COCCIDIOSIS IN ZAMBIAN GOATS AND THE LIFE CYCLE OF EIMERIAN SPECIES

A DISSERTATION

SUBMITTED TO THE

SCHOOL OF VETERINARY MEDICINE

OF THE UNIVERSITY OF ZAMBIA

IN FULFILLMENT OF THE

REQUIREMENTS FOR THE

DEGREE OF MASTER

OF SCIENCE

JANUARY 1991

BY

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DEDICATION

Dedicated to my God who has loved and protected me all through these years. May you bless everyone who reads this dissertation. AMEN.

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I hereby declare that this dissertation is my own work and that it has not been previously submitted for Degree purposes here or at any other University.

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ACKNOWLEDGEMENT

I wish to express my sincere gratitutde to Professor C.E.A. Lovelace for her tireless effort, criticism and advice during the research as well as the write-up. I wish to thank Dr. T.O.M Koomson, Dr. K. Verstraelen and Dr. F. Sabbe for the assistance and advice he provided during clinical examinations. I would like to thank Dr. M.W. Gregory for his assistance in identifying the giant and small schizonts and other helpful suggestions on the general life cycle of Eimeria species.

Special thanks go to Mr. G. O'Mahony and Mr. I. Nyirenda who gave great assistance during development of histological techniques. I must thank Mr. J. Daka for his encouragement and assistance in biochemical analysis. For assistance with the statistical analysis of my data I am very grateful to Dr. D.N. Kisauzi, Mr. E. Siwo, Mr. D.J. Banda and Mr. K. Chipampe. My thanks go also to Mr. P. Chengo for cartographic work on all my graphs. My sincere gratitude goes to Ms. A. Mukelabai for putting up with the many trials of typing this dissertation. Many thanks to the staff of the Samora Machel School of Veterinary Medicine. I must express my appreciation to Dr. R. Alders for proof-reading this dissertation.

Lastly but not least I wish to express my appreciation to my husband Samuel Kwesi and my children Yaa, Kwesi, Ama and Efua and my niece Junic for the support and encouragement I received from them throughout this study.

ABSTRACT

Goats are of economic importance in parts of Zambia because of their adaptability and that they can survive well during drought. Most goats found in Zambia are kept by peasant farmers under simple village management.

In this study, ten kids with ages ranging from 5.5 to 20 weeks and, pre-exposed to natural infection with coccidiosis were used. They were weaned, divided into two groups, and kept in a confined area. Oocyst count, body weight, temperature, blood samples for biochemical and haemotological analysis and clinical symptoms were recorded weekly. Biochemical measurements included serum total protein, albumin and globulin.

The clinical symptoms of the disease appeared after six weeks of the experiment in the first set and after a week in the second set. The kids became critically ill during the last 8-10 days before they were sacrificed. Half the number of kids were treated with the coccidiostatic drug amprolium one day before sacrifice. Post mortem was carried out and coccidial lesions were observed in the intestine. A detailed histological study was carried out on intestinal sections and the stages of the life cycle are presented. Giant and small schizonts were observed and different forms of degenerating giant schizonts were recorded. Unusual areas of the villi containing only microgametocytes were also observed.

A small survey was carried out on three goat herds to determine the prevalence of coccidia in Zambian goats. Two were commercial farms and the other was under village management. Faecal samples were collected once in the dry season (June) and once during the wet season (February), and results showed 100 per cent infection, with higher counts in the village samples.

Statistical analysis of the results of the biochemical and haematological values were looked at in relation to development of disease of coccidiosis. It was observed that total serum protein and albumin levels generally decreased. Results overall were slightly different to reported results of European goats.

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INTRODUCTION

1.1 Zambian Goats

Zambia is 750,000 Km^2 in area and supports a total population of 8 million people, as projected by Central Statistical Office in Lusaka (1985). Nearly 60% of the population live in urban centres. With the substantial decline in copper prices, Zambia is facing an economic crisis which has lead the government to place considerable emphasis on agricultural productivity.

