described the life cycle of a coccidia parasite in birds [24]. Development of the parasite in the host cells involves both asexual and sexual stages of multiplication. Destruction of host tissue as a result of parasite development and multiplication leads to the various clinical manifestations observed in outbreaks of disease. Development of the various species of chicken coccidia includes minor variations. A generalized life cycle is illustrated in figure 2.4. Infection occurs when a susceptible chicken ingests a sporulated oocyst from its environment. The sporulated oocyst contains four sporocysts, each sporocyst contains two sporozoites. The sporozoites are released by mechanical and biochemical action in the digestive tract of the chicken (Figure 2.5) [25].

Sporozoites escape from the sporocysts and oocysts, but the factors contributing to excystation have not been definitely established, pancreatic juice, in particular trypsin, is one of the factors responsible for excystation. Escape of sporozoites through any available fracture in the cyst 5-10 minutes after the cyst wall has been placed in a trypsin solution, maintained at 37° C. Hydrogen-ion concentration, bile and buffers were also found to be important factors in excystation of various species of coccidia, the effects of pH, buffers, bile and bile acids on the excystation of sporozoites of various *Eimeria* spp. including *E. tenella*, found no excystation when any of the bile acids, bovine or chicken bile was used alone without trypsin. Therefore the release of large numbers of sporozoites into the digestive tract of chickens requires the wall of the oocyst to be broken, weakened or partially the walls of many sporulated oocysts expelled through faeces to be structurally changed [26].

The liberated sporozoites invade epithelial cells in a specific zone of the intestine or caeca depending on the species. Upon entering the host cell, the sporozoite transforms in 12 to 48 hours to a feeding stage called a trophozoite. The trophozoite begins to enlarge, and the parasite nucleus

divides by a process of asexual multiple divisions known as schizogony (merogony). At this point, the parasite stage is referred to as a schizont or meront (Figure 2.6). The small parasitic stages forming within the schizont are called merozoites. The schizont ruptures when mature in third day, releasing the merozoites. Most of these invade other epithelial cells to repeat the process of development through the trophozoite and schizogonous stages. The merozoites from the second schizogonous cycle again penetrate the epithelial cell of the host. Some or all may go through a third schizogonous cycle, depending on the species, before formation of male (microgametocytes) or female (macrogametocytes) gametocytes. The male gametocyte matures and ruptures, releasing a large number of minute biflagellate microgametes. The macrogametocyte grows to form a macrogamete [27].

A thickened wall forms around the macrogamete, forming a zygote when the macrogamete is fertilized by a microgamete. This stage is the young or immature oocyst. The prepatent period varies with each species depending on the time required for each schizogonous cycle and the number of cycles. The oocyst ruptures the host cell when mature and passes out of the bird in the droppings. Under suitable environmental conditions, four sporocysts, each containing two sporozoites, are formed within the oocyst after about 24 hours [27].

2.6.1 Special features of *E. tenella* life cycle

This parasite develops in the cells of the caeca. Infections may be characterized by the presence of blood in the droppings and by high morbidity and mortality [23].

2.6.2 *In vitro* cultivation

Important prerequisites for the successful *in vitro* cultivation of *E. tenella* in primary chick kidney cells (PCKC) are optimal conditions for the controlled growth of PCKC and the coccidia parasite, i.e. the use of suitable nutrient media, concentrations and quality of fetal calve serum as well as the production of ultrapure sporozoite

suspensions. It has been possible to film the complete life cycle of *E. tenella* *in vitro*. Motion pictures of all endogenous and exogenous developmental stages of the parasite are demonstrated the invasion of sporozoites into host cells and their further development to schizonts of the first, second and third generation by multiple asexual reproductions (schizogony), the maturation of female and male gamonts to respective gametes, and finally the formation of zygotes [28].

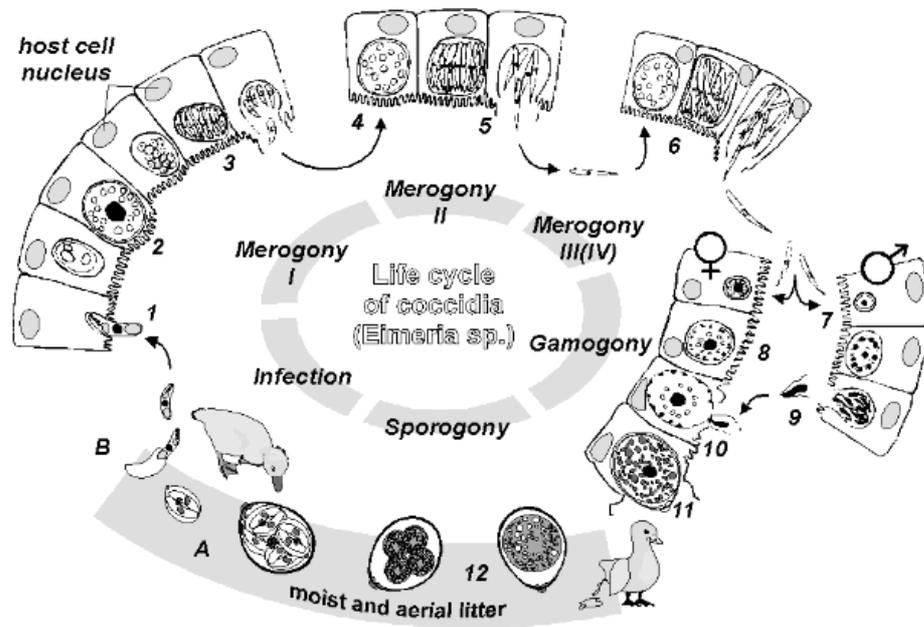


Figure 2.4: Life cycle of *E. tenella* [29]

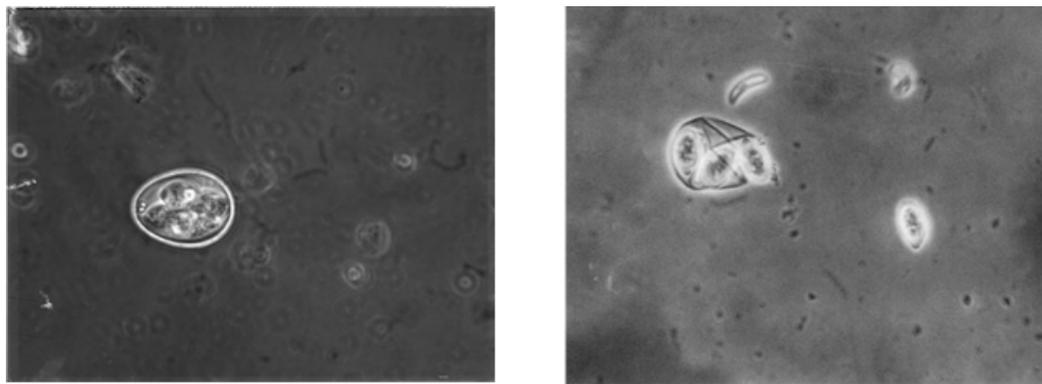


Figure 2.5: Sporulated oocyst (left), released sporocysts and sporozoite (right) [7]

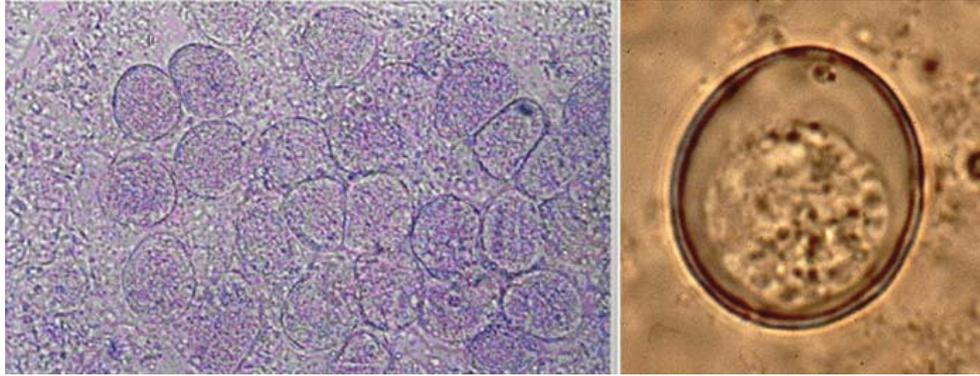


Figure 2.6: *E. tenella* schizonts 10 x (left) and unsporulated oocyst 100 x (right) [30]

2.7 Transmission

Chickens become infected with *Eimeria* spp. by ingesting infective oocysts (eggs) from litter, soil and contaminated feed and water. The infected birds excrete oocysts into their faeces and are a source of infection for other birds. As *Eimeria* spp. can survive for long periods in infected birds and the environment [31]. The oocysts in faeces become infective through the process of sporulation in about two days [32].

Birds in the same flock may ingest the oocysts through litter pecking or the contamination of feed or water. Although no natural intermediate hosts exist for the *Eimeria* spp. many different animals, insects, contaminated equipment, mice, wild birds, and dust can spread oocysts mechanically. Oocysts generally are considered resistant to environmental extremes and to disinfectants, although survival time varies with conditions oocysts may survive, for many weeks in soil, but survival in poultry litter are limited to a few days because of the heat and ammonia released by composting and the action of molds and bacteria. Viable oocysts have been reported from the dust inside and outside broiler houses, as well as from insects in poultry litter. The darkling beetle, common in broiler litter, is a mechanical carrier of oocysts. Transmission from one farm to another is facilitated by movement of personnel and equipment between farms and by the migration of wild birds, which may mechanically spread the oocysts. New farms may remain free of coccidia for most of the first grow out of chickens until the introduction of coccidia to a completely susceptible flock. Such outbreaks, often more severe than those

experienced on older farms, are often called the new house syndrome. Oocysts may survive for many weeks under optimal conditions but will be quickly killed by exposure to extreme temperatures or drying. Exposure to 55°C or freezing kills oocysts very quickly. Even 37°C kills oocysts when continued for 2-3 days. Sporozoites and sporocysts can be frozen in liquid nitrogen with appropriate cryopreservation technique, but oocysts cannot be adequately infiltrated with cryoprotectants to effect survival. Threat of coccidiosis is less during hot dry weather and greater in cooler damp weather [2]. Recovered chickens shed oocysts representing a problem in multi-age operations [33].

Factors contributing to outbreaks of clinical coccidiosis include; litter moisture content exceeding 30% due to ingress of rain or leaking waterier, immunosuppression (Marek's, IBD and Mycotoxins diseases), suboptimal inclusion of anticoccidials or incomplete distribution (poor mixing) in feed and environmental and managerial stress such as overstocking, inoperative feeding systems, inadequate ventilation [34].

2.8 Epidemiology and economic impacts

Coccidiosis remains one of the major disease problems of poultry in spite of advances made in prevention and control through chemotherapy, management and nutrition. *E. tenella* and *E. necatrix* are the most pathogenic species [35].

The disease causes high mortality, morbidity and adverse effects on the growth of infected birds [36]. The incidence of coccidiosis in commercial poultry has increased due to higher stocking densities and intensive husbandry practices [37]. Coccidiosis occurs worldwide and is a major cause of mortality and suboptimal growth and feed conversion efficiency in immature flocks unless appropriate preventive measures are implemented. The cost of anticoccidial feed additives and treatment is estimated to exceed 400 million US\$ annually in all poultry producing areas of the world [34].

Hence, particular vaccines may be designed for rearing standard broilers for up to about 6 weeks or for breeding stock [38]. Coccidia have been found wherever

poultry are raised. The spread of this parasitic disease is enhanced by poor bio-safety and management practices. While the *Eimeria* spp. that are known to infect chickens, typically a poultry facility will contain only 1-3 species at a time. In the United States (US) the species *E. acervulina*, *E. maxima* and *E. tenella* are found most often. However, reports of increased incidence of *E. mitis* and *E. praecox* have been surfacing. There is evidence that protection against the major species of coccidia will allow for the emergence of minor species in a poultry operation. Thus, vaccines using oocyst based or subunit must provide protection against other species that are pathogenic for chickens [39]. In Pakistan whereas in layers and breeders, *E. tenella* showed the highest prevalence, 38.88 and 65% respectively [31].

The disease is often diagnosed in birds brought to diagnostic laboratories but the vast majority of cases are diagnosed in the field and handled by poultry service personnel. The current expense for preventive medication exceeds 90 US\$ million in the US. Surveys in North and South America revealed coccidia present in almost all broiler farms. Very high percentages of positive flocks were also, reported from Europe [2]. Northern Argentina prevalence was 14% in 1986. Presence of all known *Eimeria* spp. recorded in France 1996, Sweden 1990 and Korea 1984. Several studies in Japan leave little doubt of the presence of all species in country. Australian studies also show full complement of species [40].

