Survey on *Eimeria* spp. infecting Sheep in the Red Sea State, Eastern Sudan

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Dedication

I dedicate this work to the soul of my late father and to the rest of my family. My mother, sister and brothers.
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Abstract

This study was carried out to determine the prevalence of Eimeria spp. that infect sheep in the Red Sea State, their prevalence and the influence of age, season, sex and location on infection rate and oocyst output.

One thousand and two hundred faecal samples were collected from apparently healthy sheep in pasture and around water points over a period of 12 months from January to December 2002. The faecal samples were collected from three different geographical areas viz., Halaib (in the northern part of the State), Port Sudan town
(in the middle part) and Tokar delta (in the southern part of the State).

Ten species of *Eimeria* were detected in this study; these are *E. ovina* (75%), *E. ovinoidalis* (54%), *E. parva* (53%), *E. faurei* (39%), *E. ahsata* (38%), *E. marsica* (26%), *E. crandalis* (16%), *E. intricata* (12%), *E. pallida* (7%) and *E. granulosa* (5%).

One thousand and thirty seven samples were found to be infected with the *Eimeria* spp. with an overall prevalence of 86% throughout the year. The highest prevalence occurred in March (98%) and the lowest prevalence occurred in June and August (70%). No significance difference was shown between individual months.

According to age, adult sheep had significantly lower prevalence (63%) than lambs (93%) and yearlings (89 %.). However, lambs expressed significantly higher mean of oocyst output (5617 opg), while yearlings and adults showed lower means of oocysts output 2989 and 2428 opg, respectively.

The study indicated that no significance difference on the prevalence of *Eimeria* infection in sheep during the cold wet season and the hot dry season (94% and 71%, respectively). On the other hand, cold wet season showed significantly higher mean of oocyst output (4762 opg), when compared with hot dry season (2361 opg). Sex didn't show significant difference neither on prevalence nor on oocyst output. The prevalence in male and female was 90% and 84% and the mean oocyst output was 4736 and 3627opg, respectively.

Tokar area showed high rate of infection (90%), followed by Port Sudan (86%) and Halaib area (80%). No significance difference
in prevalence of *Eimeria* spp. was found to occur between the three locations. However, Halaib showed significantly lower mean of oocyst output (2679 opg) when compared with Tokar and Port Sudan 4909 and 4412 opg respectively.

Temperature, relative humidity and rain fall were found to affect prevalence of *Eimeria* infection in sheep. The results indicated that the high prevalence of infection occurred when ambient temperature is low and relative humidity is high.

Eighty three percent of examined sheep showed mixed infection with more than one species of *Eimeria*, while 17% showed pure infection (one species of *Eimeria*).

Molecular identification of sheep *Eimeria* spp. based on PCR assay and DNA sequencing indicated that multiple infection is the common type in natural infection. As PCR products showed amplification of more than one species and DNA sequence was not possible to be read. These results just confirmed the infection with sheep *Eimeria* spp.

**CHAPTER ONE**

**INTRODUCTION AND LITREATURE REVIEW**

1.1: Introduction

Coccidiosis is the disease of major economic importance in all animals and can be significant problem in the youngs of all animal species (Blood and Radostitis, 1989., Urquhart *et al.*, 1996). The disease is also most important where sheep are housed or confined in
small areas, in particularly; young sheep kept in over crowded pens or on irrigated pastures during winter months (Blood and Radostitis, 1989; Maingi and Munyua, 1994).

Different species of *Eimeria* parasitize the alimentary tract of sheep and mixed infections with a number of *Eimeria* spp. are common in natural infections (Vercruysse, 1982; O'Callaghan *et al.*, 1986; Osman *et al.*, 1990, and Abakar, 1996).

In the Red Sea State sheep plays an important role in the economy of the families. They are bred primary as a source of meat which is preferred to that of other livestock, for its milk which is consumed within household or processed into clarified butter, and for other social trans-actions such as marriage payment (Oxfam, 1990).

Most of sheep in the State are thin-tailed desert sheep. The Beja sheep is common and is raised by the major different tribes namely: Beja, Beniamer and Rashaida.

The type of husbandry in the state depends on extensive system of grazing where sheep spend all the day on the pasture and around water points and return back to the camp in the evenings (Oxfam, 1990).

The climate in Red Sea State is characterized by being dry hot during summer and cold rainy during winter. High proportions of herds congregate in the state during winter (October to April) where the pastures and water are available, while in summer (May to September) the total number of sheep significantly drop, due to the movement of herds outside the State searching for pastures, or due to selling in different markets (Oxfam, 1990).
Sheep in the Red Sea State are affected by different diseases among which external and internal parasites were most important (Anon, 2000). Clinical out breaks of coccidiosis in sheep were reported as one of the major internal parasite infections (Anon, 2000, 2001). The infection is characterized by mild to severe diarrhea, emaciation and reduction in productivity.

As sheep in the State are regarded as the main source of protein for the area population, the need to increase the protein sources in the State requires understanding any disease element such as coccidia infection which can limit the production of small stocks. Information on Eimeria spp. that infect sheep is absent. Therefore, the study was designed to:

1- determine the prevalence and intensity of coccidia infection in sheep in Red Sea State.
2- to identify Eimeria spp. occurring in the sheep using morphological and molecular characterizations.
3- to study some factors that might influence prevalence of Eimeria spp. infection in sheep reared in the State.

1.2: classification of Eimeria
Coccidia are intracellular protozoan parasites of vertebrates and invertebrates that parasitize gastrointestinal tract and other organs such as liver and kidney (Levine, 1973). The majority of coccidia of veterinary importance belong to families Eimeriidae and Sarcocystidae (Soulsby, 1982).

Coccidia are classified by Levine et al. (1980) under the phylum Apicomplexa which has a characteristic by structure known as apical complex that is only visible under the electron microscope.
Generally, it consists of polar ring (s) which is present in some stages, rhoptries, micronemes, conoid and subpellicular tubules. Cilia and flagella are absent except in microgamete of some group (Levine, 1973; Soulsby, 1982).

The classification of Levine et al. (1980) was as follows:-

- **Phylum:** Apicomplexa (Levine, 1970)
- **Class:** Sporozoea (Leuckart, 1879)
- **Subclass:** Coccidia (Leuckart, 1879)
- **Order:** Eucoccidiida (Leger and Duboscq, 1910)
- **Suborder:** Eimeriina (Leger, 1911)
- **Family:** Eimeriidae (Minchin, 1903)
- **Genus:** *Eimeria* (Schneider, 1881)

In the late eightieth, Sleigh (1989) and Cox (1991) (Cited by Tenter, 2002) classified coccidia under the Phylum: Sporozoa, Class: Coccidea and the Order: Eimeriida. Their classification was based on phenotypic characters which include the morphology of available parasite stages and host specificity. Furthermore, according to information derived from phylogenetic reconstruction based on 18S rRNA gene sequences, Cavalier–Smith (1993) and Corliss (1994) classified coccidia under Phylum: Apicomplexa.

Recently, Mehlhon (2001) classified coccidia according to molecular character as follow:-

- **Phylum:** Alveolata
- **Subphylum:** Apicomplexa
- **Class:** Coccidea

**Order:** Eimeriida

**1.3: Morphology of Eimeria spp.**
The most common shapes of coccidia are spherical, subspherical, ovoid or ellipsoidal (Soulsby, 1982). The oocyst wall is composed of one or two layers and is generally clear and transparent; however, it may be yellowish or green in colour (Levine, 1973; Soulsby, 1982).

Several species of coccidia oocyst posses a micropyle at one extremity, this being the pointed end. The micropyle may be covered by a micropylar cap, and occasionally there may be a dome-shaped projection of the oocyst wall to the exterior in the form of polar cap. There may be a refractile polar granules and residual body in the oocyst (Levine, 1973; Soulsby, 1982). In sheep coccidia there are no oocystic residual bodies in the oocyst (Christensen, 1938).

Coccidial oocysts sporulate outside the host and sporulation is affected by certain environmental factors such as temperature, moisture and free access to oxygen (Kheysin, 1972). The sporulated oocyst of an eimerian species contains four sporocysts. The sporocysts in genus *Eimeria* are more or less elongated or ovoid forms with one end more pointed than the other, known as steidia body (Soulsby, 1982). Each sporocyst contains two sporozoites, each having a granular cytoplasm and central nucleaus. The sporozoites are usually sausage or comma-shaped and contain one or more clear proteinaceous globules (refractile bodies, eosinophlic globules) of unknown function (Levine, 1973. Soulsby, 1982).