Zambia has long been known as a cattle rearing country and owing to a combination of climate, vegetation, a previously healthy economy and traditional preference, the husbandry of sheep and goats has always been a peripheral enterprise, (see Table 1.1 and 1.2). The lack of emphasis on goats has existed across the board, the government preferring to concentrate the majority of the limited inputs and manpower allocated to the livestock sector on the development of the much larger cattle industry. However, there is a current shortage of beef and the utilisation of Zambia's sheep and goat resources are now being studied as a potential source of animal protein. There are about 2 million cattle in Zambia in the commercial sector. This can be compared with 400,000 goats (Ministry of Agriculture and Water Development Annual Report 1987) - (see table 1.2), of which of only 1-2% are kept by commercial farmers.

Table 1.1

APPENDIX IV A.

LIVESTOCK POPULATION IN DIFFERENT PROVINCES - TRADITIONAL SECTOR

PROVINCE	BULLS	COWS & HEIFERS	OXEN & TOLLIES	CALVES	TOTAL CATTLE	SHEEP	GOATS	TOTAL-SHEEP & GOATS	PIGS
CENTRAL	8474	135,537	78,376	51,863	274,653	212	12,117	12,329	4,829
COPPERBELT	837	9,907	3,612	2,004	17,360	288	2,279	2,567	1,580
EASTERN	5614	122,026	77,354	50,420	257,014	9,847	149,271	159,118	133,327
LUSAKA	783	17,654	9,729	7,444	35,561	329	11,136	11,459	1,227
LUAPULA	529	4,512	960	1,488	7,488	6,216	15,082	21,698	1,309
NORTHERN	5569	49,217	10,191	16,855	81,852	6,762	11,504	18,266	2,030
N. WESTERN	4229	37,593	2,060	10,156	55,024	2,120	12,499	14,619	2,301
SOUTHERN	16192	442,794	280,519	178,994	918,769	3,727	175,964	225,842	45,078
WESTERN	14097	259,879	114,404	96,248	494,729	-	2,847	5,456	4,092
TOTAL 1987	56236	1,091,179	577,744	415,942	1,142,454	29,601	400,997	478,159	196,310
TOTAL 1986	77914	1,077,858	536,750	400,141	2,054,792	29,143	417,423	446,566	174,709
INCREASE	-	13,321	40,994	15,801	87,662	458	-	31,593	21,601
DECREASE	21678	-	-	-	-	-	16,425	-	-

Table 1.2

APPENDIX IV B.

LIVESTOCK POPULATION BY PROVINCE - COMMERCIAL SECTOR

PROVINCE	BULLS	COWS & HEIFERS	STEERS	CALVES	TOTAL CATTLE	SHEEP	GOATS	PIGS	HORSES
CENTRAL	1,113	30,321	39,382	38,368	144,854	[illegible]	[illegible]	[illegible]	-
COPPERBELT	819	27,197	7,726	[illegible]	42,122	[illegible]	[illegible]	[illegible]	12
EASTERN**	46	1,334	486	1,173	3,449	210	-	-	-
LUSAKA*	-	-	-	-	-	-	-	-	-
LUAPULA**	73	2,136	215	1,037	3,466	-	-	-	-
NORTHERN	260	1,810	7,127	1,996	11,073	1,269	736	842	-
N. WESTERN	123	1,978	343	696	3,140	-	-	-	4
SOUTHERN	5,156	108,195	35,013	43,852	192,157	7,956	5,675	4,321	337
WESTERN	-	-	-	-	-	-	-	-	-
TOTAL 1987	10,695	229,971	79,252	86,197	400,097	17,225	13,234	28,023	46
TOTAL 1986	11,039	222,760	69,187	82,289	388,225	15,589	12,222	24,780	111
INCREASE	-	1,211	10,065	3,808	11,862	1,636	1,012	3,248	359
DECREASE	344	-	-	-	-	-	-	-	-

* Data not provided

** Used 1986 data.

About 45% of the total goat population is kept in the Gwembe Valley area of Southern Province, and 30% are found in Eastern Province. In both these areas, sheep and goats are of importance because of their tolerance to trypanosomiasis. In Luapula Province there are almost no cattle (Cossins and Bennet 1978) and mainly goats, (fig 1.1).