In Saudi Arabia, The prevalence of the infection was 80% among the house-reared chicks while no infection was reported among the farm chicks [41]. In china the prevalence of the infection rate of identified *Eimeria* spp. was 90%, 88%, 72%, 68%, 60%, 26%, and 8% for *E. tenella*, *E. praecox*, *E. acervulina*, *E. maxima*, *E. mitis*, *E. necatrix*, and *E. brunetti*, respectively [42]. Coccidiosis is one of the most important and common diseases that affect poultry, it results in a great economic loss all over the world [43].

Worldwide, coccidiosis causes more than 3 US\$ billion in damages each year. Prevention of the spread of the disease among broilers is primarily based on hygiene measures and adding anti-coccidiosis drugs to feed or drinking water [44]. The

impact of disease on animal agriculture is typically assessed in quantitative terms. In poultry industry, these terms include for example lost revenues; costs of vaccination, prevention, eradication, decontamination and restocking. These have been referred to as negative inputs [45].

In Ethiopia coccidiosis used to be the most important cause of mortalities in all farms. Incidences of the disease were as higher as 80%, usually occurring in the form of outbreaks, poultry coccidiosis caused by for example *E. acervulina*, *E. necatrix*, *E. maxima* and *E. tenella* is endemic in all parts of the country and affects mainly young growing birds. In Ethiopia, coccidiosis is causing significant poultry losses; coccidiosis was identified to be a major cause of both direct and indirect losses in all farms. Losses occurred in the form of mortalities, coccidiostat costs, reduced weight gains, reduced market value of affected birds, delayed off take and reduced egg production in layers. The disease also contributed to culling. Quantification of economic losses resulting from coccidiosis in small scale and large-scale poultry farms [46].

The total cost of coccidiosis in chickens in the United Kingdom in 1995 was estimated to be at least 55 US\$ million of which 98.1% involved broilers (80.6% due to effects on mortality, weight gain and feed conversion, and 17.5% due to the cost of chemoprophylaxis and therapy). The costs of poor performance due to coccidiosis and its chemical control totaled 4.54% of the gross revenue from UK sales of live broilers [47].

2.9 Clinical findings

Clinical signs of coccidiosis are due to destruction of the intestinal epithelium and frequently, the underlying connective tissue of the mucosa. This may be accompanied by hemorrhage into the lumen of the intestine. Signs may include discharge of blood or tissue, catarrhal inflammation and dehydration. Serum protein and electrolyte levels may be appreciably altered [48]. Signs range from decreased growth rate to a high percentage of visibly sick birds, severe diarrhea and high

mortality. Feed and water consumption are depressed, weight loss, development of culls and increased mortality may accompany outbreaks [49].

The effects of coccidiosis are due to a number of factors, all of the observed effects are related to disruption of the epithelial cells lining the intestine by the release of parasite stages. While infection with high doses of some *Eimeria* spp. (*E. tenella* and *E. necatrix*) may cause death to chickens, usually the effects are insidious and are not apparent to the poultry farmer until the chickens are sent to market. The main effects that cause economic losses are a decreased weight gain due in part to the malabsorption of nutrients through the gut wall. This effect causes an increased feed conversion ratio, which is the amount of feed converted into body weight, because feed that is consumed is used inefficiently. Chickens that are infected with high levels of coccidia display symptoms such as droopiness and emaciation and may never achieve weight gain equal to their uninfected counterparts (Figure 2.7) [6].



Figure 2.7: Clinical signs of coccidiosis [50]

Coccidiosis is generally acute in onset and is characterized by depression, ruffled plumage, and diarrhea. Birds infected with *E. tenella* show pallor of the comb and wattles and blood-stained caecal droppings [34].

Several factors influence the severity of infection, some of these include; the number of oocysts eaten, generally an increase in the number of oocysts eaten is

accompanied by an increase in the severity of the disease. Different strains of a species may vary in pathogenicity, environmental factors affecting the survival of the oocysts, Site of development within the host; coccidia that develop superficially are less pathogenic than those that develop deeper. Young birds are generally more susceptible than older ones and nutritional status of the host, poorly fed birds are more susceptible to the infection. Coccidiosis in chickens is generally classified as either intestinal or caecal. Most serious cases of intestinal coccidiosis are caused by *E. necatrix*. Caecal coccidiosis is due to *E. tenella*. Coccidiosis occurs most frequently in young birds, old birds are generally immune as a result of prior infection. Severe damage to the caeca and small intestine accompany the development of the coccidia. Broilers and layers are more commonly infected, but broiler breeders, turkey and pheasant are also affected. Coccidiosis generally occurs more frequently during warmer (May to September) than colder months (October to April) of the year [22].

2.10 Pathogenicity

Coccidiosis often lead to disturbances lowering nutrients absorption and lead to disturbances in the ions and osmotic balance of the gut epithelium [51]. Oocysts infect the cells of intestinal lining, replicate and cause them to burst, change the gut morphology, reducing gut length and truncating the intestinal villi [52]. Ingested small number of oocyst lead to a subclinical infection with no obvious diagnostic symptoms and subsequent immunity to reinfection. Pathology is largely associated with destruction of the epithelial lining of the infected part or intestine which results in reduced ability for the digestion and absorption of nutrient by the bird [53].

Excystation of *E. tenella* sporozoites was more rapid in chicks aged 4, 5, and 6 weeks than in those 0, 1, 2 and 3 weeks [54]. The mucus gel layer overlying the gastrointestinal epithelium plays an important role in host–pathogen interactions, the initial interaction between *E. tenella* and host cells of the intestinal epithelium must occur across this mucus interface. The relationship between *E. tenella* and avian mucin was examined; in particular, the effect of purified intestinal regional mucin on parasite adherence and invasion in vitro, secreted mucin from the chicken duodenum

and caeca was purified by density gradient centrifugation and gel chromatography. *E. tenella* adherence to chicken duodenal mucin was detected, whereas adherence to caecal or bovine mucin was not shown. Parasite invasion into epithelial cells was not influenced by bovine mucin, whereas chicken mucin purified from the duodenum and caeca significantly inhibited invasion. Inhibition of *E. tenella* invasion into cells by mucin from the duodenum was marginally greater than that of the caeca, but this was not significant [55].

The species important in broiler production include *E. tenella* (90%), *E. maxima*, *E. acervulina*, and *E. mivati*, the species important in breeder and layers are *E. burnetti* and *E. necatrix*. Seven species infect turkeys, the big three of concern are *E. meleagritidis*, *E. adenoides* and *E. gallapovonis* [56].

E. tenella is the well-known cause of caecal or bloody coccidiosis, invades the two caeca and in severe cases may also parasitize the intestine above and below the caecal junction. Lesions of *E. tenella* are divided into +1, +2, +3 and +4 scores.

E. tenella +1 is shown in figure 2.8, few scattered petechiae, which are reddish or purple in color, are seen on the unopened caeca. There is no thickening of the caecal wall, the caecal contents usually show a normal brownish color, although a slight amount of blood may be present, mild clinical signs may show in infected chickens[7].

E. tenella +2 is shown in figure 2.8. Petechiae, which are apparent on the serosal surface, are somewhat more numerous, bleeding, which appears on the fifth to seventh day of infection, is more marked on the mucosal surface than in a typical +1 score. In this example, bleeding is slightly more severe than in the usual +2. Except for the presence of some blood, the caecal contents are normal, another more reliable characteristic in judging severity is the amount of thickening of the caecal wall, which is slight in this case. Clinical signs are apparent in infected chickens with these degrees of infection [7].

E. tenella +3 is shown in figure 2.9, bleeding is more severe, with clotting appearing in the distal end of the pouch. The clot becomes hardened as the sloughed mucosal surface joins the bloody material to form a core. There is an absence of normal caecal contents since the caeca become practically nonfunctional. Marked thickening of the caecal wall has occurred. The serosa of the unopened caeca shows the petechiae as coalesced and eroding the entire surface. Huddling, chilling and bloody droppings constitute clinical signs [7].

E. tenella +4 is shown in figure 2.9, severe bleeding, a much thickened caecal wall, and eroding of the mucosal surface show up on the fifth day of infection. The unopened caeca is distended, with blood at the distal end, but is contracted and shortened. Chickens huddle and sometimes let out a high-pitched call, chickens cease feeding and drinking, death may come suddenly beginning on the fifth day, reaching the greatest number on the sixth and extending through the seventh to the tenth day of infection. By the sixth to eighth day, the caecal core is hardened and may persist for another week or more. The core may take on a more whitish cast with a huge accumulation of sloughed mucosal surface material. Microscopic examination of scrapings would show many oocysts. Purple areas denoting the presence of gangrene and rupture of the caecal wall may occasionally occur at this stage, dead birds are scored +4 [7].



Figure 2.8: *E. tenella* infection scored + 1(left) and + 2 (right)

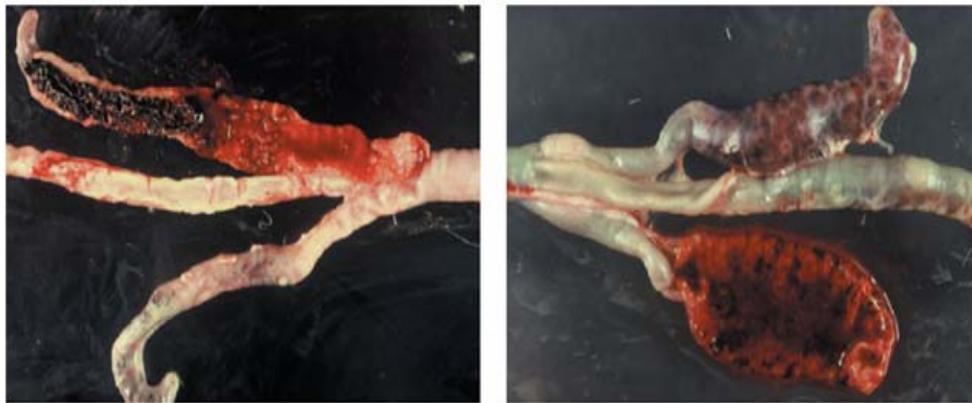


Figure 2.9: *E. tenella* infection scored + 3 (left) and + 4 (right)

2.11 Diagnostic methods

2.11.1 Clinical and postmortem inspection

E. tenella is the best known of poultry coccidia, because of the easily recognizable lesions and often-spectacular losses it causes in commercial broilers or layer pullets. This species inhabits the caeca, causing a severe disease characterized by bleeding, high morbidity and mortality, lost weight gain, emaciation, loss of skin pigmentation, and other signs. Diagnosis is dependent upon finding caecal lesions with prominent blood and often-firm bloody cores and accompanying clusters of large schizonts and oocysts [2].

2.11.2 Faecal examination

Identification of *Eimeria* spp. oocysts in faeces is an easy and cheap way to diagnose many *Eimeria* spp. infections and to get an impression of the infection level, direct smear method and both qualitative and quantitative techniques can be done to faecal sample.

Direct smear method

Identification of coccidia oocysts is possible by using a direct smear method, where a thin smear of emulsified faeces is examined under a microscope. Direct microscopic examination of intestinal mucosa can only be used in animals, which have been culled or found dead. It can be used to find the intracellular and extracellular stages of coccidia and other protozoa.

Qualitative techniques for faecal examinations

A large number of different procedures are available for demonstrating coccidia oocysts in poultry faeces. The most widely used principle for concentration of parasite oocysts is flotation. Coccidia oocysts have a specific gravity, which is lower than that of plant residues in the faeces, the oocysts may be separated from other faecal particles by mixing the faeces with a fluid (saturated NaCl + glucose) in which the oocysts float, these procedures include test tube flotation and simple flotation.

Quantitative techniques for faecal examinations

The qualitative flotation techniques, which are used for nematode eggs, cestode eggs and coccidia oocysts, have been elaborated to become quantitative, when the eggs are allowed to float in a special counting chamber, called the McMaster chamber. Many modifications exist, and a Simple McMaster Technique and slightly more elaborated Concentration McMaster [1].

2.11.3 Hemagglutination inhibition assays

Intracellular *Eimeria* sporozoites are observed in epithelial cells, it is attached on the cell surface of the epithelial cells. The carbohydrates present on sporozoites and lectin-binding sites on the surface of sporozoites were detected by means of peroxidase-conjugated lectins. By investigated parasite lectins of *E. tenella*, *E. acervulina* and *E. maxima* using hemagglutination inhibition assays, different stages of these parasites have specific surface sugar lectins were found. The lectins found

on the surface of the sporozoites play a role in determining the site of infection within the intestine of the host [57].

2.11.4 Enzyme-linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) was adapted to detect antibodies to *Eimeria* spp. It is simple test and reliable methods for the determination of the exposure status of chickens to *Eimeria* spp. and detecting specific IgG and IgM antibodies in serum samples. The indirect ELISA assay may be possible to discriminate between chickens actually infected with *Eimeria* spp. (as indicated by high levels of antiparasite IgM), chickens which have been repeatedly exposed to *Eimeria* (as indicated by high levels of antiparasite IgG) and unexposed birds. The applicability of this ELISA, using sporozoite antigen of *E. tenella* to practical situations was substantially confirmed [58].