**1.4: Life cycle of *Eimeria* spp.**

The life cycle of all members of family Eimerridae is divided into three phases: sporulation, infection and schizogony, and finally, gametogony and oocyst formation (Urquhart *et al.*, 1996).
Lapage, (1965), Baker (1969), Soulsby, (1982), and Urquhart et al. (1996) described typical life cycle of coccidia. They stated that under optimum environmental conditions of humidity, temperature and oxygen the unsporulated oocyst develops to form sporulated oocyst, which contains four sporocysts each of which contains two sporozoites, is referred to as the infective stage (Soulsby, 1982 and Urquhart et al., 1996).

After the sporulated oocyst is taken by the animals into the digestive tract with contaminated feed or water, the sporocysts are then liberated either mechanically or by at least 15% CO₂ (Jackson, 1962) and the sporozoites, activated by trypsin and bile, leave the sporocyst. In most species, each sporozoite penetrates an epithelial cell. In side the epithelial cells they may round up and grow or they may be carried by macrophages elsewhere in the body, depending on the species (Urquhart et al., 1996). The growing from is known as trophozoites. Most of them start nuclear division thus developing a schizont, which differs in its size with species (from about 10 to several hundred microns in diameter). Cytoplasmic divisions then occur, to produce the small nucleated organisms known as merozoites. A variable number of merozoites are produced within the host cell (16 up to thousands), the schizont is then mature, the host cell and the schizont rupture and the merozoites escape to invade neighboring cells. Schizogony may be repeated, the number of schizonts depending on the species. Schizogony terminates when the merozoites differentiate to male and female gametocytes. The female (macrogametocytes) simply grow until they reach full size to fill the parasitized cell while, the male (microgametocytes) undergo
repeated division to form a large number of flagellated uninucleate organisms, they swim away in reach of macrogametes. A microgamete penetrates a macrogamete and unites with it to form the zygote (fertilization). The resulted zygote lays down a thickened wall around itself and forms the oocyst, which contains a single cell (sporont).

Oocysts are then passed with the faeces. The prepatent period varies considerably and may be as short as five days in poultry and up to 3-4 weeks in some ruminant (Urquhart et al., 1996).

1.5: Epidemiology of ovine coccidiosis.

Coccidiosis is most important disease where animals are housed or confined in small areas (Blood and Radostitis, 1989). All domestic animals are susceptible, but coccidial species are in general host specific and infection does not pass readily from one animal species to another nor does cross immunity occur between species of coccidia (Levine, 1973; Soulsby, 1982; Blood and Radostitis, 1989).

Clinically, the disease is most common in cattle, sheep and poultry. Faeces of clinically affected or carrier animals are the source of infection. Infection is acquired by ingestion of contaminated feed and water or by licking the hair coat contaminated with infected faeces (Blood and Radostitis, 1989). Oocysts passed in the faeces require suitable environment conditions to sporulate. Moist temperatures and cool conditions are best for sporulation while high temperatures and dryness impede it (Friend and Stockdale, 1980). The optimum temperature for sporulation of most *Eimeria* spp. is 28-31°C, it is inhibited by high temperature above 35°C and low temperature -5°C (Kheysin, 1972). The sufficient
moisture in the environment is necessary for oocyst development. A humidity deficit causes wrinkling of oocyst wall due to water loss, so that sporogony can not proceed normally (Kheysin, 1972). Oxygen is also necessary for oocyst development and sporulation appears to occur normally when the oxygen tension reduced to 10% of normal, however, decrease below 10% causes a lengthening in sporulation time. In complete absence of oxygen, no development takes place (Marquardt et al., 1960). Direct sun light is detrimental to oocyst survival, and unsporulated oocysts are more susceptible than sporulated oocyst to destruction by it. However, other radiation sources such as X-rays, gamma, ultrasonic waves and low acceleration voltage electron were found to attenuate oocysts without destroying them (Marquardte et al., 1960; Khysin, 1972). Under favorable conditions the sporulated oocyst may survive for up to two years (Apayne, 1977; Blood and Radostitis, 1989)

Lambs are usually affected between four and seven weeks of age with a peak infection around six weeks (Pout et al., 1966; Urquhart et al., 1996). The outbreaks reported, occurred where ewes and lambs were housed in unhygienic conditions (Urquhart et al., 1996). Most animals in groups become infected but only a minority develops clinical thus the infection rate is high but the rate of clinical disease usually low (10-15%) but outbreaks affecting up to 80% may occur (Niillo, 1970). The mortality rate may also be high in lambs or calves with no previous exposure to coccidia after suddenly being introduced to high level of infection (Urquhart et al., 1987). Coccidiosis occurs sporadically in lambs at about 6 weeks of age
when oocyst output is very high in healthy as well as in diseased lambs (Gregory et al., 1983).

Lambs can be infected soon after birth (before 4 weeks) of age from three possible sources of infective oocysts, a) oocysts surviving into old faecal contamination of the lambing area arising from previous occupation, b) fresh oocysts constantly passed by ewes and c) fresh oocysts passed out by the lambs (Pout, 1973). The faecal oocyst burden is high at 4 weeks of age but gradually declines so that by 5 months of age the oocysts count is similar to that of their parent ewes, (Pout et al., 1966). Nillo (1970b) reported that acute coccidiosis may occur in animals of any age when their resistance is affected by intercurrent disease or inclement weather.

Coccidiosis occurs commonly under intensive husbandry when young animals come directly from off range into feedlots where over crowding and stress factors are obvious (Osman et al., 1990; Abakar, 1996). However, over crowding of pastured animals on irrigated pasture or around surface holes in drought conditions may cause heavy infections (Richardson and Kendal, 1963). Under field conditions infection with more than one species of *Eimeria* was the rule in 94% of small ruminant (Vercruysse, 1982). Likewise, Catchpole *et al.*, (1975); Marquadt, (1976) and Abakar, (1996) reported that most naturally acquired coccidia infections were mixed infection. A single species of *Eimeria* may be major pathogenic as *E. ovinoidalis* in sheep (Gregory *et al.*, 1989; Abakar, 1996) but the others may contribute to the severity of the disease (Catchpole *et al.*, 1975).
Feeder lambs and calves brought in to feedlots from sparse grazing may carry a few oocysts, which build up in heavy infestations in lots especially if conditions are moist. In such situations, clinical signs of the disease usually appear about a month after the animals are confined. However, young lambs on pasture may shed large numbers of oocyst for long period, which may be a factor in the development of large coccidial populations (Blood and Radostitis, 1989).

Pout (1974) studied the effects of nutrition and nutritional status in lambs as predisposing factors and showed that early weaned lambs at 21 days of age followed by experimental infection resulted in failure in growth. Field observations indicate that early weaned lambs are more susceptible to coccidiosis than those weaned at later date, this may be a reflection of the lack of natural immunity in the lambs, but dietary stress in early weaned lambs contribute to the disease (Blood and Radostitis, 1989).

1.6: Enteric coccidia of sheep

Pallida (Christensen, 1938), E. parva (Kotlan, Mocsy and Vajda, 1929), E. punctata (Landers, 1955) E. weybrigensis (Norton, Joyner and Catchpole, 1974).

1.7: Pathogenicity of ovine coccidiosis

The pathogenicity of coccidia depends on number of factors these are: the size of infection dose of ingested oocysts, the number of host cells destroyed per infecting oocyst, location of parasite in the host tissue and within the host cell, the degree of infection, the age and general condition of the host and degree of a acquired or natural immunity (Levine, 1973; Pellerdy, 1974; Jubb et al., 1985).

The merozoite and gametocytes stages are the pathogenic stages that cause rupture of the cells they invade, with consequent exfoliation of epithelial lining of the intestine (Blood and Radostitis, 1989).

Lotze, (1952) and Pout, (1974a) reported that there may be no obvious relationship between infective does, the faecal oocyst production and clinical disease. This suggest, that the mere presence of large numbers of coccidia oocyst does not constitute diagnosis of coccidiosis, and that other pathogenic factors may be involved in conversion to clinical disease. Catchpole and Gregory, (1985) reported that the clinical effects were variable and not closely related to the infecting dose when lambs inoculated with $10^4$ and $10^5$ sporulated oocyst of E. crandalis. Likewise, Gregory and Catchpole, (1990) showed that the clinical effects were different and not closely related to inocula dose when four weeks- old lambs infected with E. crandalis. However, Gregory et al. (1989) found that the severity of the disease is being roughly proportional to the size of the inoculum.
and even 1000 oocysts of *E. ovinoidalis* and *E. crandalis* caused diarrhea and the pathogenic effect was attributable mainly to *E. ovinoidalis*.