Development of the sheep and goat industry could provide a useful diversification enterprise especially in places where the presence of tsetse flies prohibit the husbandry of cattle. This can help in the increase of meat supply to the market. Goats are also useful for the production of milk and skin. Owing to their small size, short generation interval and adaptive capacity, goats play an important role in developing countries and about 95% of the world goat population is found in these areas. Goats require low capital inputs for starting or expanding the flock and can survive in large numbers in extensive underdeveloped semi-arid overgrazed, denuded sites even on mountain slopes. Due to their size and agility goats can utilise productive areas which cannot be used for crop or other stock.

Poor grazing management, pastures of low nutritive value and insufficient water supply have adversely affected the production of beef cattle in many parts of Zambia. Unlike cattle, goats possess great adaptability and have lower nutritive requirements so that they can adjust to a wide range of environmental conditions. They can even be kept in areas of low

seasonal rainfall and unreliable water supplies. Zambia's rainy season lasts from December to March and sometimes during drought, the season is much shorter. Goats do show some selectivity in the plant species they eat and the quantity of weeds and shrubs consumed can be influenced by the stage of maturity and the height of the foliage. They are known for their ability to prevent the encroachment of such weeds as Lantana spp (Cossins & Bennett, 1978). Because of this and their general browsing tendencies, goats can be a potential factor in controlling existing vegetation.

In the West African humid zone, additional and more intensified efforts are to be made in the field of small ruminant management research (FAO 1988). These regions traditionally depended on areas to the north to meet their demand for animal protein. Trypanosomiasis has generally limited livestock production and most small ruminants in the zone are indigenous trypanosomiasis tolerant dwarf breeds (FAO 1988).

The majority of goats in Zambia are kept by peasant farmers under simple management procedures. There are four possible ways of management under village conditions:

(a) Tethering: The animal is tied to a tree. The advantages to this are that growing crops are protected, pasture is in the immediate vicinity and hence animals do not have to graze very far, they are protected from wild animals and safe from theft. Tethering has far more disadvantages however, i.e.

limited amount of grazing causing emaciation, increased risk of helminth and coccidial infection, strangulation and high mortality.

(b) Semi-Extensive: The animals are housed at night but left to graze freely during the day. Usually they return home on their own in the evening.

(b) Extensive: Animals roam freely both day and night, no shed is provided. Sometimes they shelter under a roof or a verandah at night. In the humid zone of West Africa, goats are mostly raised as scavengers and sleep where they can, except during the rains when they are confined to protect crops, (Oppong 1988).

(d) Intensive: Animals are confined in enclosures both day and night and may be released to paddocks for at least two hours during the day.

In many villages where goats are kept in Zambia, the animals are turned loose in the morning and return on their own from pasture. They are then tethered around the living quarters or put into pens constructed of thorns or in mud huts. Animals in Luangwa District (Lusaka Province) are allowed to graze on open land during the day and at night the animals sleep in huts on raised slattered floors. This has the advantage of allowing the droppings to fall on the ground giving easy cleaning and collection of manure and reduces reinfection of parasites contained in the faeces (Lovelace 1988). In this type of management (village) a group of farmers make an agreement to

employ a single herdsman who combines all the individual flocks in the morning and takes them out for grazing in a single large flock. At sunset he returns the animals to the village where they are separated into individual flocks and moved to their respective pens.

With the present need for more animal protein for feeding the steadily increasing human population of the world and the ability of goats to produce this protein under both extensive and intensive conditions, goats have to be integrated with other forms of animal production. Improvements required for better management of goats are training programmes for farmers, veterinary extension workers, regular marketing of animals and transport. Barns, sheds, shelters and shade pens should be constructed to make the environment more favourable for animals. Commercial farm houses with lights, ventilators, watering facilities, drains, feed racks and troughs etc., could be built for goats at a commercial scale. Overcrowding and use of dark, damp, poorly ventilated houses are to be avoided. Concrete or brick floors which can be rapidly and frequently cleaned need to be installed. In the most intensive conditions goats should be turned out to graze in a field for part of the day.

1.1.1 Nutrition

Goats are useful for producing fresh clean milk for families in rural areas where keeping of cows would be difficult. Goats can also provide a good source of animal protein through their meat.