ELISA analyses of serum pools having varying protective capacities revealed good correlations between passive protection and levels of anti-unsporulated oocyst, anti-sporulated oocyst, anti-merozoite and anti-gametocyte antibodies . The ELISA test is an initial comparison revealed few differences in their ability to monitor the onset, kinetics and magnitude of the antibody response. Furthermore, the cross-reactivity of these antigens with sera from birds infected with chicken *Eimeria* spp. is similar. The merozoite antigen is selected for further evaluation because it was easier to prepare. Discrimination between sera from birds experimentally infected with *E. tenella* and birds maintained in an *Eimeria*-free isolation facility was excellent. The ELISA should prove useful for monitoring infectivity in vaccination programs in layer and breeder flocks and for assessing the effectiveness of bio-safety measures in broiler flocks [59].

2.11.5 Polymerase chain reaction (PCR)

A polymerase chain reaction (PCR) assay, based on the amplification of internal transcribed spacer regions of ribosomal DNA, was developed for the chicken coccidian species-specific primers for the detection and discrimination of all *Eimeria* spp. that infect the domestic fowl is now available. The PCR assay provided a faster,

more simplified read-out compared to staining of amplified bands in an agarose gel with ethidium bromide [60].

The unsporulated oocysts of *Eimeria* spp. are difficult to differentiate. For identification of *Eimeria* spp and variations in genomic DNA two primers corresponding to highly conserved regions of the 18S ribosomal DNA of the coccidian forward primer (BSEF) and the reverse primer (BSER) were chosen. The internal transcribed spacer 1 (ITS-1) from within ribosomal DNA (rDNA) genes was investigated to differentiate chicken intestinal coccidian to the genus and species level. The spacer separates the 3' end of the 18S ribosomal RNA gene from the 5' end of the 5.8S rRNA gene within individual rDNA transcription units. The five chicken *Eimeria* genus species-specific primers was designed as markers to identify species and sequences from the vaccines coccivac-B and coccivac-D and from a Taiwanese strain of *E. tenella* (Table 2.3). The cross-reaction would occur with the DNA from chicken intestinal contents and muscles, and the detection limit of PCR tested with pureline oocysts of *E. tenella* [61].

Table 2.3: Specific primers derived from rRNA sequences of *E. tenella* based on sequences derived from GenBank

Name	Position	Sequence	T _m Calculated annealing temperature)	GC (%)Content of deoxy-guanidine and deoxy-cytidine
T1	2311–2330	5_-CGC TGC TGG TTT TAC AGG TT-3	60.0	50.0
T2	2773–2754	5_-GCT GAA GCA AAG TTC CAA GC-3	60.0	50.0

Several PCR based assays targeting different regions of the *Eimeria* genome have been described, such as the 5S rRNA, the small subunit rRNA, the sporozoite antigen gene EASZ240/160. Nevertheless, the practical implementation of these methods in routine diagnostics and epidemiological studies of chicken coccidiosis has been limited, and the assays must still be regarded as experimental [62].

2.11.6 A real-time diagnosis system

Using digital images of oocysts, an important goal in image analysis is to classify and recognize objects of interest in digital images. Objects can be characterized in several ways, e.g. by identifying their colors, textures, shapes, movements and position within images. Coccimorph, a realtime system accessible through a web interface, (available at <http://puma.icb.usp.br/coccimorph>). Coccimorph allows the user to upload an image, detect the contour interactively and obtain a real-time classification. The framework of this system is divided into the following levels:

- Database: This level stores the feature vectors that compose the data set. Micrographs and isolated images are also stored and can be visualized through a web interface.
- Application: This is the developmental level of the system, which is divided into three modules: import subsystem, analysis subsystem, application and web server.
- Client: This level is oriented to interact with the end-user, allowing for the visualization and uploading of images for diagnostic purposes.

The analysis subsystem represents the kernel of the system and is responsible for the image pre-processing, feature extraction and pattern classification. This module was entirely developed, resulting in a rapid response of the system during the image-processing step, thus permitting a real-time processing through the web [63].

2.12 Treatment

2.12.1 Types of medication

Shuttle program:

Shuttle or Dual programs use of one product in the starter and another in the grower feed is called a shuttle program in the US and a dual program in other countries. The shuttle program usually is intended to improve coccidiosis control. Intensive use of the polyether ionophore drugs for many years produced strains of coccidia in the field that have reduced sensitivity to the ionophores. Either it is a common practice to use another drug such as nicarbazin or halofuginone in the starter or grower feed to bolster the anticoccidial control and take some pressure of the ionophore. The use of shuttle programs is thought to reduce buildup of drug resistance. In 1988, approximately 80% of the US producers used some type of shuttle program [2].

In which two compounds usually a synthetic agent (such as Incarbazin) and Ionophore (such as Salinomycin) are employed successively in single flock, during 1999 in the US, shuttles involving synthetic drugs followed by Ionophores were employed by approximately 25% of broiler complexes [64].

Ionophore program:

Can be divided to three categories

- A lower concentration in the starter than the grower feed
- A higher concentration in the starter than the grower feed
- The same concentration used in the starter and the grower feed [64].

Ionophore products:

Ionophores are the main group of poultry feed additives the polyether antibiotics commonly called Ionophores, six compounds have become available (Monensin, Laslocid, Salinomycin, Narasin, Maduramycin and Semduramycin), the mechanism of action of all Ionophores is very similar since they mediate the transport of mono and divalent cations through the membrane of the parasite, resulting in disturbance of its osmotic balance. Ionophores can be divided into three groups according to the precise of action and chemical structure; monovalent (Monensin, Narasin and Salinomycin), monovalent glycoside (Maduramycin and Semduramycin) and divalent (Laslocid). Laslocid and Maduramycin are more effective against *E. tenella* than Monensin, Narasin and Salinomycin [65].

Synthetic products:

These are synthesized drugs, which include variant groups of completely different chemical classes:

- Amprolium is good against *E. tenella* but is not very effective against *E. acervulina* and *E. maxima*.
- Nicarbazin is broad-spectrum anticoccidial, it is used in colder seasons or climatic areas and the drugs should not be used in birds older than 20 days because the possibility of strong growth depression.

- Robendine is safe broad-spectrum anticoccidial but it must be used with caution because of its potential fast resistance build up.
- Halofuginone and Lerbek effects on *E. tenella* are coccidiostatic activity and no coccidiocidal effect, but good for control of *E. acevurlina*.
- Clinacox (Diclazuril) broad-spectrum activity against all *Eimeria* species, its low potential for resistance development, especially *E. tenella* and *E. maxima*. In addition, use for clean up program after the use of Ionophores [65].

Sulfonamide products:

In 1948, sulfaquinoxaline was introduced commercially as a poultry coccidiostat. It was not the first sulfonamide found active against *Eimeria* spp. in poultry, but its practical success in disease control firmly established the routine incorporation of anticoccidial drugs in poultry feed. In this way, the drug exerted a major impact on the worldwide production of poultry meat [66].

Veterinarians regularly use sulfonamides for therapeutic, prophylactic or growth promoting purposes in laying hens. In Japan, sulfamonomethoxine, sulfadimethoxine, and sulfaquinoxaline are mainly used for prevention or treatment of poultry coccidiosis, and are generally co-administered in feed. The treatment of hens with sulfonamides supplemented feed may result in sulfonamides residues being present in market eggs if these drugs have been improperly administered or if the withdrawal time for the treated hens has not been observed. To assure the food safety for consumers, the European Union has set a maximum residue limit for sulfonamides in foods of animal origin such as meat, milk, and eggs [67].

Improper use of these veterinary drugs in laying hens is of great concerns because the drug residues are turning up in eggs, an indispensable food for the consumers because it is highly nutritious, cheap and readily available. A rigid residue monitoring of sulfonamides in eggs is therefore an important specific activity to guarantee the food safety. Discharging the waste of organic solvents is also a severe problem on the world scale. From the viewpoint of the effect of organic solvents to

environments and analysts, analytical methods for the monitoring should avoid the use of organic solvents [68–69].

The feeding of 2,500 parts per million (ppm) sulfaquinoxaline causes a severe anemia in chickens with hemorrhages on the legs, breast muscle, and in abdominal organs [48]. Toxicity is more likely to be observed when medication is given in the water during hot weather. Feeding 300-ppm sulfaquinoxaline to growing chickens for 8 weeks reduced the weight gain of female birds but adverse, effects were not observed when sulfaquinoxaline was administered to growing chickens at 500-ppm in various feeding schedules. Continuous feeding of 125-ppm sulfaquinoxaline was highly efficacious in preventing naturally acquired caecal and intestinal coccidiosis. The overall efficacy advantages of sulfaquinoxaline in comparison with other sulfonamides were attributed to the fact that it is more readily absorbed than other sulfonamides when given in the feed. *E. tenella* results resistance to sulfonamide, which become not efficacious [7].

2.12.2 Testing anticoccidial

Three types of tests are generally used to study anticoccidial drugs in broiler birds. These are; Battery tests (7–14 days) tests with birds in wire cages, Standard grow-out (6–8 weeks) tests in floor pens and Full-scale tests in commercial facilities. Each type has a different objective and value to the investigator for example; the battery test is used most effectively to measure the efficacy of an anticoccidial drug against a variety of field isolates of coccidia. This is an efficient and relatively inexpensive testing procedure. The floor pen test is an intermediate testing procedure with a primary goal of providing statistically useful performance data under controlled conditions. Individually, the predictive value of each test is limited. One cannot, for example, confidently extrapolate performance data in a seven-day battery test to market weight, nor can one predict from a few commercial trials the efficacy of an anticoccidial agent in preventing the lesions of major species of coccidia. As a whole, when properly conducted, the tests complement one another by providing a comprehensive picture of the efficacy, safety and economic value of an anticoccidial agent [7].

2.13 Nutrition during outbreak

Dietary levels of various feed components can influence coccidial infections in poultry. In addition, it has been suggested that the physical structure of the diet may play a role in the development of the disease [70].

Betaine, Ortrimethylglycine is natural product produced by most living organisms. Sugar beet concentrates large amounts of betaine and it is extracted in an industrial process, due to its three reactive methyl groups and its bipolar structure. The bipolar nature of betaine makes it important for osmoregulation, which assists in the balance of water within cells, generated by changes of concentration of charged particles in solution [71]. A dietary addition of betaine in birds infected with *Eimeria* spp. including *E. tenella* reduced coccidiosis lesions in the gut and increased performance. .this effect of betaine was more pronounced when acocciostat was also present in the diet. When betaine and salinomycin were fed together, weight gain and feed conversion efficiency were significantly improved, compared to when they were fed singly [70].

Fatty acid, polyunsaturated fatty acid (PUFA) in animal tissues Linoleic acid (n-6) is converted to Arachidonic acid (AA), and alpha-linolenic acid (n-3) converted to Eicosapentaenoic acid (EPA). EPA and AA are the precursors of Eicosanoids, important regulators of various immuno responses and feed ingredients high in n-6 fatty acids (corn, vegetable oil and poultry fat). Fish oil, fish meal and linseed are the major dietary sources of n-3 fatty acids. In practical broiler diets, normally several ingredients high in n-6 fatty acids are included, whereas the content of n-3 fatty acids is mostly limited. The addition of cod liver oil is beneficial to bird infected with coccidiosis. The birds feed the fish oil diet displaying lower weight losses and mortality than those feed the diet without fish oil. The diet high in n-3 fatty acids shows reduced lesions scores associated with coccidiosis caused by *E. tenella* because n-3 fatty acids in the diet can improve the development of resistance against coccidiosis infection [70].

Vitamin, Biotin is necessary for the development of the intracellular stages of the parasites. Thiamine, nicotinic acid, folic acid and riboflavin are also required for the successful completion of the lifecycle within the host. However, the use of vitamin-deficient diets has limitation. Various vitamins are also known to be associated with the way that the host deals with the disease. Vitamin k has antihemorrhagic properties and effective with hemorrhagic associated with *E. tenella* and *E. necatrix* infections. Increased level of vitamin A and E enhance the immune system by enhancing the proliferation response of lymphocytes to mitogens [72].

Protein, lower protein levels appear to reduce trypsin production, which is essential for excystation of the parasite. However, it is inevitable that at low protein levels performance would be sever affected, decrees lesion scores and pancreatic enzymes inhibitors [72].

Whole grains and dietary fiber, birds feed diets high in dietary fiber produced significantly fewer oocysts than those feed low fiber diets. Grains structure has no effect on coccidiosis but some suggested that the feeding of whole wheat was responsible for the decreased oocyst yields due to increased in gizzard size and activity, and that this was responsible for the mechanical destruction of oocysts before they could reach their infected sites [72].