In heavy infections with *Eimeria* spp. in which the developing schizonts or gamonts found in deep in the mucosa or submucosa, the destruction is so severe that hemorrhage occurs while in light infections the effect on the intestinal mucosa is to impair local absorption (Urquhart *et al.*, 1996). In the species of *Eimeria* which develop superficially, the infection results in villous atrophy that characterized by reduction in epithelial cells high and a diminution of the brush border. These changes result in a reduction of the surface area available for absorption and consequently reduced feed efficiency (Urquhart *et al.*, 1996). Similar observations were reported when Pout (1974a) studied the effect of *E. ovina* and *E. crandalis* in naive lambs and found that these species caused massive reduction in surface of intestinal mucosa and loss of villus architecture. Moreover, Gregory and Catchpole, (1990) showed that inoculation of lambs with various levels of doses of *E. crandalis* caused extensive loss of epithelial cells of lower jejunum, destruction and erosion of caecal mucosa which cause the diarrhea. These changes were associated with the first and second generations of merozoites and gamonts. Recently, Taylor *et al.* (2003) reported that lambs infected with *E. crandalis* showed loss of surface epithelial cells and villus atrophy that associated with pro-gamonts stage.

Infection with more than one species of *Eimeria* was common in the field and intra specific reactions between different species can result in exacerbation of total effects or modified activity of induced
species (Blood and Radostitis, 1989). This pattern was studied by Catchpole et al. (1975) who showed that mixed infection with E. weybridgeienses, E. ahsata, E. ovina, and E. ovinoidalis in sheep caused extended patency and increased oocysts production of the first three species; however, clinical symptoms were observed only in lambs that received E. ovinoidalis. Generally, E. ovinoidalis is considered to be one of the most pathogenic species in sheep (Gregory et al., 1989 and Abakar, 1996). E. crandalis may also consider as pathogenic species (Gregory and Catchpole, 1990 and Taylor et al., 2003).

Stress factors and environmental conditions were also playing part as predisposing factors in the pathogenicity of coccidia. Many researchers have indicated that the environmental and stress due to weaning are associated with subsequent outbreaks of bovine coccidiosis (Marquardt, 1962; Parker and Jones, 1987). Moreover, treatment with corticosteroids can convert subclinical infections in lambs and calves into peracute clinical forms (Niillo, 1970; Stockdale and Niillo, 1976; Gasmir, 1991; Abakar, 1996).

1.8: Clinical symptoms of ovine coccidiosis

The first sign of clinical coccidiosis is usually the sudden onset of severe diarrhea with foul smell and fluid faeces containing mucus and/or blood and progressive body weight loss (Blood and Radostitis, 1989). The blood may appear as dark tarry staining of the faeces or as flakes, or the evacuation may consist of large clots of fresh, red blood. The degree of hemorrhagic anaemia is variable
depending on the amount of blood lost. Pale mucus membranes, weakness, staggering, dyspnea, dehydration and recumbency were observed by Osman, *et al.* (1990). Inappetance, dullness, pallor of mucus membrane and slight pyrexia were reported by Abakar (1996).

### 1.9: Necropsy findings of ovine coccidiosis

Necropsy findings included congestion, hemorrhages and thickening of the muocosa of large and small intestine (Blood and Radostitis, 1989). Villus atrophy of the proximal ileum was reported by Pout, (1974a) in both natural and experimental infection in lambs. However, Osman *et al.* (1990) reported the gross lesions of clinical coccidiosis in lambs as thickening and edema in mucus membrane of small intestine and whitish foci along the surface of small intestine. Moreover, Abakar (1996) reported the thickening of the wall of small intestine, congestion, hemorrhage in large intestine, hydro-pericardium, hydro-peritoneum and hydrothorax in experimentally infected lambs.

Loss of surface epithelial cells and villus atrophy in the small intestine were associated with the first generation meronts and severe diffused crypt hyperplasia was associated with progamonts in the small and large intestine in lambs experimentally infected with *E. crandalis*(Gregory and Catchpole, 1990).

Many researchers studied the effect of coccidiosis on morphological and biochemical changes in blood components. They included changes in numbers of red blood cells (RBCs), white blood cells (WBCs), values of hemoglobin (Hb), packed cell volume (PCV), and level of proteins in the plasma. Hayat, *et al.* (1990)
found that in 15 lambs 2-4 months-old infected with $10^5$ sporulated oocysts of multiple species of coccidia, the total erythrocytes counts, PCV, Hb concentration, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and total live weight were decreased, whereas erythrocyte sedimentation rate (ESR) and mean corpuscular volume (MCV) increased in infected lambs. Abakar, (1996) reported that experimentally infected lambs with multiple species of sheep *Eimeria* showed decrease in red blood cells (RBCs), serum protein and serum albumin, whereas (WBCs), (PCV), (MCV), (MCH) and serum globulin increased. Serum globulins and plasma alkaline phosphatase were reduced in lambs experimentally infected with *E. crandalis* (Catchpole and Gregory, 1985).

1.10: Diagnosis of ovine coccidiosis

Coccidiosis in sheep and goats can be diagnosed from a combination of history, clinical signs, gross lesions and necropsy findings, and microscopic examination of the intestinal mucosa and faeces. However, recognition of coccidia in the lesions at necropsy is necessary for positive diagnosis (Levine, 1973).

The presence of oocysts in the faeces does not necessarily that the disease is due to coccidia. Oocysts counts of $(10^5/gm)$ may occur in the faeces of normal animals, therefore, the observation of large numbers of oocyst in the faeces of lambs affected with diarrhea may not, it self constitute diagnosis of coccidiosis (Pout, 1976). However, acute coccidiosis may be present before any oocysts appear (Gregory *et al.*, 1983). This is possible that large numbers of
sporozoites may cause destruction of the tissues to that produced merozoites fail to find or invade new cells to develop.

1.10.1: Molecular characterization of coccidian parasites

The development of molecular biology has added available tools that detect parasite molecules, which are important in veterinary diagnostic parasitology (Zarlenga and Higgins, 2000).

In the past, the studies done on occurrence of *Eimeria* spp. had relied either upon consideration of traditional characters, or in combination with methods such as the electrophoretic variation of enzymes (as in avian *Eimeria* spp.). Recently, approaches that make use of variation with sequences of DNA have been reported and PCR-based assay has been described that offers a chance for *Eimeria* identification methodology (Viljoen *et al*., 2002). The development of new DNA-based diagnosis assays could facilitate the rapid and convenient identification of coccidia. The application of the polymerase chain reaction (PCR) method (Erlich *et al*., 1991) is revolutionizing the diagnosis of disease causing organisms. Welsh and McClelland (1990) and Williams *et al.* (1990) (Cited by MacPherson and Gajadhar, 1993) described a novel PCR procedure that did not require previous knowledge of target sequence.

Later, most of *Eimeria* studies that based on PCR- assay were done on identification or discrimination of chicken *Eimeria* spp. or other genera of the family Sarcocystidae such as *Toxoplasma* (Burg *et al*., 1989; Savva and Holliman, 1990) and *Sarcosyris* spp. (Kibenge *et al*., 1991) in bovine sarcocystosis. However, studies on molecular characterization of *Eimeria* spp. of sheep were few.
Studies of (PCR) for discrimination of chicken *Eimeria* spp. were carried out with different scientists using different techniques such as intra- and intra specific random amplified polymorphic DNA (RAPD) marker (Fernandez *et al.*, 2002), a novel multiplex PCR based on sequence- characterized amplified region (SCAR) markers (Fernandez *et al.*, 2003), comparing internal transcribed spacer 1(ITS-1) sequences and ITS-1 PCR methods (Lew *et al.*, 2003) and PCR assay (Tsuji *et al.*, 1997). They manged to differentiate of approximate eight species of chickens *Eimeria* viz., *E. acervulina, E. brunetii, E. mitis, E. maxima, E. praecox, E. tenella, E. mivati* and *E. hagani*.

PCR-based also was used to differentiate between three procine *Eimeria* spp. and *Isospora suis* in pigs.

Seven *Eimeria* species that infect five different hosts included chickens, rats, mice, cattle and sheep were differentiated by the polymerase chain reaction using random amplified polymorphic DNA (RAPD) (MacPherson and Gajadhar. 1993).

The only molecular study on *Eimeria* spp. of sheep was done by Berriatua *et al.*, (1995) who reported the identification of *E. ovinoidalis* and *E. crandalis*, using DNA probes from pure culture of each species.