There is an increasing gap existing between the available supplies of animal protein and the quantities that are required by man. Efforts to close this gap must be intensified and all possible methods must be employed including the use of goat meat and milk. Otherwise the deficiency will continue to affect the health, physical output and social, economic and political development of the people in the areas affected.

Traditionally it is believed that livestock and particularly goats can find their own feed requirements and that grass and fodder have the same nutritive value irrespective of species or stage of maturity. Yet climate and seasonal insufficiencies of feed and water can have adverse effects on the animals. There has been very little research work done on goats hence less information is available on goat nutrition especially in Zambia. The major resources in extensive systems are uncultivated browse and grass. Goats in particular are able to select the most nutritious plants and parts of plants, obtaining a reasonably balanced diet throughout the year. Farmers may assist by lopping branches that would otherwise be out of reach of livestock and by providing water to animals at night.

Crop residues should be made available to the animals. Where there is a shortage of forage a residue of low nutritional value will assume a greater importance than when animals are left to scavenge around the village. The remaining stems and leaves after harvesting maize, groundnuts or any other crop can be fed to the animals.

Tropical grasses mature rapidly and crude protein levels can fall as low as 2% in the dry season (Atta-Krah and Reynolds 1988). Deep rooted browse trees, however, show less variation in protein level throughout the year and browse is often the only source of green foliage in the dry season. Fodder trees contribute to soil conservation and fertility maintenance. Animals under confinement or tethering often receive browse fodder collected from fallow lands. The International Livestock Centre for Africa (ILCA) has, since 1980, been working on two leguminous fodder trees Leucaena leucocephala and Gliricidia sepium in the development of small holder fodder production systems. Both species are very well adapted to humid and sub-humid tropical conditions and are capable of nitrogen fixation (ILCA 1979).

The energy requirements for maintenance rises from 17Mcal/day for a 40kg goat to 29Mcal/day for a 80kg goat. The requirements during the last two months of pregnancy increase to 34Mcal/day and 46Mcal/day respectively (Ryder 1986). Energy requirements increase rapidly during late pregnancy. Kids of dairy breeds can be expected to gain an average of 225g/day for the first six months of life. Males grow faster and have heavier weights than females, while goats castrated soon after birth grow quicker with heavier weights than entire males (French (1970).

When born, kids are single stomached animals with digestion of milk taking place in the abomasum. From the end of the second week of life, kids start nibbling hay etc., and the rumen starts to grow until it occupies 75% of abdominal capacity. Kids should suckle their dams for the first four days of life so as to get the nutritive and immunological benefits of colostrum. Kids of meat producing goats continue to suckle their dams until they are weaned. During weaning it is important that kids receive good quality fodder and are fed concentrates. Under natural extensive conditions weaning often coincides with the onset of the dry season, when the animal must rely on fibrous, mature herbage and this may retard growth.

Many commercial farms provide supplementary feed such as sun-dried hay which is fed as roughage, with dairy concentrates and fibrous agriculture by-products. Residues like cotton seed husks, rice straw, mollases, sugar cane and orange pulps can be given to goats to supplement their feed especially during the dry season. A study on 24 Somali-Arabian goats was carried out in Somalia on the effects of varying digestible energy fed in pregnancy with body weight and birth weight after feeding the goats on hay. Body weight of pregnant goats and kid birth weight increased with the level of digestible energy. Hay and total feed intake tended to decrease as digestible energy in the concentrate increased and also as goats approached parturition. This seems to suggest that the increase was not significant, for a low digestible energy level (29.3Mcal/kg) in the concentrate

was adequate for daily digestible energy requirements during th last six weeks of pregnancy (Hassan 1989). The dairy concentrate intake should be of good quality not quantity.

1.1.2 Goat Reproduction

Some of the advantages of goats are their survival ability, their economic productive capabilities in often hard environments and their powers of transmitting these characteristics to their offspring. It should be possible to intensify goat production in less developed and remote areas of countries like Zambia, to help to deviate from the existing heavy dependency on cattle.