2.14 Immunity

2.14 .1 Natural immunity

The surface layer involved with the digestive, respiratory and reproductive tracts is referred to as epithelium and the underlying tissue the lamina propria. The combination of these two tissues forms the mucosa. Mucosal membrane is considered as the largest organ system in vertebrates. To protect the body from infection within the mucosal immune systems of the gut, respiratory and reproductive tracts have highly developed lymphoid tissues such as the gut-associated lymphoid tissue (GALT) and bronchial-associated lymphoid tissues (BALT). In addition, there are well-developed immunological activities that provide essential protection in the different parts of these systems. Within the gut, especially,

there are different immunological requirements in different locations, because of the nature of the different local conditions and the specialized functions within different regions. In mammalian species, the GALT contain more lymphocytes than secondary lymphoid tissues, such as the spleen and lymph nodes. It is likely that this is also the case in avian species. The mucosal surfaces have a number of common features. Since each forms a major barrier between the external environment and internal milieu, they provide an important portal of entry for pathogens. This is especially the case with the gut and respiratory tract where the continuous movement of external substances, nutrients and air, respectively, and the need to transport or exchange essential molecules across the mucosal surface for organs to function properly and the animal remain healthy. Some organisms (mainly bacteria) may reside and have a beneficial effect on digestive processes, while pathogenic organisms can replicate in the mucosal epithelial cells or cross the mucosal surface to enter the body proper and cause disease [73].

A small-scale, low-density production system can allow a low level of exposure to coccidia, which permits the chicks to develop immunity without triggering the disease. However, birds may not pick up enough parasites to cause immunity. In addition, immunity is only species-specific; exposure to one type of coccidia will not protect a chicken from the other species that can infect it [74]. Oral inoculation of *E. tenella* led to parasite invasion of the intestinal caeca and caecal tonsils, protective immunity to *E. tenella* infection produce intestinal lymphocytes and gamma interferon [75].

Previous applications used vaccination to protect broilers via maternal antibodies, protein complex extracted from gametocytes stage of *E. maxima* elicited maternal protection and enabled young chicks to exposed *Eimeria* spp. without usual signs and consequences of coccidiosis, protection was heterologous against *E. tenella* and *E. acervulina* as well as against the homologous [76].

2.14.2 Vaccination

The application of attenuated vaccines for the prevention of chicken coccidiosis has increased exponentially in recent years. In *Eimeria* spp. infections, protective immunity is thought to rely on a strong cell mediated response with antibodies supposedly playing a minor role. However, under certain conditions antibodies seem to be significant in protection. Furthermore, antibodies could be useful for monitoring natural exposure of flocks to *Eimeria* spp. and for monitoring the infectivity of live vaccines [77].

Western blotting analysis of parasite antigens prepared from the lining of caeca infected with the attenuated strain of *E. tenella* revealed two dominant antigens apparently associated with trophozoites and merozoites that were present at high concentrations between 84 and 132 hours post-infection. When cryosections of caeca infected with *E. tenella* were probed with IgY purified from immune birds the most intense reaction was observed with the asexual stages. Western blotting analysis of proteins of purified sporozoites and third generation merozoites and absorption of stage-specific antibodies from sera suggested that a large proportion of antigens are shared by the two stages. The time-courses of the antibody response to sporozoite and merozoite antigens were similar but varied depending on the inoculation regime and the degree of oocyst recirculation [77].

In the past, most broiler producers have controlled coccidiosis by providing anticoccidial drugs in poultry feed; this approach is becoming less desirable in light of growing public concern about food safety. Presently, vaccination consists of infecting young poultry with a known dose of live coccidian parasites. This vaccination will immunize poultry against the disease [78]. Avian coccidia are highly immunogenic and primary infections can stimulate solid immunity to homologous challenges. Therefore, it would seem obvious that vaccines could offer excellent alternatives to drugs as a means controlling coccidiosis [21].

Live vaccine for coccidiosis control have been used to a limited degree by the poultry industry for about 60 years, their effectiveness hinges on the recycling of initially very low doses of oocyst and the gradual buildup of solid immunity. They

have been used primarily to protect breeder and layer flocks. However, their use, particularly in broiler flocks, is increasing. Live vaccine contains attenuated or not coccidial strains, an advantage of attenuated vaccines is that they have low reproductive potentials, thus avoiding crowding in the specific mucosal areas of infection and resulting in the development of optimal immunity with minimal tissue damage. It is believed that the drug-sensitive, attenuated strains and wild, native strains interbred, reducing both virulence and drug resistance in local population. Thus, the useful period of anticoccidial drugs could be extended by rotating their application with live vaccine [78].

A low-molecular-weight immunogenic antigen with a single immunodominant epitope was reported to be present in all endogenous stages of *E. tenella*. Metabolic antigens from developing sporozoites, merozoite antigens and gamete antigen all elicit various degree of protective immunity. A delivery mechanism for coccidial vaccines that produces optimum resistance to challenge infection has yet to be determined. Immunogenic *Eimeria* antigens have been administered as isolated proteins with adjuvants, as recombinant antigens in live vectors such as nonpathogenic strains of *Escherichia coli*, *Salmonella enteric, serovar and typhimurium*, poxviruses, fowlpox virus and turkey herpesvirus and by direct plasmid DNA injection with various degree of success[78].

A species-specific immunity develops after natural infection [79]. The degree of which largely depends on the extent of infection and the number of reinfections. Protective immunity is primarily a T-cell response. Commercial vaccines consist of low doses of live, sporulated oocysts of the various coccidial species administered at low doses in day-old chicks. Because the vaccine serves only to introduce infection. The vaccine strains of coccidia may or may not be attenuated. The self-limiting nature of Coccidiosis is used as a form of attenuation for some vaccines, rather than biological attenuation, Layers and breeders that are maintained on floor litter must have protective immunity. Often, they are given a suboptimal dosage of an Anticoccidial drug during early growth, with the expectation that immunity will continue to develop from repeated exposure to wild types of coccidia. This method

has never been particularly successful because of the difficulty in controlling all of these factors [80].

removing coccidiostats from feed and using vaccination is useful and Sweden is one of the world's leading countries in the area of removal of feed additives the potential problem associated with removal of coccidiostats and that is necrotic enteritis, caused by the gram-positive bacterium *Clostridium perfringens* [81]. Anticoccidial vaccines may not induce complete immunity in chickens with lowered immunocompetence due to stress, including certain viral diseases [82].

Types of Vaccines

Coccidial vaccines licensed in the US include Coccivac, Immucox and Advent vaccine. These vaccines can actually cause some lesions and occurrence of coccidiosis in birds because they are not attenuated or weakened in some way. It is a controlled occurrence, but it may be necessary to treat for secondary gut disease, using antibiotics or alternatives such as probiotics. In contrast, coccidiosis vaccines used in Europe are attenuated. They are altered because the coccidia used in the vaccine are designed to mature quickly and have a short life cycle and low fertility. They are not pathogenic disease causing and are more costly to produce than the nonattenuated vaccines. They include Paracox, Livacox, and Viracox, which are marketed in other countries but not currently in the US. More types of vaccines are likely to be developed, because the government approval process is much cheaper for vaccines than for anticoccidial drugs. Anticoccidial vaccines include mixtures of species of *Eimeria* that affect chickens. It is especially important to include the three types that cause the most damage in chickens; *E. acervulina*, *E. maxima*, and *E. tenella* [83].

Methods of application vaccine include;

Spray cabinets; these are used at hatcheries on day-old chicks, resulting in 90 to 95 percent of chicks exposed to the vaccine.

Edible gel; gel pucks are placed in transport crates or on the floor of the house when the chicks arrive.

Feed spray; vaccines are mixed with water in a garden pressure-sprayer and sprayed on a 24-hour supply of feed [83].

The chicks should be slightly water-starved to encourage them to drink. Since oocysts are heavy and fall to the bottoms of drinkers, they are mixed with a suspension agent to keep them evenly distributed. This method can be used for older chicks. Vaccines cannot be given through proportioners or nipple drinkers. It is important to apply vaccines uniformly to ensure the birds get equal exposure. If birds receive too much of a nonattenuated vaccine, the parasites can cause lesions. If attenuated vaccines are not given in adequate doses, the birds will be susceptible to field strains of the coccidia. The environment must allow the oocysts to sporulate, since the goal of vaccination is to introduce the parasite in small numbers. Litter should be damp but not wet after vaccination; birds excrete fresh oocysts onto the litter. Birds then eat these (second cycle) oocysts. Two cycles of replication are needed for good protection. Since the vaccines contain live oocysts, they should not be frozen. Do not give drugs and vaccines to the same flock, they are opposed to each other. Table 2.4 shows some commercial coccidiosis vaccines in poultry [83].

Table 2.4: some commercial coccidiosis vaccines in poultry [84]

Composition	Strain	Target Specie	Country	Company	Commercial Name
Eac, Emax, Emit, Eten	Non-attenuated	Broilers	USA	SPAH	Coccivac B
Eaden, Emeleag, Egallop, Edispersa	Non-attenuated	Poultry	USA	SPAH	Coccivac T
Eac, Emax (x2), Eten, Emit	Attenuated	Broilers	UK	SPAH	Paracox 5
Eac, Emit, Ebru, Ehag, Emax, Eprae, Eten, Enec	Non-attenuated	Breeders	USA	SPAH	Coccivac D
Eac, Emax (x2), Eten, Emit, Enec, Eprae, Ebru	Attenuated	Breeders (Chickens)	UK	SPAH	Paracox
Eac, Emax, Enec, Eten	Non-attenuated	Broilers	Canada	Vetech	Immucox I
Eac, Emax, Eten	Attenuated	Broilers	Czech Republic	Biopharm Res. Inst.	Livacox T
Eac, Emax, Eten, Enec	Attenuated	Breeders	Czech Republic	Biopharm Res. Inst.	Livacox Q
E.meleagrimitis and E. adenoides	Non-attenuated	Turkey Breeders	Canada	Vetech	Immucox for Turkeys
Eac, Emax, Eten	Non-attenuated	Broilers	USA	Viridus Animal Health	Advent
Emax	Non-attenuated	Broilers	USA	Elanco	VAC M
Eac, Emax (x2), Eten	Non-attenuated	Broilers	-----	Intervet	Nobilis Cox ATM
Eac, Emax, E. mitis, E. prae, E. ten.	-----	Broilers	Spain	Hipra	Hipracox Broilers
Eac (RA), Emax (MCK ₊₁₀), Eten (Rt ₃₊₁₅), Enec (mednec ₃₊₈)	Attenuated	Breeders & layers	Australia	Bioproperties	Eimeriavax 4m
Eac, Emax, Ehagani Eten, Enec, Eprae, Ebru, Emivati	Attenuated	Broilers	Korea	Cavac	Cocci – Vac
Eimeria acervulina, E. maxima (x2), and E. tenella	-----	Broilers	USA	Pfizer	Inovocox

Eac: *E. acervulina*, **Emax:** *E. maxima*, **Emit:** *E. mitis*, **Eten:** *E. tenella*, **Eaden:** *E. adenoides*, **Emeleag:** *E. meleagrimitis*, **Egallop:** *E. gallapovonis*, **Edispersa:** *E. dispersa*, **Ebru:** *E. brunetti*, **Ehag:** *E. hagani*, **Eprae:** *E. praecox* and **Enec:** *E. necatrix*.

In ovo vaccination

Developed by the Poultry Health Division of Pfizer Animal Health, is delivered via in ovo administration and will provide a new tool for the broiler industry to help control one of the global poultry industry's most prevalent and costly diseases. Inovocox is administered in ovo to 18 or 19 day-old incubated broiler chick eggs via an in ovo injection system (Figure 2.10). The in ovo administration of Inovocox helps ensure that every bird receives a uniform dose for effective protection. This technology is based on more than a decade of research, involving millions of birds to evaluate Inovocox for efficacy and safety. The Inovocox vaccine contains highly immunogenic, anticoccidial-sensitive, sporulated oocysts of *E. acervulina*, *E. tenella* and two strains of *E. maxima*. These originated from field isolates, which were screened and selected for their ability to help protect against challenge when administered in ovo, and for their sensitivity to anticoccidial drugs. Pre-hatch exposure to coccidial organisms will allow birds to develop early immunity to the disease [85].

Early and uniform flock immunity to coccidiosis helps provide control of clinical and subclinical coccidiosis and may result in more uniform growth and development of the flock throughout the grow-out. Inovocox has no significant effect on hatch rate. Performance trials show Inovocox-vaccinated flocks will help provide attractive weight gain, feed conversion and settlement costs. In addition, Inovocox vaccine may be used as a year-round coccidiosis control program, or as part of an annual rotation program. One dose of Inovocox helps provide broiler birds with life-long immunity against coccidiosis, the new vaccine is a useful addition to the use of in ovo injection systems, which already is utilised on a large scale in the broiler industry. It seems that Inovocox will be a new convenient, efficient and precise method of coccidiosis protection [85].