**1.11: Immunity to *Eimeria* spp.**

Infected animals develop a degree of resistance to further infections with the same *Eimeria* species. However, immunity to coccidia is seldom absolute and recovered animals are often continuously re-infected with different species so that they carry light infections which don’t harm them but, make them a source of
infection for the young (Levine, 1973; Pout, 1976; Soulsby, 1982 and Gregory, et al., 1983). Rose, (1973) reported that resistance to *Eimeria* may commence during the primary infection and exposure of the host to massive numbers of early stages (schizonts), may stimulate immune mechanisms that are effective against late stages (gametocytes). Resistance to coccidia infection is thymus dependent, and is largely mediated by T-cell-promoted intra cellular killing which seems to be directed mainly against asexual stages in the life cycle (Jubb et al., 1985).

The degree of resistance to infection depended on the species involved, the age of the host and the severity of the primary infection (Apyne, 1977).

Gregory and Catchpole, (1989) reported that a heavy single inoculation with $10^4$ oocysts of each of *E. ovinoidalis* and *E. crandalis* in lambs up to 4 days of age caused no clinical disease. However, inoculation at 7, 14 and 21 days of age caused softening of faeces and reduction in weight gain. First inoculation at 28 days of age resulted in severe diarrhea and weight loss. Furthermore, challenge with $10^5$ oocysts of each species at 42 days of age caused severe coccidiosis with 50% mortality in susceptible control lambs, while immunized groups (previously infected) showed diarrhea and weight loss with no mortalities. This suggest that the later the immunization, the less severe clinical signs.

Small repeated inocula produced stronger immunity than the same number of oocysts administered in single dose (Joyner and Norton, 1973). Chapman, (1974) reported that two subsequent challenges with $10^5$ oocysts *E. ovinoidalis* in lambs 3 months of age
experimentally infected failed to cause re-infection and concluded that both natural infections acquired at pasture and artificial infections acquired by experimental inoculation result in immunity to the challenge dose. Rose, (1970) demonstrated the presence of antibody in the sera of animal infected with coccidia and showed that all stages of *Eimeria* spp. are affected with serum (from immunized animals), but the early schizogony stages are more susceptible and has greater effects. Further more Rose, (1973) reported that the inoculation of hyper immune serum (immune globulin) in chicken resulted in reduction of pathogenic effects of *E. maxima* in chicken.

### 1.12: Treatment and control of ovine coccidiosis

Coccidiosis is self-limiting disease and clinical signs subside spontaneously when multiplication phase of the parasite has passed.

The drugs used in coccidiosis can be divided into treatment and control drugs. In treatment drugs, the intension is completely to clear the protozoan from the animal, while in control drugs the idea is to use the drug as coccidiostat, which interferes with the life cycle of coccidia (Apyne, 1977).

Amprolium is a very good prophylactic drug of coccidiosis in feedlot lambs, in 50 mg /kg, of body weight given to lambs in ration for 21 days resulted in rapid reduction in oocysts production and clinical cure (Baker, Walters and Fisk, 1972). Christensen and Foster (1943) studied the effect of sulfaguanidine in control of ovine coccidiosis under conditions of moderate exposure to infection and found that ,daily doses of 1gm in lambs before artificial inoculation gave adequate protection, while doses of 3gm daily given after
development of high oocyst production, seemed to interfere with production of normal oocysts. Lambs fed monensin mixed in a complete fattening ration at concentrations of 5, 10, or 20 ppm were protected against death, impaired body weight gain, and diarrhoea due infections of *E. ovinoidalis* and *E. ahsata*. Monensin given at concentrations of 10 ppm reduced oocyst passage and, at concentration of 20 ppm almost completely controlled oocyst passage (Bergstrom, and Maki, 1976). Recently, Abakar (1996) reported that monensin had a good prophylactic activity in experimentally infected lambs with six *Eimeria* species. Mixture of amprolium at 62.5 mg/kg, body weight and ethopabate at 3.2 mg/kg for 14 days reduced oocyst counts to very low levels within five days, and treated lambs gained 22kg, over a 21days period (Rose, 1986). Shumard (1959) stated that treatment with nitrofurazone (Furacin) at 0.008%, 0.01% and 0.133% in drinking water prevented mortality and reduced morbidity resulting from experimental infection with mixed level of *E. ovinoidalis*, *E. ovina*, *E. intricata*, *E. parva*, *E. faurei* and *E. pallida*.

Gjerde and Helle, (1991) studied the effect of toltrazuril against coccidiosis in naturally infected lambs and found that a single oral dose of toltrazuril at 20 mg/kg body weight given on day 7 after turn out on pasture proved to be highly efficacious in preventing clinical coccidiosis by reduction of oocyst output to very low level and prevented the development of diarrhoea. Alzieu *et al.* (1999) reported the economic benefits of the prophylactic administration of diclazuril (Vacoxan) in lambs naturally infected with *Eimeria* spp. They found that the growth rate and feed conversion rates of lambs
treated one or twice is better than untreated lambs. Lately, Taylor et al. (2003) showed that diclazuril (Vacoxan) appeared to have a direct effect on several stages of *Eimeria* life cycle, in particular, the large first-generation meronts and gamont stages. The therapeutic benefits of diclazuril treatment appeared greatest when given early in the infection before damage to the intestine occurs.

Control of coccidiosis depends largely upon hygiene and avoidance of overcrowding and as far as possible. Young animals should be separated from adults which provide the source or infection. Pens should be kept dry, cleaned out frequently and bedding disposed off, so that oocysts don’t have time to sporulate and become infective. Feed and water troughs should be high enough to avoid faecal contamination.

In groups of lambs at pasture, efficient control can be exercised by frequent rotation of fields. Special attention must be given to flocks where environmental conditions are conducive to spread, especially if the ewes have been exposed to the disease previously.

**1.13: Prevalence of coccidia in sheep in the world**

The coccidia have world-wide distribution, and distribution of various species of *Eimeria* is limited only by availability of the host, as no vector is needed for the transmission of the infective stage. Sheep coccidiosis seems to be world-wide in distribution and that it has been reported in Europe, Americas, Australia and Africa.

In Europe, five species of *Eimeria* were recorded in Italy by Battelli and Poglayen, (1980). These were *E. ahsata*, *E. ovina*, *E. ovinoidalis*, *E. parva*, and *E. intricata*. In North West Germany,
Barutzki et al (1990) reported ten species and new five species were detected. Those were: *E. crandalis*, *E. weybrigdensis*, *E. faurei*, *E. granulosa* and *E. pallida*. In England and Wales, similar ten species were detected and *E. weybrigdensis*, was found the most frequent species (Catchpole et al., 1975).

In the United States, 69% of apparently healthy sheep were found to have coccidia oocyst in their faeces. *E. ovina* was the most prevalent species.

In Australia O’callaghan et al. (1986) reported that 80% of sheep were positive for coccidia and eleven species of *Eimeria* were identified, ten species were similar to those previously detected. *E. punctata* was the new species observed.

In Kenya, the prevalence of the disease in sheep was (42.7%) and (45.2%) during dry and wet seasons, respectively, and eight species of *Eimeria* were recognized (Maingi and Munyua, 1994).

1.14: *Eimeria* infection in the Sudan
1.14.1: *Eimeria* of sheep

In the Sudan, seven species were reported for the first time from clinical cases of sheep in Khartoum Province (Osman, et al., 1990). The prevalence of these species was as follows: *E. ovina* (40%), *E. intricata* (23%), *E. ahsata* (13%), *E. parva* (7%), *E. crandalis* (7%), *E. ovinoidalis* (7%), and *E. pallida* (3%). Later, Abakar, (1996) conducted coccidian infections survey in various part of the Sudan and indicated the presence of eleven species in Sudanese sheep. He added a new four species namely *E. faurei* (28%), *E. marsica* (13%), *E. granulosa* (8%), and *E. Punctata* (0.03%). The over all prevalence of infection was 59%. Recently,
Abakar et al. (2001) reported the prevalence of 67% of enteric coccidia in sheep in south Darfur (Nyala) and eight species were detected.

In the Red Sea State, cases of sheep coccidiosis represented 13% and 33% of sheep diseases diagnosed at Port Sudan Veterinary laboratory during years 2000 and 2001, respectively, (Anon, 2000, 2001).

1.14.2: *Eimeria* of goats

Different reports of *Eimeria* infection of goats were documented in the Sudan from different localities by Osman et al., 1979a; El gazuli et al., 1979b; Osman, 1988; Elghli and Elhussein, 1995; Elrabie, 1999 and Abakar et al. 2001. The prevalence of these infections was variable in a range of 6% to 82% of examined animals.

1.14.3: *Eimeria* in cattle

Nine species of bovine *Eimeria* were reported in Khartoum State (Gasmir, 1991). These were *E. zurrnii* (42%), *E. bovis* (40%), *E. canadensis* (25%), *E. cylindrica* (16%), *E. aubernensis* (9%), *E. alabamensis* (8%), *E. wyomingensis* (5%), *E. ellipsoidalis* (4%), and *E. subspherica* (1%). The overall prevalence in the state was 14% (Gasmir, 1991).