Goats are polyoestrous and can breed all the year round in the tropics. Oestrous lasts 2-3 days and the duration of the cycle is around 21 days; matings are promiscous. The gestation perio averages 148 days (Wilson 1957). Conception can occur throughout the year depending on what type of management is carried out by the farmer. Results from other African countrie illustrate this. In Malawi a study was carried out in six villages around Bunda College and Lifidzi Ranch in Salima. At Salima Ranch first mating time was controlled until goats reached an average age of 12 months, but under village management there was no such control. The season of birth of the dam affected the age at first kidding in the village because of the way the goats were managed under the village system. In the hot wet season, goats were tethered both night and day which

restricted breeding activities (to protect crops). During the dry season goats were loose and mixed freely thus allowing increased breeding activities (Karua 1989). In South West Nigeria under traditional conditions, there was no control of the reproductive processes and does were mated for the first time when they achieved puberty. This meant that goats produced young all the year round (Wilson et al 1988). In Swaziland, traditional system kids are born all the year round with a peak in the winter months of May to July. Lebbie et al (1989) observed that births in the dry season were at a disadantage due to poor quality and limited quantity of feed since little supplementary feeding is practised in the Swazi traditional system. Average litter size in goats is 1.50 kids, depending on management. Managemegt effects also influence the kidding interval and birth weights, the kidding interval being longer in the villages than on ranches according to studies done by Karua on Malawian goats (Karua 1988).

Cross breeding has many advantages, such as improved milk yields, increased number of progeny born at each parturition, better meat producers and skin quality. In Zambia, Boer goats from South Africa were introduced at three places including Liempe farm in Lusaka in 1971 for cross breeding with the local goats (Savage 1977).

Kids are the most fragile and perishable animals in a goat flock as they are prone to disease. Therefore weaning should be well calculated and well done at this tender age.

1.1.3 Parasitic Infections of Goats

Parasites are a major constraint to goat production. There is a high kid mortality due to parasitic gastro-enteritis, particularly haemonchosis. A study carried out on two Zambian commercial farms recorded a number of nematodes. Species indentified were: Haemonchus contortus, Oesophagostomum colombianum, Strongyloides papillosus, Trichuris spp, Cooperia spp and Trichostrongylus colubriformis. Other parasite species found included Stilesia hepatica, Cysticercus tenuicollis and Moriezia expansa (Muimo 1989). In Zambia, during the rainy season, climatic conditions become more favourable for worm infestation resulting in increased infection rates. The infection is transmitted as eggs from adult animals to the young ones as both graze the same pasture. Parasitic gastro-enteritis causes anaemia, diarrhoea, emaciation resulting in reduced weight gains, increased mortalities and increased production costs.

The loss to Kenya's agricultural sector due to haemonchus in sheep has been estimated at US $26 million (Preston and Allonby 1979). In Kaboboya Goat Scheme in Fiji 50% of the goat population died during the first 3 months of the project due to an outbreak of haemonchosis (Restrepo and Preston 1989). In Columbia there have been cases where all goats have died from parasite infestation (Restrepo and Preston 1989). In the Solomon Islands worms are a major problem in intensive pasture fed goats and the principal species are Haemonchus, Trichostrongylus, Ostertagia, Oesophagostomum and Moriezia,

(Simpson 1984). In the Australian goat industry it is believed that parasites are the most important cause of loss of goats (Baxendell 1984).

Treatment with drugs alone is not a satisfactory way of controlling the number of worms. There is a need to provide feed which is not contaminated by worm eggs, dry bedding (slatted floors) or non edible litter that is changed frequently. These ensure that the natural life cycle of the parasite is broken. Tethering should be discouraged since the animal is likely to pick up the larvae during feeding.

The other diseases observed by other research workers in goats were associated with ticks, nasal discharges, viral disease and pneumonia, the last being prevalent in wet season when damp conditions in the mostly open and unprotected pens rendered the animals more susceptible to attacks.