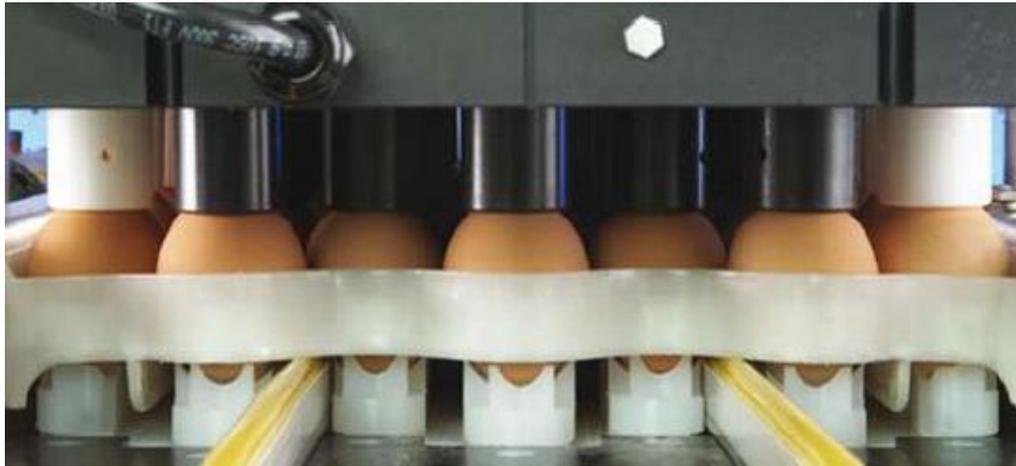


Figure 2.10: In ovo injection system

2.15 Potential hazards to human beings through anticoccidial residues in broilers meat

Anticoccidial drugs play an important role in animal production, especially in intensive broiler production. They are used for disease prevention and therapy, as well as for their growth-stimulating effect. These drugs contribute to the recovery of animals from protozoal endoparasites, increase breeding productivity and decrease economic losses caused by coccidiosis. However, mass and long-term administration of these substances has brought problems connected to the occurrence of unfavorable residues in animal products for human consumption. The residues of anticoccidial drugs represent a potential risk to human health. Proper administration of these substances will ensure minimal content in animal products that will minimize health risks. To protect the health of consumers against the entry of residues of anticoccidial drugs into the food chain, it is necessary to monitor drug residues in animals for food production and for valid veterinary hygienic legislation to pay appropriate attention to this group of drugs [86].

Some anticoccidial drugs such as Ionophores are not used in human medicine due to their potent cardiovascular effects. Ensure that recommended withdrawal periods are observed, it has been suggested that residues of ionophores in food could cause adverse health effects in humans as a result of their cardiovascular toxicity. Since

poultry litter is extensively applied to land as manure ionophores and their degradation products may readily enter the soil and water environment. Few studies have been published regarding the environmental fate of ionophores and thus it is difficult to assess their potential impact. Biodegradation studies have indicated that monensin is degradable under aerobic conditions with or without manure and in manure piles within 33 days. Degradation in manure piles under anaerobic conditions was less extensive. It should be assumed that the microbiological activity of soil will be affected, at least initially following application of ionophore containing manure and this may affect nutrient release. Direct effects on plants are not expected except that an inhibitory effect on apple pollen has been reported for monensin. Ionophores may cause irritation and allergic reaction in humans and protective clothing and dust masks should be used whenever there is a risk of exposure [87].

Alarming human health hazards, the emergence of resistant strains of bacteria in birds and passage of these or other resistant factors via food chain from birds to human beings. Use of antibiotics at sub-therapeutic levels in broiler feeds may lead to the development of resistant strains of bacteria in the bird. While consuming the meat containing residues of antibiotics over protracted period may lead to emergence of resistant gut flora and pathogens in human beings such as *E.coli* and *Salmonella* spp. Production of harmful effects from direct toxicity or from the allergic reactions (hypersensitivity reactions) in persons already sensitized to them. Some drugs and or their metabolites possess carcinogenic potential e.g.sulphamethazine residues containing meat preserved with sodium nitrate may develop a triazine complex that has a considerable carcinogenic potential. Prolonged ingestion of tetracycline present in the broiler meat has detrimental effects on teeth and bones in growing children. It is pertinent to mention that except for some tetracyclines, most therapeutic antibiotics are relatively heat stable and resist both pasteurization and cooking process [88].

Adverse effects on the cartilage development in children may result if the broiler meat contains quinolone residues. Drug residues may destroy the useful microflora of gastrointestinal tract, especially in children and hence lead to enteritis (diarrhea, dysentery) like problems. Super infections that refer to as fresh invasion or re-

infection added to an already existing infection. Candidiasis caused by *Candida albicans* is a classical example of the untoward consequence of the use of antibiotics. Residues of chloramphenicol are known to cause bone marrow depression and problems like anemia in consumers [88]. Table 2.5 show withdrawal period for some anticoccidial drugs. In addition, there are many safe veterinary drugs and none withdrawal period like, amprolium [89].

Factors that leading to the occurrence of antibiotics residues in animal products are; failure to observe drug withdrawal period, extended usage or excessive dosages of antibiotics, non-existence of restrictive legislation or their inadequate enforcement, poor records of treatment, failure to identify treated animals, lack of advice on withdrawal periods, off-label use of antibiotics, availability of antibiotics to lay persons as over the counter drugs in the developing countries, the addition of antibiotics as milk preservatives during hauling from the centre of production (villages) to the centers of consumption (cities or factories) and Lack of consumer awareness about the magnitude and human health hazards associated with antibiotic residues in the food of animal origin [88].

Table 2.5: Preventive anticoccidials approved by Food and Drugs Administration (FDA) for use in feed formulation [2]

Trade or Empirical Name	First Approval by FDA	Drug Withdrawal (Days before Slaughter)
Sulfaquinoxaline, 0.015—0.025%	1948	10
Nitrofurazone, 0.0055%	1948	5
Arsanilic acid or sodium arsanilate, 0.04% for 8 days	1949	5
Butynorate, 0.0375% for turkeys	1954	28
Nicarbazin, 0.0125%	1954	4
Furazolidone, 0.0055—0.011%	1957	5
Nitromide, 0.025% _ sulfanitran, 0.03% _ roxarasone 0.005%	1958	5
Oxytetracycline, 0.022%	1959	3
Amprolium, 0.0125—0.025%	1960	0
Zoalene, 0.004—0.0125%	1960	5
Amprolium, 0.0125% _ ethopabate, 0.0004/0.004%	1963	0
Buquinolate, 0.00825%	1967	0
Clopidol or meticlorpindol, 0.0125—0.025%	1968	0 days at 0.0125 5 days at 0.025%
Decoquinatate 0.003%	1970	0
Sulfadimethoxine, 0.0125% _ ormetoprim, 0.0075%	1970	5
Monensin, 0.01—0.0121 %	1971	0
Robenidine, 0.0033%	1972	5
Lasalocid, 0.0075—0.0125%	1976	3
Salinomycin, 0.004—0.0066%	1983	0
Halofuginone, 3 ppm	1987	5
Narasin, 54—72g	1988	0
Madurimicin, 5—6 ppm	1989	5
Narasin _ nicarbazin, 54—90 g	1989	5
Semduramycin, 25ppm	1995	0

Chapter Three

Materials & Methods

3.1 Introduction

This chapter contains the procedures followed throughout the study. It includes a complete description of the methodology of the study, the study site, the population, the sampling, the instrumentation and the research design. Moreover, it describes the statistical analysis used for the study findings.

3.2 The methodology of the study

The study design was cross sectional to determine the prevalence of caecal coccidiosis among broilers in Gaza strip to estimate the prevalence of *Eimeria tenella* and its seasonal occurrence in the study area. Questionnaire survey was also conducted to collect information from farmers of poultry farms regarding the general conditions and bio safety procedures about poultry in the study area.

3.3 The study area

The study was conducted in Gaza strip, which is internationally recognized as part of the Palestinian territories.

3.4 The study population

The community of the study consists of all broilers in Gaza strip five governorates; Rafah, Khanyonis, Middle, Gaza city and North, during the three months of September, October and November in the year 2009. Farmers breed 1.5 million broilers per month that is 4.5 million in 3 months period.

3.5 The sampling

This study is cross sectional and because the prevalence of coccidiosis in chicken farms has not been reported in Gaza strip, the prevalence of infection in each farm was assumed to be 50%. The calculated sample size was 385; using a 95%, level of confidence and 5% desired absolute precision [90]. In the present study, random sampling was used to collect 390 broilers. A total of 390 intestinal gut samples

(caeca) of broilers were collected from poultry shops in the markets of Gaza strip in September, October and November 2009. For each shop, 10 birds were collected, which represents a farm. Table 3.1 shows the distribution of the samples among governorates and table 3.2 by months.

Table 3.1: The distribution of the collected samples in Gaza governorates

Group	Frequency	Percent
North	60	15.38
Gaza	150	38.46
Middle	60	15.38
Khanyounis	60	15.38
Rafah	60	15.38
Total	390	100

Table 3.2: The distribution of collected samples by months

Group	Frequency	Percent
September	40	10.26
October	200	51.28
November	150	38.46
Total	390	100

3.6 The variables of the study

The study included the following variables:

The independent variables represented in

1- Tests:

Direct smear scraping

Test tube flotation

2 - Months: September, October and November

3 - Governorates; Rafah, Khanyonis, Middle, Gaza city and North

4 - Weight of birds

3.7 Examination and identification of *E. tenella*

All the collected caecae were kept in an icebox for 8 - 12 hours in the laboratory at 4-8 °C. All samples were examined by qualitative techniques according to Section for Parasitology, Institute of Veterinary Microbiology The Royal Veterinary and Agricultural University, Copenhagen, Denmark and Animal Production and Health Division, Food and Agriculture Organization of the United Nations Rome, Italy [1]. Clinical disease using postmortem for each bird was recorded. The caeca of gastrointestinal tract was grossly examined carefully.

3.7.1 Qualitative techniques

A large number of different procedures are available for demonstrating coccidia oocysts in poultry faeces. Two methods, which provide qualitative or at the most semi quantitative results will be described below. The most widely used principle for concentration of parasite eggs is flotation. As most nematode eggs, cestode eggs and coccidia oocysts have a specific gravity which is lower than that of plant residues in the faeces. The oocysts may be separated from other faecal particles by mixing the faeces with a fluid in which the oocysts float, while the plant particles sink. Unfortunately, the specific gravity of helminthes eggs varies, while most nematode and cestode eggs will float in saturated NaCl. Some nematode and cestode species have eggs which will float only in fluids with higher specific gravities, such as saturated MgSO₄ or saturated NaCl + glucose (as used below). Among poultry helminthes, little is known about this fact, and therefore it is recommended to standardize and continuously use only one of the flotation fluids with high specific gravity, e.g., the saturated salt and sugar solution.

3.7.1.1 First test: Direct smear scraping

All the caecae were opened, their contents were evacuated, and direct scraping for caecal mucosa was done. Direct microscopic examination of intestinal mucosa can only be used in animals, which have been culled or found dead. It can be used to detect the intracellular and extracellular stages of coccidia, other protozoa and small nematodes.

Procedures

A deep scraping was done of the suspected mucosa with one end of the slide. The material was spread in a very thin layer on a new slide and covered with a cover slip and the thin smear was examined microscopically using 100 x magnification (attached in the appendix).

3.7.1.2 Second test: Test tube flotation

All the caecae were opened and their contents (faeces) were collected in a beaker. The faeces were macerated and the suspension was filtered through a muslin cloth and allowed to float. The oocysts in the flotation were separated by flotation method in saturated sodium chloride and sugar solution. They were examined microscopically and the species were identified on the basis of shape and size of oocysts. This is a simple qualitative flotation technique for the detection of nematode eggs and coccidia oocysts in the faeces.

Procedures

Approximately 3 g faeces (weigh out or measure with pre-calibrated teaspoon) transferred to plastic container 1, fifty ml of flotation fluid was poured into plastic container 1 by means of the measuring cylinder, faeces and flotation fluid was mixed thoroughly with a stirring device immediately after stirring.

The faecal suspension poured through a tea strainer or a single layer of cotton gauze into plastic container 2, the retained faecal debris discarded, and poured the strained faecal suspension from plastic container 2 into a test tube immediately.

Test tube was placed in a vertical position in a test tube rack, the test tube was topped up with the faecal suspension, so that it has a convex meniscus at the top and a cover slip was placed on the top of the test tube.

The test tube was leaved for about 20 minutes. The coccidia oocysts floated and thus accumulated just beneath the cover slip, the cover slip was lifted off vertically from the tube together with the adhering flotation fluid. Some of the accumulated coccidia oocysts are within the adhering fluid, and the cover slip was transferred very

carefully in order to retain as many oocysts as possible. The cover slip was placed on a microscope slide, and the sample was examined microscopically at 40-100 x magnification using a light microscope (attached in the appendix).