1.14.4: *Eimeria* of camels

Coccidia in sudanese camels had only been investigated in the eastern region of the Sudan by Yagoub, (1989). Only three species were isolated, these were: *E. cameli*, *E. dromedari*, and *E. rajasthani*.

1.14.5: *Eimeria* of chicken
There were five species reported in chicken in Khartoum province by Mohamed et.al. (1990), these were: \textit{E. tenella} (53\%), \textit{E. maxima} (18\%), \textit{E. mivati} (15\%), \textit{E. praecox} (10\%), and \textit{E. brunetti} (5\%).

1.14.6: 	extit{Eimeria of rabbits}

Five species of \textit{Eimeria} were reported in rabbits in Khartoum State, these were: \textit{E. magna} (37\%), \textit{E. preforans} (20\%), \textit{E. irresidua} (20\%), \textit{E. stiedae} (17\%), and \textit{E. coecicola} (6\%) (Omer et al., 1991).

CHAPTER TWO
MATERIALS AND METHODS

2.1: Study area

This study was carried out in the Red Sea State which occupies the northern east corner of the Sudan, between longitudes 17-22°N and latitudes 23-38°E. It is bordered by Egypt in the north,
Eritrea and Kassala State in the south, Rive Nile State in the west and the Red Sea in the east (Anon, 2002). The total area is about 210410 km². The principal types of livestock found in the state are cattle, sheep, goats, camels, donkeys and poultry. The animal population in the state is composed of approximately 661 thousands sheep, 512 thousands goats, 212 thousands camels and 56 thousands cattle.

The state was divided to four provinces, Halaib in the northern part, Tokar in the southern part, Port Sudan in the middle and Sinkat in the western part of the State. Sheep population in the Red Sea State is concentrated in the southern part of the State.

2.2: Samples

A total of 1200 faecal samples were collected monthly during the period from January to December 2002 from apparently healthy sheep. Animals were of different age groups and from different locations within the Red Sea State.

Five hundred and twelve samples were collected from sheep in Tokar area, 298 samples from Port Sudan town and 390 samples from Halaib area.
Fig (1): Map of the Red Sea State

Five hundred and nine faecal samples were collected from lambs (< year), 468 were from yearlings (1-2 years) and 223 samples were from adult sheep.

Seasons were taken as cold wet season (October to April) and hot dry season (May to September).
Samples were collected directly from the recta of the sheep into plastic bags, labeled and kept in refrigerator at 4°C at Port Sudan Veterinary laboratory until tested. Samples from far location were put on ice until transferred to the laboratory in Port Sudan.

2.3: Examination of faecal specimens

For detection of coccidial oocysts individual faecal samples were floatated in saturated sucrose solution in test tube covered with a cover slip for 10 minutes (simple flotation technique), then examined under 10X objective of the microscope. The sucrose solution was prepared by dissolving 454gm of sugar in 355ml of hot water and left to cool at room temperature before use. The specific gravity (S.G) of the sugar was 1.3 (Kenyon and Gasmir, 2001).

2.4: Oocyst counts

Positive samples were used for determination of number of oocyst per gram (opg) using modified McMaster technique (Anon, 1977) as follows: 3 grams of faeces were mixed with 42 ml of tap water using a pestle and mortar to make up the suspension, which was strained through 80µ /square sieve to remove debris and the filtrate was collected in clean dry bowl. 15ml of this filtrate were taken into centrifuge tube, centrifuged for 2 minutes at 110 x g and the supernatant was then discarded. The sediment was emulsified by gentle agitation and saturated sucrose solution was added until the volume become equal to the initial aliquot of the filtrate. Then the centrifuge tube was inverted several times until the sediment was evenly suspended. The two chambers of McMaster slide were filled using a clean Pasture pipette. The slide was then left for a couple of minutes to allow the oocyst to float and it was then examined under
the low power (10X) of the microscope. The average numbers of oocysts present in the two chambers was multiplied by 100 to obtain the oocyst per grams (opg).

2.5: Oocyst sporulation

About 2 to 3 g of the faecal material was thoroughly mixed with tap water and passed through 100, 80, 63 μ mesh screens. The filtrate was transferred into cylinder and allowed to stand over night. The supernatant fluid was discarded and the sediment was divided into centrifuge tubes and centrifuged at 110 xg for 2 minutes and finally the sediment was suspended in shallow layer of 2.5% potassium dichromate in Petri dishes during cold-wet season and was left to sporulate at room temperature (25-27°C) under aeration until sporulation was completed (Osman et al., 1990). Cooled incubator (25-30°C) was used during hot-dry season.

2.6: Oocyst identification

The identification of sporulated oocyst was based on oocyst morphology, measurements and sporulation time.

2.6.1: Oocyst morphology

Morphological characteristics of the oocyst undertaken included the oocyst shape (ellipsoidal, spherical or ovoidal) and the presence or absence of micropyalar caps (Christensen, 1938; Morgan, 1951; Shah, 1963; Levine, 1973; Norton and Catchpole, 1976; and Anon, 1977). In addition to that photographs of ovine oocyst previously documented by Christensen, 1938; Morgan, 1951; Levine, 1973; O’callghan et al., 1986; and Abakar, 1996 were considered as an aid in the identification of oocyst.

2.6.2: Measurements of oocyst
Measurements of *Eimeria* spp. were performed in 50-100 oocysts of each species. Length and width of oocysts were measured using calibrated an eye-piece micrometer using (Olympus, Japan) microscope under objective lens 40X. The measurements were done as described by Christensen, 1938; Morgan, 1951; Shah, 1963; Levine, 1973; Norton and Catchpole, 1976; Anon, 1977 and Abakar, 1996.

### 2.6.3: Sporulation time

Every 24 hours the faecal sample in potassium dichromate were examined for detection of sporulated oocyst by placing drop of faecal material in a microscope slide and examined under the low power (10X). The progress was reported until about 90% of the oocysts under the microscopic field were fully sporulated.

### 2.7: Meteorological data

Data of temperature, relative humidity and rainfall during the study period from January to December 2002 were supplied by the authorities of Meteorologic office at Port Sudan airport.

### 2.8: Molecular characterization of coccidian parasite in sheep

This work was carried out at Wildlife Research Centre in Saudia Arabia.

#### 2.8.1: Initial purification of oocyst from faecal material

In order to send the oocyst samples to Saudia Arabia, oocysts were purified using the special modified procedure of Davis (Hammond and Long 1973) as follows:
Faecal material which was previously suspended in 2.5% potassium dichromate was homogenized in a blender, transferred to test tube and centrifuged for 3 minutes at 49 x g. The supernatant was discarded, and the sediment was resuspended in saturated sucrose and centrifuged for 3 minutes at 49 x g. The oocyst containing scum was then removed using suction pump in to flask. The residue was taken and resuspended in saturated sucrose for second collection of oocysts. The oocysts rich sucrose suspension was taken in a flask, sieved through 150 µ mesh sieve, washed with water ten times the volume of the original sucrose suspension and centrifuged for 5 minutes at 200 xg. The supernatant was then removed carefully using a vacuum pump and discarded. Two percent potassium dichromate solution was added to the sediment and kept in a refrigerator at 4°C. Four patches of samples in biju bottles were sent to Saudia Arabia for molecular characterizations.

2.8.2: Further purification of oocysts for molecular characterization

Oocysts were resuspended in 2% potassium dichromate (K$_2$Cr$_2$O$_7$), and were diluted to 1% with distilled water and washed 3-4 times in water by centrifugation at 8000 x g. 1 ml of 30% Percoll® (density: 1.13 g/ml, Pharmacia LKB Biotechnology, Uppsala, Sweden) was added to the sediment and centrifuged at full speed for 15 min. The supernatant was discarded and the purified oocysts were resuspended in 750 µl of phosphate buffered saline (PBS), vortexed, and then washed by centrifugation for 5 minutes at 10000 x g.

2.8.3: DNA Extraction
DNA was extracted from the oocysts using the QIAGEN extraction kit (QIAGEN GmbH, Hildesheim, Germany). Briefly, oocysts were first digested by the addition of 180 µl of ATL buffer and 25 µl of proteinase K and incubation at 55° C for 2-3 hours. After digestion, 200 µl of AL buffer was added and then the suspension was incubated for 10 minutes at 70° C before 200 µl of absolute ethanol were added and vortexed. These were transferred into the Qiagen column and centrifuged for 1 minute at 6000 x g. The column was washed once in 500 µl of washing buffer (AW1), then in 500 µl of washing buffer (AW2) each by centrifugation for one minute at 6000 x g. DNA was eluted by the addition of 200 µl of an elution buffer, incubated for 1 minute and then centrifugated as above.