1.1.4 Coccidiosis

Coccidiosis is a disease that affects all types of livestock kept in an intensive environment. The disease is caused by the genus Eimeria in the Class Sporozoa. The family Eimeridae contains 17 genera (Levine 1988), of which the vast majority are found in vertebrates. Most species of Eimeria parasites infect epithelial cells of the intestinal tract, though other species have their life cycle in other organs such as liver, lung,

kidney and testis. Dykova and Lom (1981) named 31 species of Eimeria that develop outside the digestive tract. Members of this family are highly host specific, i.e. species that infect goats are not infectious to sheep (Gregory and Norton 1986). The infection is confined to a certain section of the intestine or tissue. Some of the important differences between Eimeria species are size of the oocyst, prepatent period and sporulation time, reproductive capacity, pathogenicity and immunogenic properties.

All goats probably get infected with coccidia at some stage of their life, but only a small percentage of them suffer from coccidiosis. This disease mostly affects kids and can be responsible for diarrhoea, weight loss, poor feed conversion and sometimes death.

There are at least nine different species of Eimeria (Gregory and Norton 1986) that can cause coccidiosis in goats, though some are more pathogenic than others. Goats become infected when they eat food contaminated with coccidial oocysts. This is a relatively resistant stage of the parasite which is passed in the faeces of infected animals. Each oocyst ingested can result in millions of new oocysts being produced. Because of this massive production of oocysts, the environment can become heavily contaminated within a short time particularly when conditions are warm and moist. Adult goats develop a strong resistance to coccidial infection but kids are highly susceptible. Coccidiosis is most common in intensively reared kids usually around the time of weaning.

1.2 Life Cycle of Eimeria Species

Members of this subclass Coccida (Soulsby, 1982) have a single host, schizogony and gametogony taking place within the host cells and sporogony occurring outside the host's body. The infective stage occurs when a susceptible animal consumes a sporulated oocyst from its environment. The sporulated oocyst contains four sporocysts, each sporocyst containing two sporozoites. In the case of chickens the sporozoites are released by mechanical and biochemical action in the digestive tract (Pattilo 1959). According to Long and Millard (1979), in chickens inoculated with oocysts, only a small proportion of sporozoites reach the liver. Other workers have established that passage of sporulated oocysts of Eimeria species through the gizzard is necessary to damage the oocyst wall enabling the release of sporozoites (Guyonnet, Johnson & Long 1989). This was tested by infecting oocysts of E. acervulina, E. maxima and E. tenella into the duodenum. Lotze & Leek (1968) recovered some oocysts containing motile sporozoites from the faeces of birds given E. tenella by mouth. Goyonnet et al (1989) discovered that birds inoculated directly into the intestine developed coccidia lesions. The oocysts released sporozoites which then completed their endogenous cycle in the intestine although the manner of release of sporozoites from oocysts was not clearly established. Doran & Farr (1962) suggested that coarse food materials within the intestine exerted some pressure on the oocyst wall causing it to break in a similar way to the gizzard.

Eimeria species are believed to be site specific. Augustine (1989) studied Eimeria invasion *in vivo* and suggested that specific interaction between the sporozoites and the target host cells may initiate the invasion process. Sporozoites of the avian *Eimeria* have been observed to invade the same sites in a foreign host bird as in the natural host. This indicated that the site specificity could be a response to characteristics that are shared by a number of hosts. These workers discovered that there are molecules on the surface of cells in the intestinal epithelium that act as receptor or recognition sites for sporozoite invasion. There is also evidence for reciprocal receptors on or within the sporozoites that may participate in cellular invasion (Augustine 1989).

Many studies have been done on the invasion of the sporozoite into the host cells. Coccidia, being members of the phylum Apicomplexa, are characterised as having an apical complex that includes polar rings, rhoptries, micronemes, conoid and subpellicular microtubules. It is proposed that these structures are involved in the invasion of host cells by motile stages of the parasite. Invasion begins with the contact of apical end of the motile stage with the host cell membrane, in front of the advancing parasite and ends with the sealing off of the membrane at the site of parasite entry (Augustine 1989). Constriction of *Eimeria* sporozoites at the site of entry of the host cell has been reported by many investigators. This constriction may constitute the close junction between host cell

and invading parasite. The parasite then enters the cell by "capping" the junction down its length in a manner similar to the way that specific surface bond receptors are capped down the parasite body (Russel and Sinden 1981).