3.7.1.3 Clinical coccidiosis

Using postmortem examination, *E. tenella* infections are found only in the caeca and can be recognized by accumulation of blood in the caeca and by bloody droppings. Caecal cores, which are accumulations of clotted blood, tissue debris and oocysts, may be found in birds surviving the acute stage. In this study, chicken that showed caecal core were recorded as clinical cases

3.7.1.4 Identification of *E. tenella* using measurement of oocysts

Length and width were measured for 50 oocysts to determine the shape and size of oocyst using Eyepiece graticules, stage micrometers and recording for each positive case.

3.8 Questionnaire

Information collected at the time of sampling included farmer's name, address, farm location, flock age, flock size, use of coccidiostats in the feed for that flock and previous coccidiosis infection. The questionnaire was conducted on 30 farmers having direct practice in poultry production, 6 farmers in each governorate. The questionnaire was developed and used to gather information regarding general production system in the study area, farmer's treatment practices and knowledge of disease were also recorded. The questionnaire included three parts, demographic, bio-safety procedures and farmer's knowledge of the disease. Three local experts evaluated the questionnaire (attached in the appendix).

3.9 Documentation and storage

Canon 7.1MP camera model no: Power Shot A470 was used to document the study and 106 (50 %) positive samples kept in 10 % formalin.

3.10 The statistical analysis

Data were collected and computed by using version 11 of Statistical Package for Social Science, (SPSS). Frequencies, mean, standard deviation and chi square were the main statistical treatments made in the present study.

Chapter Four

Results

4.1 Parasites detection

4.1.1 Prevalence

Frequency, percent, direct smear scraping to detected *Eimeria tenella* schizont and test tube flotation to detected *E. tenella* oocysts (Figure 4.1) were used to determine the prevalence of caecal coccidiosis.

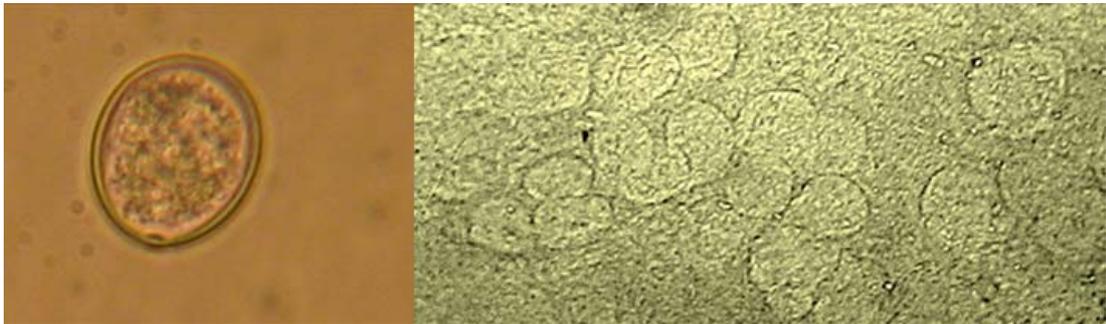


Figure 4.1: *E. tenella* oocyst 100 (left) x and shizonts 10 x (right)

From 390 broilers caecal samples examined, 212 (54.4%) and 129 (33.1) were infected as shown by direct smear scraping and test tube flotation respectively. In addition, clinical coccidiosis was observed in 14 birds (3.6 %). From 39 stocks, it was found that 22 (56.4%) stocks and 17 (44.7 %) were positive by using direct smear scraping and test tube flotation respectively, while clinical coccidiosis appeared in four stocks only (10.3 %) (Figure 4.2).

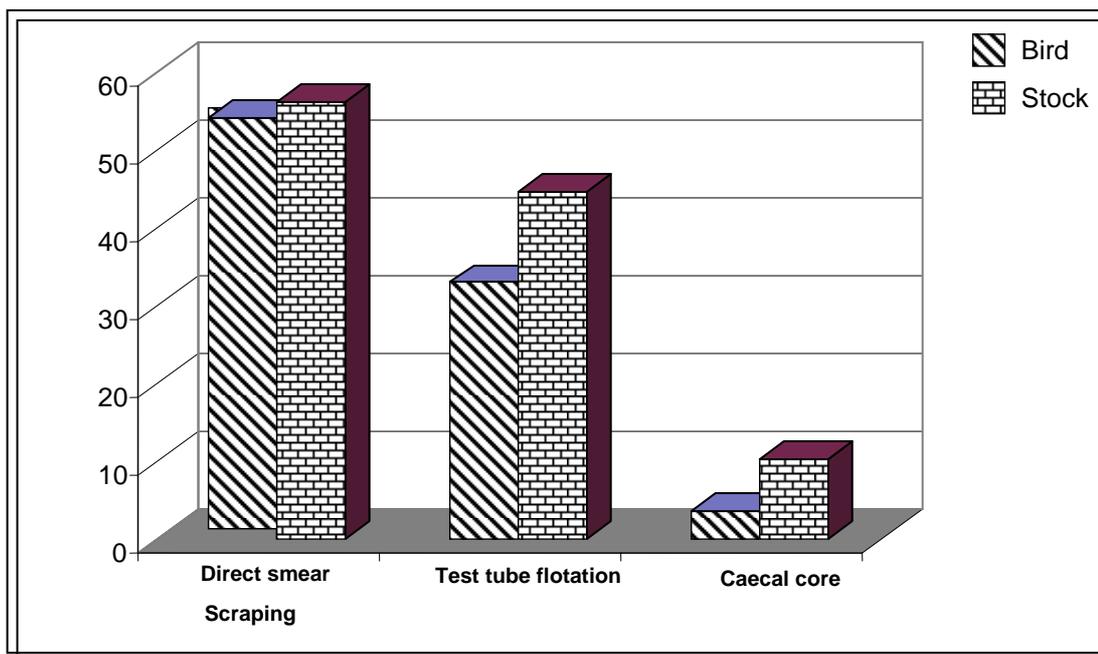


Figure 4.2: Prevalence using direct smear scraping, test tube flotation and clinical coccidiosis

Accumulation of blood in caecae were seen in infected birds of clinical coccidiosis (Figure 4.3).



Figure 4.3: Clinical coccidiosis using postmortem

4.1.2 Differences of prevalence among Gaza governorates

Chi-square test to determine the differences of prevalence between Gaza governorates was used. Middle governorate represented the highest prevalence (80%), while in North governorate the prevalence was (66.7%), Khanyounis governorate (50%), Rafah governorate (45%) and Gaza city governorate (44.7) using direct smear scraping. North showed the highest prevalence (43%), Gaza city (42%),

Middle (35%), Khanyounis (20%) and Rafah (11.7%) using test tube flotation. Clinical coccidiosis which was found in 14 birds shows that the North governorate had the highest prevalence 10 (16.7%), Gaza city governorate 3 (2%) and the Middle governorate 1 (1.7%) (Table 4.1).

Table 4.1: Differences of prevalence among Gaza governorates

Test	PLACE	- ve		+ ve		Total	Pearson Chi-Square	Df	Sig. (2-sided)	Sig. level
		Count	%	Count	%					
Direct smear scraping	North	20	33.3	40	66.7	60	27.821	4	0	0.01
	Gaza	83	55.3	67	44.7	150				
	Middle	12	20.0	48	80.0	60				
	Khan-Younis	30	50.0	30	50.0	60				
	Rafah	33	55.0	27	45.0	60				
Test tube flotation	North	34	56.7	26	43.3	60	25.407	4	0	0.01
	Gaza	87	58.0	63	42.0	150				
	Middle	39	65.0	21	35.0	60				
	Khan-Younis	48	80.0	12	20.0	60				
	Rafah	53	88.3	7	11.7	60				
Caecal core	North	50	83.3	10	16.7	60	35.851	4	0	0.01
	Gaza	147	98.0	3	2.0	150				
	Middle	59	98.3	1	1.7	60				
	Khan-Younis	60	100.0	0	0.0	60				
	Rafah	60	100.0	0	0.0	60				

4.1.3 Differences of prevalence due to weight

Chi-square test was used to determine the differences of prevalence due to weights after dividing the broilers to four groups. It was found that broiler group from 1.6 to 1.9 kg of weight showed the highest prevalence (63.9%) followed by 1.3 to 1.5 kg (62.1%) and the 1 to 1.2 kg (20%) and no prevalence in 2 kg of weight using direct smear scraping.

Broiler group from 1.3 to 1.5 kg of weight showed the highest (45%) followed by 1.6 to 1.9 kg (31.1%) and the 1 to 1.2 kg (20%) and no prevalence in 2 kg of weight using test tube flotation.

Clinical coccidiosis was only found in groups 1.3 to 1.5 and 1.6 to 1.9 kg with prevalence (9.3%) and (6%) respectively (Figure 4.4).

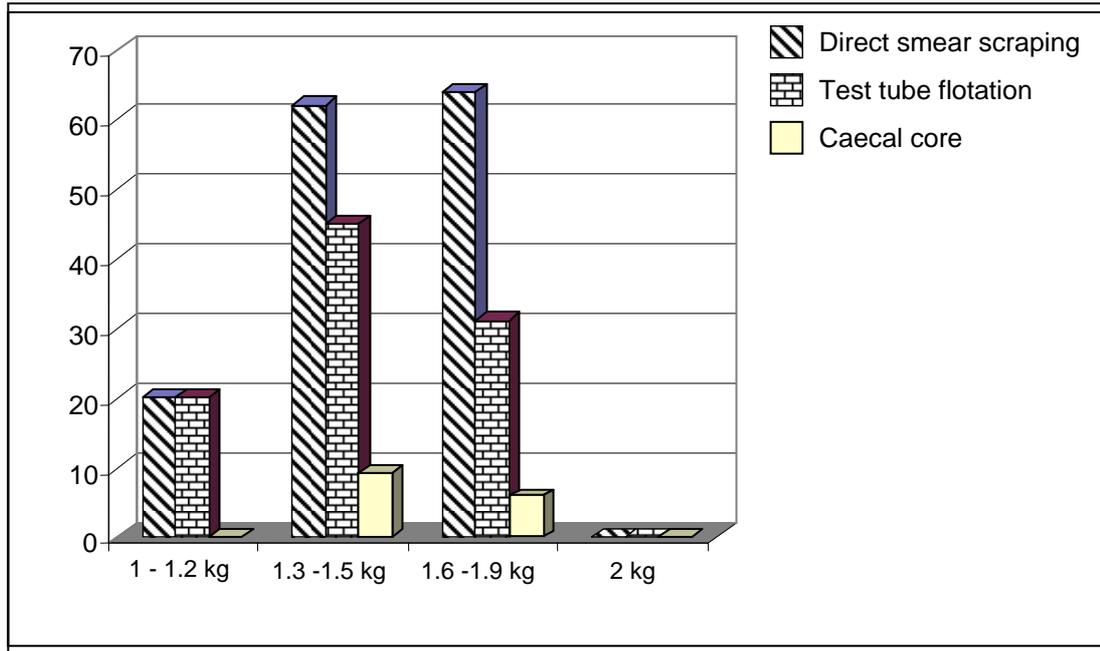


Figure 4.4: Differences of prevalence due to weight

4.1.4 Differences of prevalence during collection period in Gaza strip

Chi-square test was used to determine the differences of prevalence during the collection period, where the disease was observed during the entire period of the study in Gaza strip. The prevalence was noted high in September (75 & 72.5%), October (66 & 31.5%) and November (33.3 & 24.7%) using direct smear scraping and test tube flotation respectively. Clinical disease found only in October (14 cases 7%) (Figure 4.5).

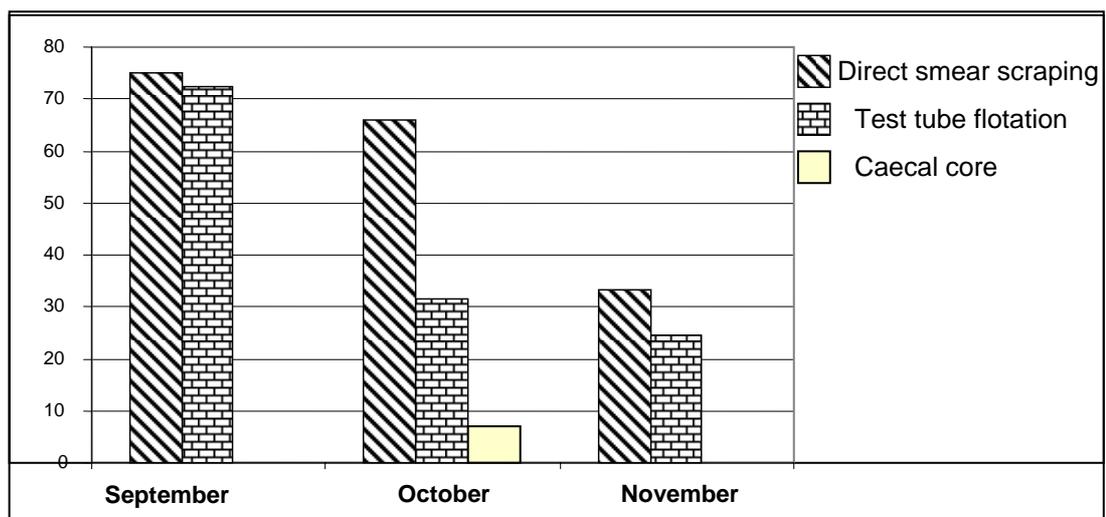


Figure: 4.5: Differences of prevalence during collection period in Gaza strip

4.1.5 Differences of prevalence during collection period in Gaza city

Chi-square style was used to determine the differences of prevalence among the months. The disease was observed in all the period of the study in Gaza city. The prevalence was highest in September (75 & 72.5%) followed by October (34 & 28%) and November (33.3 & 33.3 %) using direct smear scraping and test tube flotation respectively. Clinical disease was found only in October (3 cases 6%) (Figure 4.6).