Alternatively, oocysts were purified and collected by simple floatation using saturated NaCl solution. The top layer was carefully removed and washed in distilled water several times. DNA was isolated from these oocysts the same way as above.

2.8.4: Polymerase Chain Reaction (PCR)

DNA fragments spanning the full length of the 18S rRNA region (small subunit ribosomal RNA, ssurRNA) were generated by PCR from DNA isolated from different samples of purified oocysts. This was done by using primers located at the 5’ and 3’ ends of the region, corresponding to regions of the 18S rRNA that are highly conserved among eukaryotes (Dams et al., 1988; Ellis et al., 1995; Medlin et al., 1988). The sequences of the primers were as follows:

AP1: 5’ AAC CTG GTT GAT CCT GCC AGT 3’
AP2: 5’ TGA TCC TTC TGC AGG TTC ACC TAC 3’
PCR was performed in a final volume of 25 µl that contained purified oocyst genomic DNA, 2.5 µl 10x PCR buffer (16 mM (NH₄)SO₄, 67 mM Tris-HCl [PH 8.8], 1.5 mM MgCl₂), 1 µM of each primer, 200 µM dNTPs each. Taq DNA polymerase (0.5 Units-Bioline, UK) was added to the reaction mixture when its temperature first reached 94°C.

Cycling was performed in a Hybaid DNA thermal cycler (Teddington, UK) with the following parameters: initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 second and extension at 72°C for 30 seconds, then a final extension at 72°C for 2 minutes.

The PCR product was checked by electrophoresis in 1% agarose/ ethidium bromide (0.5 µg/ml) gel, visualized using ultraviolet light and photographed using a digital camera (Syngene, UK).

2.8.5: Preparation of PCR product for sequencing

To 20 µl PCR products, 10 µl of 30% (in 1.5M NaCl) polyethylene glycol (PEG) were added, vortexed and left to stand at room temperature for 10 minutes. These were then centrifuged for 8 minutes at 14000 x g. The supernatant was carefully removed with a pipette 300 µl of 75% ethanol were added to wash the pellet, which was spinned for 4 minutes at maximum speed. Ethanol was discarded, tubes were dried then the pellet was resuspended in a small volume of 10 mM Tris.

2.8.6: DNA Sequencing
Sequencing was performed using ALF II (automatic laser fluorescent DNA sequencer) using Cy5 dye-labeled ddNTP terminators. Four tubes were labelled A, C, G and T for the respective termination mix. To each tube 4 µl of 1.1 mM dNTP, 2 µl of Cy5-ddNTP and 16 µl of distilled water. For each template 4 tubes were labeled then 2 µl of each of the above mixtures were added to the respective tubes. To each tube, 1.0-20.5 µl of template DNA was added according to the concentration of the DNA, 4 pmol (picomole) of the sequencing primer, 3.5 µl of the sequencing buffer and 1µl of DNA polymerase. Sequencing reaction was done under the following conditions: 95º C for 30 seconds, 50º C for 30 seconds and extension at 72º C for 80 seconds for 30 cycles. The resultant sequencing reactions were further purified by ethanol precipitation and pelleted. Pellets were resuspended in 6 µl of formamide loading dye, vortexed vigorously, preheated at 70º C for 2 minutes and then loaded into polyacrylamide sequencing gel.

2.9: Statistical analysis

The prevalence of Eimeria spp. and prevalence of infections according to age groups, seasons, sex and location was calculated as percent of faecal samples containing coccidia oocysts using EPI 6 statistical program. The effect of age, seasons, sex and location on prevalence of Eimeria infections was analysed using Chi-squire. The data of oocyst counts of Eimeria spp. and the effect of the some factors on oocyst counts were analysed using analysis of Variance (ANOVA). A value of ($P \leq 0.05$) was considered significant.

CHAPTER THREE
RESULTS

3.1: *Eimeria* species recovered from sheep in Red Sea State

Ten species of *Eimeria* were identified on the basis of their morphological characteristics, measurements and sporulation time exhibited by oocysts. The species recovered and their prevalence is as follows: *E. ovina*, *E. ovinoidalis*, *E. parva*, *E. faurei*, *E. ahsata*, *E. marsica*, *E. crandalis*, *E. intricata*, *E. pallida* and *E. granulosa*. The prevalence of identified *Eimeria* species is shown in Fig. 2.

Mean dimensions of oocysts of isolated species and their sporulation time are presented in Table (1). Photomicrographs of isolated species are shown in Figs (3a-3j).

3.2: Molecular characterization

All of the four sheep samples amplified well and the expected size of DNA fragment was detected. The PCR product around 1800 bp was detected which is the right size of the 18S rRNA gene in *Eimeria* species (Fig 4). It was not possible to read the obtained sequences because it showed a pattern of mixed sequences.

3.3: Prevalence of *Eimeria* spp. infection in sheep

One thousand and thirty seven samples were found to contain the parasite, with an over all prevalence of 86%. The monthly prevalence was generally high and showed variation that ranged between 98% in March and 70% in June and August. These findings are presented in Fig: (5). No significant difference on prevalence was shown to occur between different months.
Fig 2: Prevalence of Eimeria spp. in sheep in the Red Sea State (%)

- E. ovina: 75%
- E. ovinoidalis: 54%
- E. parva: 53%
- E. faurei: 26%
- E. ahsata: 38%
- E. marsica: 26%
- E. crandalis: 16%
- E. intricata: 12%
- E. pallida: 7%
- E. granulosa: 5%
<table>
<thead>
<tr>
<th>Species of Eimeria</th>
<th>Length (in micron)</th>
<th>Width (in micron)</th>
<th>Sporulation time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>E. ovina</td>
<td>26.23 ± 4.14</td>
<td>(18.8-35)</td>
<td>18.48 ± 2.75</td>
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<td>25.68 ± 2.33</td>
<td>(22.5-30)</td>
<td>19.62 ± 1.70</td>
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<tr>
<td>E. ahsata</td>
<td>31.32 ± 3.21</td>
<td>(25-37.5)</td>
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<td>29.98 ± 3.51</td>
<td>(24.8-37.5)</td>
<td>21.94 ± 2.29</td>
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<td>17.97 ± 1.96</td>
<td>(13.5-20)</td>
<td>15.96 ± 1.87</td>
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<td>(17.5-27.5)</td>
<td>18.60 ± 1.68</td>
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<td>19.64 ± 1.37</td>
<td>(16.6-22.5)</td>
<td>15.08 ± 1.90</td>
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<td>E. intricata</td>
<td>45.42 ± 3.60</td>
<td>(40-54)</td>
<td>33.64 ± 2.67</td>
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<tr>
<td>E. pallida</td>
<td>16.36 ± 2.26</td>
<td>(12.5-20)</td>
<td>12.67 ± 1.87</td>
</tr>
<tr>
<td>E. granulosa</td>
<td>27.60 ± 4.07</td>
<td>(22.5-35)</td>
<td>20.96 ± 3.21</td>
</tr>
</tbody>
</table>
Fig (3a): *E. ovina* sporulated oocyst, ellipsoidal, with micropylar cap. (40x)

Fig (3b): *E. ahsata* sporulated oocyst, ellipsoidal with a dome-shaped polar cap. (40x)
Fig (3c): *E. parva* sporulated oocyst, spherical to subspherical, no polar cap (40x)

Fig (3d): *E. faurei* sporulated oocyst, ovoid (hen's egg shaped), no polar cap (40x)

Fig (3e): *E. crandalis* sporulated oocyst, spherical to broadly ellipsoidal, visible micropylar cap (40x)
Fig (3f): *E. ovinoidalis* sporulated oocyst, ellipsoidal, no polar cap (40x).

Fig (3g): *E. intricata* sporulated oocyst, ellipsoidal with distinct light–coloured polar cap (40x).
Fig (3h): *E. marsica* sporulated oocyst, ellipsoidal, inconspicuous polar cap (40x).

Fig (3i): *E. pallida* sporulated oocyst, ellipsoidal, no polar cap (40x).

Fig (3j): *E. granulosa* sporulated oocyst, urn- shaped with polar cap (40x).
Fig.4: PCR product of *Eimeria* species amplified from 4 sheep samples and the first lane indicates the 100 bp DNA ladder. Lanes 2-5 indicate the PCR resulted form the sheep samples. Lane 9 is the PCR blank with no PCR product.
Fig 5: Prevalence of *Eimeria* infection in sheep in Red Sea State during the period January-December 2002

3.3.1: Factors affecting the prevalence and oocyst out put
Several factors were found to have an influence on the rate of prevalence and oocyst output of *Eimeria* infections. These included the age, seasons, sex and location.