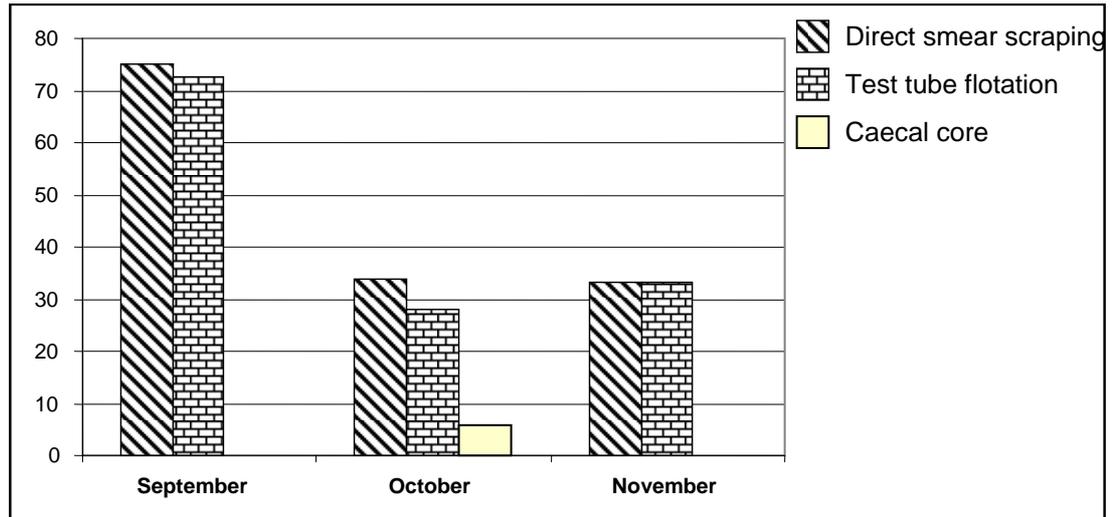


Figure: 4.6: Differences of prevalence during collection period in Gaza city

4.1.6 *E. tenella* oocyst size

Minimum value, maximum value, mean and standard deviation were used in identification of *E. tenella* oocysts using Eyepiece graticules, stage micrometers to determine the size of oocysts; *E. tenella* oocysts size are varied (Table 4.2), and the mean is 17.8 x 22.2 μm (Table 4.3).

Table 4.2: Difference in sizes of *E. tenella* oocysts

Oocyst	Size- μm		Oocyst	Size- μm	
	Length	Width		Length	Width
1	26	21	26	22	18
2	22.5	17.5	27	25	18
3	25	17.5	28	23	19
4	23	18	29	20	18.5
5	22	20	30	20	16.5
6	22	18	31	21	16.5
7	20	19	32	24	16.5
8	20	17	33	21	17
9	21	18	34	20	17
10	21	20	35	21	16.5
11	24	18	36	21	17
12	22	19	37	25	17
13	21	18	38	23	18
14	23	19	39	24	19
15	22	16.5	40	22	18
16	21	18	41	24	19
17	24	16.5	42	21	18
18	21	17	43	21	17
19	24	18	44	24	18
20	20	16.5	45	24	17
21	24	19	46	23	17
22	22	19	47	19.5	18
23	21	17	48	23	18
24	22	16.5	49	22	18
25	20	17	50	24	17

Table 4.3: Minimum value, maximum value, mean and standard deviation of *E. tenella* oocyst

Measurement/ μm	N	Minimum	Maximum	Mean	Std. Deviation
LENGTH	50	19.50	26.00	22.220	1.648
WIDTH	50	16.50	21.00	17.830	1.043

4.2 Questionnaire

4.2.1 Description of bio-safety procedures

Bio-safety procedures in broilers houses were poor, 27 (90%) semi closed farms and 3 (10%) opened. It was found that 73.3% with farm door and 26.7 % without. A total of 73.3% of farms were found to be not protected against wild animals and birds and 26.7 protected, 83.3 % of farmers did not have disinfection pool. It was found that 46.7 % of farms allowed trucks loaded with birds or their manure to access the farm. While 73.3 % allowed trucks loaded with bird's feed to access the farm. Storage feed inside poultry houses consisted 66.7 % (Figure 4.7). It was found that 86.7 % used disinfection materials to clean the farm but 100 % did not know its concentration and also 100 % thought the health procedures are important (Table 4.4).



Figure 4.7: Storage feed inside poultry houses

Table 4.4: Bio-safety procedures in broilers houses

Questions	Yes	%	No	%
1. Is the farm semi closed?	27	90	3	10
2. Is the farm protected against wild animals and birds?	8	26.7	22	73.3
3. Farm gate?	22	73.3	8	26.7
4. Disinfection pool?	5	16.7	25	83.3
5. Trucks loaded by birds or their manure access to the farm?	14	46.7	16	53.3
6. Trucks loaded with bird's feed access to the farm	22	73.3	8	26.7
7. Is there special store for poultry feed or inside the hunger?	10	33.3	20	66.7
8. Do you use disinfection materials to clean the farm?	26	86.7	4	13.3
9. Do you feel that health procedures are important for you?	30	100	0	0

In the present study, 63.3 % of the farmers reported the veterinary services teams did not wear protective personal equipments (PPE) when they enter the farm, 33.3% sometime and 3.4% did. Ten percent added new stock before marketing the previous one. Forty percent did not leave two weeks or more periods between stock and the next, 36.7 % observed this period and 23.3% observed it frequently sometime (Figure 4.8).

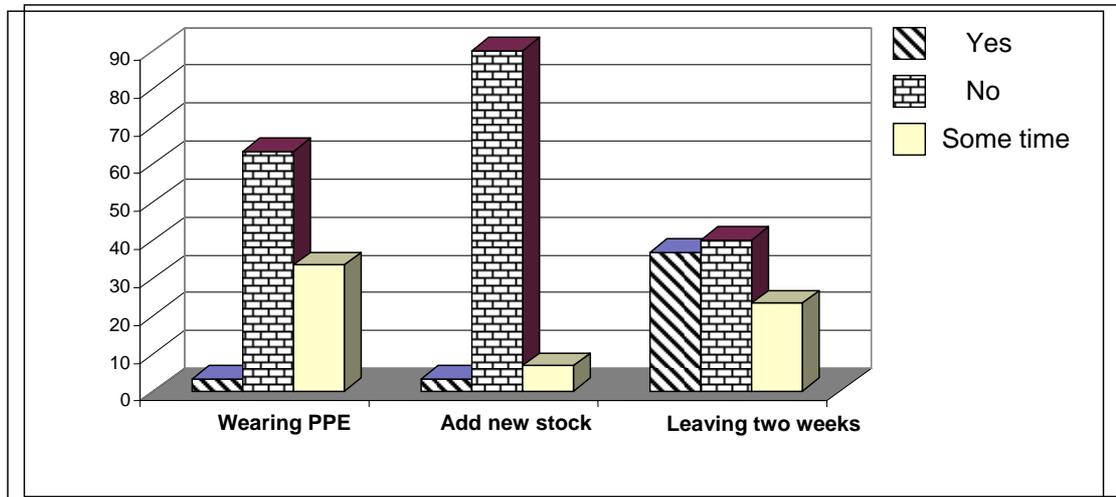


Figure 4.8: Bio-safety procedures in broilers houses

4.2.2 The farmers' knowledge of coccidiosis

Knowledge of disease was scarce among the broilers farmers where 50 % reported that they hear about coccidiosis, thinking that coccidiosis affects the health of birds. They used anticoccidial drug, did not use coccidiosis vaccination and the veterinarian performed postmortem examination for birds before and they suspected coccidiosis (Table 4.5).

Table 4.5: The farmers' knowledge of coccidiosis

Question	Response	Frequency	Percent	
Did you hear about coccidiosis?	Yes	15	50	
	No	15	50	
	Total	30	100	
The farmer who responded Yes				
Questions	Yes	%	No	%
1. If yes, do you think that coccidiosis is affecting the health of birds?	15	100	0	0
2. If yes, do you use anticoccidial drug?	15	100	0	0
3. Do you use coccidiosis vaccination?	0	0	15	100
4. Did veterinarian performed postmortem for birds before and he suspected of coccidiosis?	15	100	0	0

Sixty percent of them used the same anticoccidial drug in reinfection in the same stock, 73.3 % thought that coccidiosis causes economic losses, 46.7 % removed anticoccidial drug at least 3 days before marketing, 40 % sent samples for veterinary laboratories and received positive reports for coccidiosis, 6.7 % refer to governmental veterinary services and 93.3 % to private veterinarians (Figure 4.9).

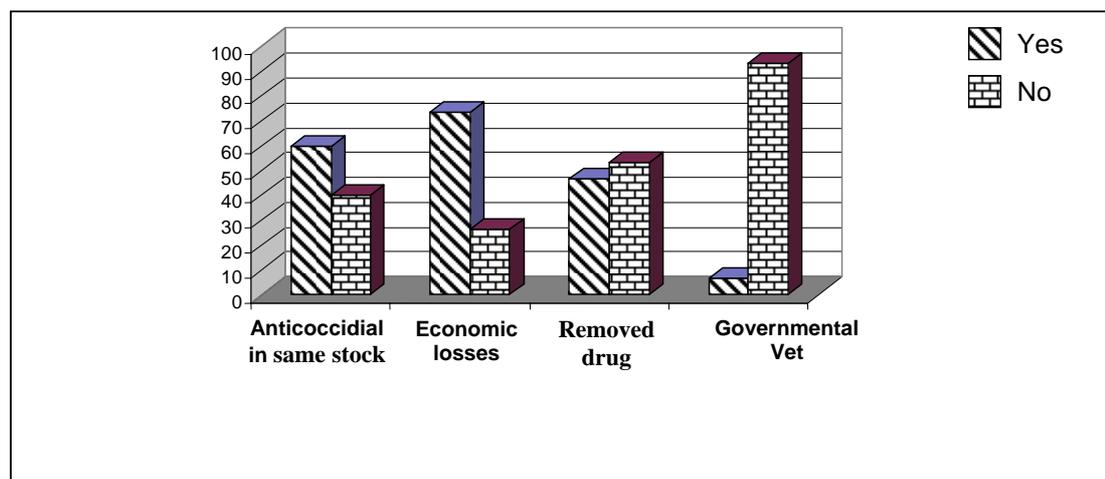


Figure 4.9: The farmers dealing with coccidiosis

Chapter Five

Discussion

5.1 Parasite detection

5.1.1 Approved technique

Direct smear scraping test, is more sensitive than test tube flotation to detect sub-clinical coccidiosis because the first was used to detect the parasite stage in the caecal mucosa and the second one detects the parasite oocysts in faeces or intestinal contents. Oocyst might be absent during certain periods of the disease, the test tube flotation may give false negative results [23]. In this study, the prevalence using test tube flotation was lower than in direct smear scraping. This may be explained by the fact that the timing of inspection was not appropriate to discover the oocysts.

5.1.2 Prevalence in individual birds and stocks

The prevalence in the present study in individual sample and in all stock as one sample was very near (54.4%) and (56.4%) respectively which give significance of this study to determine the prevalence of the disease using both methods because the birds in same farm are homologous. Clinical disease which was recognized by caecal cores. *E. tenella*, the well-known cause of caecal or bloody coccidiosis invades the two caecae and in severe cases may also parasitize the intestine above and below the caecal junction. Only 14 cases, which were diagnosed, might be due to the marketing before this stage, which appears on the fifth to seventh day of infection.

5.1.3 Differences of prevalence among Gaza governorates

There was a decrease in the prevalence in the samples collected from north to south and which can be referred to early rainfall during the study period and percent of rainfall, which is more in the North which played a role in the high level of moisture which is important for oocyst sporulation [22]. The appearance of highest prevalence of clinical disease in North governorate which were 10 from 14 birds found in the study site support this hypothesis.