### 3.3.2: The effect of age on the prevalence of infection

The infection was more prevalent in lambs (93%) followed by yearling (89%), and then adults (63%). There was significantly lower prevalence rate in adult sheep when compared to lambs and yearling. This is shown in Fig. (6).

### 3.3.3: Effect of seasons on the prevalence of infection

The prevalence was ranged between (94%) in the cold wet season (757 samples out of 804 were positive), to 71% in the hot dry season (280 out of 396). No significant difference in prevalence occurred between the two seasons. Fig (7) expresses these findings.

### 3.3.4: Effect of sex on the prevalence of infection

The prevalence of infection with *Eimeria* spp. was 90% (455 samples out of 507) and 84% (582 out of 693 samples) in male and female sheep respectively. No significant difference occurred between different sexes. This is shown in Fig.8.

### 3.3.5: Effect of location on the prevalence of infection

The infection rate in sheep in Tokar area 90% (461 samples out of 512 were positive), 86% in Port Sudan (264 out of 298) and 80% in Halaib area (312 out of 390). No significant difference on prevalence rate was found to occur between different locations. Fig.9 expresses the prevalence rate in relation to location.

### 3.4: Oocyst output

The mean of oocyst shedding was highest in February (12237opg), while the lowest mean was in September (1474opg). Fig.10 shows the mean of oocyst out put shedding during different months.
Fig 6: prevalence of *Eimeria* infection in sheep according to age group

- <one year: 93%
- 1-2 years: 89%
- > 2 years: 63%
Fig7: Prevalence of *Eimeria* infection in sheep according to season.
Fig 8: Prevalence of *Eimeria* infection in sheep according to sex
Fig 9: Prevalence of *Eimeria* infection in sheep according to location.
Fig 10: Monthly mean of oocyst output in sheep during the period January – December 2002.
The oocyst shedding was generally low as 46% of sheep examined sheded between 100-1000 opg, 34% sheded between 1100-5000 opg, 13% sheded between 5100-10000 opg, 7% sheded between 10100-50000opg and only 1% sheded more than 50000 opg of faeces. The over all oocyst per gram of faeces ranged between 100-100200 opg with general mean 4113.6 opg. These finding are expressed in Fig.11.

3.4.1: Effect of age on oocyst output

Lambs sheded the highest numbers of oocysts that ranged between (100-100200 opg) with a mean of 5617±11991.2 opg, yearling animals sheded between (100-60000 opg), with a mean of 2989 ± 6183.5 opg and the adult sheded between (100-40000 opg), with a mean of 2428 ± 4774.4 opg. A significantly higher mean of oocyst out put was sheded by lambs. Results are presented in Table. 2.

3.4.2: Effect of season on oocyst output

Examined animals showed the highest mean oocyst shedding of 4762 ± 10433 opg during cold-wet season, while a mean of 2361±4569.7 opg occurred during hot dry season. Cold wet season showed significantly higher mean of oocyst counts when compared to hot-dry season. This is shown in Table. 3.

3.4.3: Effect of sex on oocyst output

The mean of oocyst sheded by male and female was 4736 ± 10605.1 opg and 3627 ± 8078.5 opg, respectively. No significant differences in oocyst output occurred between the two sexes. Table. 4 shows this result.

3.4.4: Effect of location on oocyst output

Tokar and Port Sudan showed a high mean of 4909 ± 9959.9 opg and 4419 ± 10791.3 opg respectively, while a low mean of 2609 ± 6286 opg oocyst output occurred in Halaib. Which was significantly
lower when compared to Port Sudan and Tokar. The mean oocyst output according to location is shown in table. 5.

Fig 11: Percentage distribution of infected sheep according to oocyst per gram (opg) distribution
**Table 2:** The Mean± SD of oocyst output in faeces in sheep according to age

<table>
<thead>
<tr>
<th>Age</th>
<th>Number positive</th>
<th>Oocyst (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambs</td>
<td>474</td>
<td>5617 ± 11991.2a</td>
</tr>
<tr>
<td>Yearling</td>
<td>421</td>
<td>2989 ±6183.5b</td>
</tr>
<tr>
<td>Adult</td>
<td>142</td>
<td>2428 ± 4774.4b</td>
</tr>
</tbody>
</table>

Means ± (SD) with the same letter are not significantly different

**Table 3:** The Mean ±SD of oocyst output in faeces in sheep according to season

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Number positive</th>
<th>Oocyst (mean) ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold wet</td>
<td>757</td>
<td>4762 ± 10433a</td>
</tr>
<tr>
<td>Dry hot</td>
<td>280</td>
<td>2361 ± 4569.7b</td>
</tr>
</tbody>
</table>

Means ± (SD) with the same letter are not significantly different
Table 4: The Mean ±SD of oocyst output in faeces in sheep according to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number positive</th>
<th>Oocyst (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>455</td>
<td>4736 ± 10605.1a</td>
</tr>
<tr>
<td>Female</td>
<td>582</td>
<td>3627 ± 8078.5a</td>
</tr>
</tbody>
</table>

Means ± (SD) with the same letter are not significantly different

Table 5: The Mean ± SD of oocyst output according to locations

<table>
<thead>
<tr>
<th>Location</th>
<th>Number positive</th>
<th>Oocyst (mean ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tokar</td>
<td>461</td>
<td>4909 ± 9959.9a</td>
</tr>
<tr>
<td>Port Sudan</td>
<td>264</td>
<td>4419 ± 10791.3a</td>
</tr>
<tr>
<td>Halaib</td>
<td>312</td>
<td>2609 ± 6286 b</td>
</tr>
</tbody>
</table>

Means ± (SD) with the same letter are not significantly different
3.5: Correlation between climatic conditions and prevalence of infection

The relationship between meteorological data (temperature, rainfall, and relative humidity) showed increased higher infection rates when temperature degrees are low and high relative humidity and rainfall. Fig.12 expresses this relation.

3.6: Type of infection with Eimeria spp.

The results indicated that 83% of examined animals had multiple Eimeria spp. infection, while 17% had single Eimeria spp. Infection due to two species was found in 18%, three different species occurred in 24%, four species occurred in 19%, five to six species occurred in 17%, while only 5% of total populations were found to be infected with more than six species of Eimeria. The type Eimeria of infections is shown in Fig.13.
Fig 12: Correlation between climatic conditions and prevalence of *Eimeria* infection in sheep in Red Sea during the period January to December 2002
Fig 13: Type of infection with *Eimeria* spp. in sheep in Red Sea Stat
CHAPTER FOUR
DISCUSSION

The present study was carried out in Red Sea State, Eastern Sudan in sheep kept under extensive system of management where animals spend the whole day in pasture and around water holes or khor banks then return to the feedlots in the evenings. The objective of the present work was to study the occurrence of species of *Eimeria* in sheep in Red Sea State and to determine the prevalence and intensity of coccidia infection in sheep in the state.

Ten species of *Eimeria* were detected in sheep in this study. They were similar to those detected by Abakar (1996) with only exception that *Eimeria. punctata* was not observed in this study. This is probably due to the fact that in the previous study, the examined sheep were from different part of the Sudan, while the data of the present study was confined to the Red Sea State only. Moreover, the samples of this study were collected from local breed in the state.

The criteria used for identification of the oocysts mainly depended on certain morphological features like presence or absence of polar cap or micro pyle and the size of the oocyst. Other less precise characters were used to differentiate between oocysts which are basically similar in appearance, such as *E. crandalis* and *E. ovina*, the difference in the straightening of the sides of *E. ovina*, while *E.*
pallida was differentiated from *E. parva* by the difference in shape and appearance (Catchpole *et al.*, 1975).

Molecular characterization of coccidian parasite in sheep using PCR and DNA sequences was used in this study to confirm the identification of *Eimeria* species that was based on morphological characters. The results confirmed the existence of sheep *Eimeria* species in the faecal samples. As multiple infections is common in natural infection, PCR products showed amplification of more than one species of sheep *Eimeria*. DNA sequences were not possible to be read because it showed pattern of mixed sequences. This is possibly due to the fact that the samples contained multiple species of *Eimeria*, therefore, different DNA from different oocysts were extracted and probably interfered with sequencing reaction. Moreover, the primers used for molecular characterization are general primers that are highly conserved among eukaryotic and not species- specific (Dams *et al.*, 1988; Medline *et al.*, 1988; Ellis *et al.*, 1995). The inability to get pure species from this study was certainly made this criteria failed in differentiation between the various species.

Generally, no much information available in molecular characterizations of sheep *Eimeria* in the world. This is probably due to the fact that DNA of some species of sheep *Eimeria* is semi- similar in nucleotides sequences and molecular length such as *E. ovinoidalis*, *E. ahsata*, *E.crandalis*, *E.weybridgetensis* and *E. faurei* (Gene bank).

The results of this study revealed a high prevalence of (86%) from apparently healthy sheep. *E. ovina* was the most prevalent species in the study area and was found during all seasons and among all age groups of sheep. This finding agrees with the findings of Osman, *et al.* (1990) and Abakar, (1996). It was followed by *E. ovinoidalis* and *E. Parva*. On the other hand, *E. faurei*, *E. ahsata* and
E. marsica were less prevalent while E. crandalis, E. intricata, E. pallida and E. granulosa were found to be the least prevalent in this study.

The present study showed that the infection was detected over all the year round with high prevalence during March, April, October and December, where sheep are congregated around water points after rain fall. Lower prevalence occurred during June, August and September, where the ambient temperature was very high and relative humidity was low, and water pools were dried out. These findings agree with Pout, (1969) and Marquardt et al. (1960) who reported that many oocysts are destroyed by dryness, exposure to direct sunlight or high temperature.

The high prevalence rate of sub clinical infections in Red Sea State was similar to the observations previously reported in sheep in the Sudan (Abakar, 1996; Abakar et al., 2001) and in other parts of the world (Barutzki et al., 1990; O'Callaghan et al., 1986; Vercruysse, 1982; Maingi and Munyue, 1994). Such high prevalence of sub clinical infections with Eimeria species was also common in other animals species such as goats (Vercruysse, 1982; Elrabie, 1999), cattle (Gasmir, 1991) and in camels (Yagoub, 2004; Mohamed, 2004). This may be attributed to the fact that immunity to infection with Eimeria species is not strong enough to prevent reinfection with the parasite.

Although most of examined sheep in this study harboured pathogenic Eimeria spp. These animals did not show any clinical signs of coccidiosis. This might be indicated by the fact that the occurrence of clinical signs in coccidial infection is likely depends upon the balance between the rate of development of resistance and the speed of build-up infection. This balance may be affected by other
factors such as the weather, type of management, hygiene, method of feeding, weaning and presence of other infections (Vercruysse, 1982).

The present study also indicated that some factors may affect infection rates such as age. That was confirmed by the significant high prevalence of coccidian oocyst in the young sheep than in adult. Similar observations were reported in sheep (Osman et al., 1990; O'callaghan et al., 1986; Abakar, 1996; Abakar et al., 2001), goats (Osman, 1988; Kanyari, 1993), cattle (Gasmir, et al., 1998) and camels (Yagoub, 2004; Mohamed, 2004). This could be attributed to lower resistance in the young compared to older animals.

Furthermore, the study indicated that the prevalence of infection was high during cold wet season and low during hot dry season. However, there was no significant difference between the two seasons. These findings agree with previous observations of other authors in sheep (Vercruysse, 1982; Maingi and Munyua, 1999; Abakar et al., 2001), goats (Vercruysse, 1982; Waruiru et al., 1991; Elrabie, 1999), cattle (Munyua and Ngotho, 1990) and camels (Yagoub, 2004; Mohammed, 2004). The high prevalence of infection during the cold wet season may be due to climatic conditions which were more suitable for sporulation and survival of coccidian oocysts.


Inspite of the insignificant difference in prevalence rates between different locations (Tokar, Port Sudan and Halaib), the intensity of infection was found to be high in Tokar. This may be attributed to the fact that Tokar is an agricultural area, therefore, the area might act as suitable environment for development of coccidia
oocyst. This finding is similar to the observation of Mohammed (2004) in camel in Red Sea State.

The result indicated that the infection rate was high among sheep grazing on natural pasture. This finding disagrees with Abakar, (1996) and Osman et al., 1990) who reported that the high prevalence of infection among sheep is dependant upon husbandry methods practiced. They found that sheep reared under feedlots conditions (intensive system of husbandry) within a short period of time acquire sufficient numbers of oocyst which have sporulated in the environment of feedlots. In animals which graze on natural pasture, the chance for ingestion of large numbers of oocysts within a short time is considerably low. This may probably due to some reasons that, sheep are gathering together on the pasture or around water points. Moreover, sheep are watered from contaminated water either from stagnant water in holes or from water dams, shallow wells and khor banks during rainy seasons, also that may due to the suitable weather conditions during cold-wet seasons. These findings are in agreement with that of Apyne, (1977) who reported that under extensive system of management the disease is seldomly encountered unless animals are gathering together in large numbers during branding, vaccinating camps or around water points. He also reported that the disease is favored by moist conditions, as in some tropical countries, and the disease appears clinically only in areas of high humidity and rainfall.

Also results indicated that oocysts output decrease with advance in age. Lambs had significantly high mean of oocysts, while yearlings and adults showed lower mean of oocysts shedding. This is probably due to low immunity in lambs. These findings are similar to observations of other authors (Pout, 1973; Masson, 1977; Vercruysse, 1982; O'callaghan et al., 1986; Abakar, 1996; Abakar et al., 2001)
The present study indicated that oocyst output was generally low and varied between individual months. The high mean of oocyst output occurred in February, while lower means occurred during June and September (Fig. 10). Likewise, the significantly high mean of shedding oocysts occurred during cold-wet season, while the lowest mean occurred during hot dry season. This agrees with findings of Abakar et al. (2001) in sheep in the Sudan and Omara-Opyene, (1985) in calves in the arid area in Kenya. They reported that the high counts of oocyst occurred during the wet season. This is possible due to the fact that the climatic conditions during this season were more suitable for sporulation and development of oocyst and also this season may be linked with preparturient and lambing period that might act as predisposing factor for the decrease in immunity of the animal.

Result also showed a higher mean of oocyst counts in Tokar and Port Sudan, while a lower mean occurred in Halaib. That was indicated by the significantly low oocyst output in Halaib area when compared to Port Sudan and Tokar. This finding could be explained by the prevailing harsh weather conditions in Halaib (high temperature, low humidity and few rainfall) and also may attributed to the fact that most of the samples from Halaib were collected from adult sheep (specially during dry hot season) which apparently discharge fewer oocysts because they develop some sort of resistance through repeated infections. 

The study also indicated that 83% of examined animals harboured mixed Eimeria infection. A finding confirms the fact that multiple infections with more than a single species of coccidia are the rule in natural coccidial infections. This finding agrees with the findings of Catchpole et al., 1975; Marquardt, 1976; Vercruysse,
Conclusion and Recommendations

In conclusion, the study showed that 10 species of *Eimeria* were detected in sheep in the Red Sea State and three of those are pathogenic spp.

86% of sheep examined harboured sub clinical infections. The study also indicates that infection with *Eimeria* is highly prevalent in lambs and the climatic conditions seem to be favourable for development and survival of sheep *Eimeria* in the State.

Molecular characterizations based on PCR and DNA sequences confirmed the existence of sheep *Eimeria*.

The study recommends an applied control measures to reduce the faecal contamination of water, food and the ground through:

i- Minimizing of stocking density, which will reduce the chance for acquisition of large number of oocysts by sheep.

ii- Routine medication of both adult carriers and lambs.
Moreover, the study recommends more investigations and studies molecular characterization of sheep *Eimeria* spp.

**References**


Anon, (2001). Annual report. Port Sudan Veterinary Research Laboratory, Red Sea State


Christensen, J. F. and Foster, A. O. (1943). Further studies with sulphaguanidine in the control of ovine coccidiosis. Veterinary Medicine, 38: 144-147.


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لا يمكنني قراءة النص العربي بشكل طبيعي. يرجى تقديم نص يمكنني قراءته بشكل طبيعي.
العائشة السنية: لفظ الدعوة السنية تعني في الدراسة أن توضح في النتائج، أ去买عطفelandia, عند بائع العائشة السنية، إذن لفظ السنية، إرتفاع التلاحظ، فقد النسبة، وارتفاع الحرارة، درجة (38%).

لذلك، إذا كان 38% من العائشة السنية توفر فحصًا، كان ذلك يتجاوز، بينما 71% من العائشة السنية كثيرة تحمل.

الحياة: فحص تقنية، استعمال ذلك كذلِك، العائشة السنية، بزائد، على الأوقات، السليمة، تتعلق على الفحص، ذلك، نسبة، ولكن، للنسبة، السنية، النوع، تتفاقم، النسبة، السنية، الأنواع، من الدراسة، يمكن لكل العائشة السنية، كذلك، الأوقات، السليمة، تتعلق على الفحص، ذلك، نسبة، للنسبة، السنية، النوع، تتفاقم، النسبة، السنية، الأنواع.