5.1.4 Differences of prevalence due to weight

The results of this study showed that the prevalence of the infection increased among the older chicks. Whose weight is 1.7 kg and age is 6 – 7 weeks. It was found that the prevalence increased with the weight except the weight of 2 kg which might be due to the use of medication [91], or infection free stocks. The Excystation of *E. tenella* sporozoites was more rapid in chicks aged 4, 5, and 6 weeks than in those 0, 1, 2, and 3 weeks [54].

This result was in agreement with a study in Northwest of Iran which recorded that the prevalence of infection was increased with the age of the chickens, chickens with 5 weeks of age showed the highest prevalence of infection [43], and with other in Mashhad, Khorasan, Iran who found an increased risk of infection in the broiler which was associated with the larger farm, in older chickens [91]. On the other hand, the result here was not in agreement with a study in Saudi Arabia which recorded that the younger chicks were more susceptible to the infection than the older ones [41]. This disagreement may be due to the fact that the older chickens may develop immunity due to their exposure to subclinical infection from the contaminated environment.

5.1.5 Differences of prevalence during collection period in Gaza strip

The results of this study showed that the disease was observed in all the period of the study in Gaza strip, the prevalence was highest in September because oocyst sporulation may be better in drier, rather than wetter, litter [38]. Moreover, coccidiosis generally occurs more frequently during warmer (May to September) than colder months (October to April) of the year [22].

5.1.6 *E. tenella* oocyst size

Characteristics of *Eimeria* spp. were useful in the identification of species and location of parasites in tissues in the intestine, oocyst size, shape, and color. *E. tenella* is easy to be recognizing because of the characteristics of oocysts, length 19.5 to 26 and width 16.5 to 22.8. The result of this study showed that the mean of *E.*

tenella oocyst size was 17.8 x 22.2 µm, (length 19.5 to 26) and width (16.5 to 21) µm. The findings are more or less similar to others [2].

5.2 Bio-safety procedures

Coccidiosis is one of the most important and common diseases that affect poultry, it results in a great economic loss all over the world. The disease causes a reduction in feed intake and feed conversion efficiency with subsequent weight loss, [50]. Management of poultry houses plays a significant role in the spread of coccidiosis because coccidial oocysts are ubiquitous and are easily disseminated in the poultry house environment. Further, owing to their high reproduction potential, it is very difficult to keep chickens coccidia free, especially under current intensive rearing conditions. Oocysts sporulate readily in poultry house litter. However, bacteria, other organisms and ammonia that are present can damage them and their viability can begin to diminish after three weeks [92]. Bio-safety measures such as requiring attendants to change clothes between houses can minimize the spread of infective oocysts [31].

The high prevalence of the diseases in the present study, indicate that the bio-safety procedures may be poor. Mechanical transmission of the diseases happened in all farms since the farms are semi closed or open, trucks loaded by bird feed, birds and birds manure access to the farm. Wild animals and birds play a role in transmission of the diseases from farm to others and the veterinary services teams when moving from infected farm to others can also be a reason. Reinfestation due to storage the feed inside the birdhouse, feeding with contaminated feed, reinfestation in the same farm for next stock due to shorten period between breeding stocks and useless of disinfection material. Unawareness of farmers play a role in this deteriorating situation, in addition they want to make more profits by saving in purchasing medicines, vaccines or adopt proper hygiene in the farms.

In the past, most broiler producers have controlled coccidiosis by providing anticoccidial drugs in poultry feed; this approach is becoming less desirable in light of growing public concern about food safety. Presently, vaccination consists of

infecting young poultry with a known dose of live coccidia parasites. This vaccination will immunize poultry against the disease [77]. Vaccination reduces the prevalence of disease; half farmers of broilers in study site have a good knowledge to dealing with the diseases and half were not, but all farmers in this study site were not using vaccination to protect their stocks from coccidiosis, drug resistance against coccidiosis and potential residual danger to the consumer has limited usefulness of already existing allopathic anticoccidials. *Eimeria* spp. is developing resistance against drugs, the farmers used the same anticoccidial drugs all times, none effective and none registered drugs, sulfaquinoxaline is the wildest drug used in prevention and treatment of coccidiosis, if sulfonamide-resistant founded of *E. tenella* or other species and it was not efficacious [7].

The use of disinfectant materials after an outbreak of coccidiosis protect from infections in other period of breeding [91, 93]. In our study, the farmers added new stock before marketing the previous one. They did not leave two weeks or a more period between stock and the next and had weak knowledge of disinfection materials. The hazard is that the farmers did not remove the anticoccidial drugs before days of marketing. In addition, anticoccidial drugs were additive for broilers feed and influenced the human health. However, none of the recognized species of *Eimeria* in broilers was reported among chicks in Gaza strip until now.

In this study, we confirm that, the prevalence of caecal coccidiosis among broiler in Gaza strip was 54.4%. This rate is high compared to results of a survey in northwest of Iran where it recorded 14.2%. In Mashhad, Khorasan, Iran it recorded 12% [43, 91], in Pakistan they recorded 30.6% [31], and in northern Jordan it recorded 39% [93]. In addition, the rate in this study is lower compared to the results of other survey in Saudi Arabia which recorded a prevalence of the infection of 80% among the house reared chicks while no infection was reported among the farm chicks [41]. The survey in Saudi Arabia was very important to be compared with present study; Saudi Arabia study showed that management system played a great role in the epidemiology of coccidial infection. Despite, the observed high prevalence rate of coccidiosis in the house reared chicks. Therefore, we evaluate the Gaza strip farms as

house-reared farms. The prevalence was lower than in eastern China, which recorded 90 % [42]. In Ethiopia, prevalence was 55.6% [35].

The Poor management practices in the present study, the warm weather and high moisture in study site might be a direct cause of high prevalence. In addition, one cause of this difference might be due to the different seasons in which the survey was undertaken. It was noticed that there is no apparent control on public and private veterinarians concerning the use of drugs and biological preparations in poultry. However, poultry farmers apply their own indigenous practices to treat and control the disease. Those might be improper and might be cause public health hazards. Good management including good ventilation, dry clean litter, cleaning, decontamination of drinkers and feeders and proper stocking density in the farm can control coccidiosis [94, 95].

5.3 Trouble of broilers price

The main effects that cause economic losses are a decreased weight gain may be partially due to the malabsorption of nutrients through the gut wall. This effect causes an increased feed conversion ratio, which is the amount of feed converted into body weight [50], the highest of cost input in most farms is one of the causes of price trouble.

Chapter Six

Conclusions & Recommendations

6.1 Conclusions

- 1- In the present study, the prevalence of sub-clinical caecal coccidiosis among broilers in Gaza strip was 54.4%.
- 2- The prevalence of the infection increased among the older broiler chicks.
- 3- Probability the infection increases in moisture and waterier season.
- 4- Absence of clinical signs of disease does not mean the farm is not infected; diagnosis is based on the presence of lesions at postmortem and the identification. With the aid of a microscope, using direct smear scraping is more sensitive and low coast and time.
- 5- *E. tenella* in the present study was found to cause economic losses in broilers.
- 6- Bad management such as wet litter that encourages oocyst sporulation, contaminated drinkers and feeders, bad ventilation, high stocking density, movements of trucks and persons caused infection.
- 7- Frequent use of drugs has led to widespread drug resistance.
- 8- The vaccines which develop the immunity of the bird to infection not used in study site.
- 9- There is no commitment to the withdrawal period of anticoccidial drugs among broiler farms.

6.2 Recommendations

Agriculture, Health, Economic Ministries, Municipalities, Universities and Veterinarians must cooperate specially in poultry production sector by:

- 1 – Raising awareness of farmers on the importance of bio-safety measurement, resistance of drugs, effect of vaccination, spreading grains in manure, dealing with disease, using drug, which do not have withdrawal period and importance of change the concentrated feed in last five days by grains.
- 2 - Controlling the illegal and misuse of drugs.
- 3 - Controlling the utilization of antibiotic additives in broiler feed.
- 4 - Poultry feed producers must be shows anticocccial drugs additives.
- 5 - Proper withdrawal period that should be given to the feed additives, so that the bird should not contain any residues after slaughtering.
- 6 - Proper legislation must be proposed and implemented in Gaza strip.
- 7 - Preventing antibiotics used for human therapy to be used for growth promotion purpose of poultry birds.
- 8 - Testing contaminated meat, if suspected, in laboratory for the presence of antibiotic residues.
- 9 - Raising the awareness concerning the use of drugs only when unavoidable.
- 10 - It is the responsibility of every veterinarian who administers drugs, to have in mind, that the drugs are handled in a proper way to avoid any harmful exposure to humans.
- 11 – Coccidiosis vaccine not available in Gaza strip; it is important to be used in both layers and broilers.
- 12 - Changing the strategy of poultry handling in Gaza strip by changing the poultry slaughter shops into stores of poultry meat and all slaughtering must be in central slaughterhouse and distribute the products by cooler vehicles. This step will put all poultry products under control and inspection and will protect us from the risks of contamination through the distribution the live birds.
- 13 - Conducting research on the economic significance of the poultry diseases and the hazards or drugs residues in the poultry products.

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Appendixes

Appendix 1: English Questionnaire

This is questionnaire survey of Prevalence of Caecal Coccidiosis among Broilers in Gaza strip in Partial Fulfillment of the Requirements for Master Science in Biological Sciences Degree



Demographic characters

Farm Owner..... Address

Farm Opening DateStock No.....

No Of Birds Birds Age.....

Put X in the square

Bio – safety characters

Is the farm

Opened

Semi closed

Farm gate

Yes

No

Is the farm protected against wild birds and animals?

yes

no

Disinfection pool

Yes

No

Trucks loaded by birds or their manure access to the farm

Yes

No

Trucks loaded with bird's feed access to the farm

Yes

No

Is there special store for poultry feed or inside the farm?

Yes

No

Do you use disinfection materials to clean the farm?

Yes

No

If yes what is disinfection and concentration?

It is

Do you feel that health procedures are important for you?

Yes

No

Do the veterinary services teams wearing PPE when they enter the farm?

Yes

No

Sometimes

Do you add new stock before marketing the previous one?

Yes

No

Sometimes

Do you leave two weeks or more periods between stock and the next one?

Yes

No

Sometimes

Knowledge characters

Did you hear about coccidiosis?

Yes

No

If yes, do you think that Coccidiosis is affecting the health of birds?

Yes

No

If yes, do you use anticoccidial drug?

Yes

No

Do you use coccidiosis vaccination?

Yes

No

Did veterinarian performed postmortem for birds before and he suspected of coccidiosis?

Yes

No

Do you use the same anticoccidial drug in reinfection in the same stock?

Yes

No

Do you think that coccidiosis is causing economic losses?

Yes

No

Do you stop anticoccidial drug at least 3 days before marketing?

Yes

No

Did you send samples for veterinary laboratories and you received positive reports for Coccidiosis?

Yes
No

If you notice symptoms of diseases among birds, do you refer to governmental veterinary services or private veterinarian?

Governmental
Private

Appendix 2: Arabic Questionnaire



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.....

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.....
.....

X

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	<input type="checkbox"/>	<input type="checkbox"/>	*
	<input type="checkbox"/>	<input type="checkbox"/>	*
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Appendix 3: Estimated Budget

	<i>Item</i>	<i>Unit</i>	<i>No</i>	<i>Unit Price</i> \$	<i>Total Price</i> \$
<i>Reagents and laboratory consumables</i>					
1	Beakers or plastic containers	1	20	7	140
2	Double layer cheesecloth	Box/25	20	7	140
3	Fork, tongue blades or stirring rod	Box/100	10	3	30
4	Test tube	Box/100	5	7	35
5	Formalin 40 %	1 liter	1	15	15
6	Microscope slides and covers	Box/100	10	2	20
7	Fecal sample container	Box/100	3	10	30
8	Eyepiece graticules stage micrometer	1	3	15	45
9	Pasteur pipettes	Box/500	2	15	30
10	Disposable poly ethylene sacs	Box/100	5	5	25
11	Disposable masks	Box/50	2	5	10
12	Disposable gloves	Box/100	3	5	15
13	Disposable shoes cover	Box/25	3	10	30
14	Surgical equipments	1	1	15	15
15	Flotation solution	-	-	-	50
16	Camera canon 7.1MP	1	1	200	200
Total					830
<i>Equipments</i>					
1	Microscope	Available in Islamic University Laboratories			
2	Balance				
3	Test tube rack				

Appendix 4: Study plan

Activity	May	June	July	August	September	October	November	December	Jan	February	March	April	May
	2009-2010												
Preparation of proposal	X												
Proposal and obtaining fund		X	X										
Literature survey and obtaining permission			X	X	X	X	X	X	X	X			
Pilot study													
Field work and lab analysis					X	X	X						
Data processing and analysis									X	X	X		
Evaluation of results and Final report													X