After invasion some species develop at the site of entry while others develop at sites other than the surface of epithelial cells. Five of the seven species of chicken Eimeria and E. zuernii of cattle undergo first generation schizogony within crypt epithelial cells and E. bovis of cattle develops in the endothelial cells of the central lacteal of intestinal villi (Fernando 1989). The entire life cycle of some species of Eimeria occurs at extraintestinal sites e.g. E. stiedai in the biliary epithelium of the goose kidney, E. neitzi in the uterus of the impala (McCully et al 1970), Eimeria spp of fish in epithelial and non-epithelial cells in the liver, pancreas and spleen (Overstreet 1981). Lotze et al (1964) have reported extraintestinal development of one or more stages in the life cycle of Eimeria spp that normally develop within the intestinal mucosa. The asexual and sexual stages of E. arloingi and E. christenseni were found in the mesenteric lymph nodes of sheep and goats. Both sexual stages including oocysts of E. reichenowi and E. gruis were found in bronchial epithelial cells in the lungs of cranes by Novilla et al (1981).

There has been much speculation as to how sporozoites that enter the host via villus epithelial cells reach the specific site at which they are known to undergo further development. Several

workers studied the possible routes by which E. stiedae in rabbits reached the bile duct epithelium. Horton (1967) found "structures" resembling sporozoites both in the mucosa and within the lamina propria either free or within macrophages. He suggested the route of migration from lymphatics to the liver as the portal blood vessel, with the parasitised cells or the parasites entering the blood vessels as they passed through the lymph nodes. Lawn and Rose (1982) showed evidence that host cells transporting the sporozoites of E. tenella from surface to crypt epithelium are not macrophages but resemble granulated intraepithelial lymphocytes. Recent work by Fernando (1989) with other Eimeria spp of the chicken showed that sporozoites of these species are also transported from villus to crypt epithelium within mononuclear cells. It is speculated that species of Eimeria that develop at extraintestinal sites have similar mechanisms for the transport of Sporozoites from the intestinal epithelium to their preferred site of development.

Once inside the epithelial cell or the specific site the sporozoite rounds up and multiplies asexually to become the first generation schizont. The number of generations of schizogony preceding gametogony in species of Eimeria varies with the animal infected and the Eimeria species. In species affecting chickens the number of generations differs between 3 and 4 generations (Long 1989). In goats only two have been clearly identified. The number of schizogonous generations

varies and can be changed by selection for precocious development. Essentially the final generation of schizogony which preceeds gametogony is substantially smaller than the parent schizont. Kelly and Youssef (1977) saw mature schizonts of E. vermiformis in mouse embryos containing first generation merozoites between 36-48 hours after invasion. The schizont ruptures three days after maturity releasing the merozoites. The merozoites are about 100-300 µm long containing anterior and posterior refractile granules. Each schizont can contain as many as 250,000 merozoites. There are no signs of disease at this stage unless the schizonts are numerous (Gregory & Norton 1986).

In the case of a goat the released merozoites invade other epithelial cells. These will multiply asexually producing smaller second generation schizonts but with much bigger merozoites compared to those in the first generation schizonts. The mature secondary merozoites are released five days after invasion.

Gregory et al (1987a) described a stage between merogony and gamogony, giving it the name of pro-gamont. In this stage the parasite stimulates the infected host cell to divide and it also divides itself in synchrony with the host cell. This stage divides to produce more of its kind. This is done for an undetermined number of generations. The last generation gives rise to gamonts which will develop into either a macrogametocyte

or microgametocyte. This is the beginning of the sexual phase of the life cycle (gametogony). Most of these progamonts will become female gametes (macrogametocytes) which will grow in size until they are mature. Within the macrogametocyte are eosinophilic plastic granules in the cytoplasm, these are composed of mucoprotein (Patillo et al 1955). They are used to lay down a wall around the zygote. One set forms the inner wall and the other the middle and outer wall of the oocyst. Within each microgametocyte a large number of tiny biflagellate microgametes are formed. When mature they are released and fertilize the mature macrogamete which will become a zygote. This develops into an oocyst.

The oocyst then breaks out of its host cell to enter the intestinal lumen and pass out in the faeces. Once outside the body, sporulation begins and may be completed in a day or two depending on temperature, moisture and size. Warmer temperatures increases the rate of sporulation.

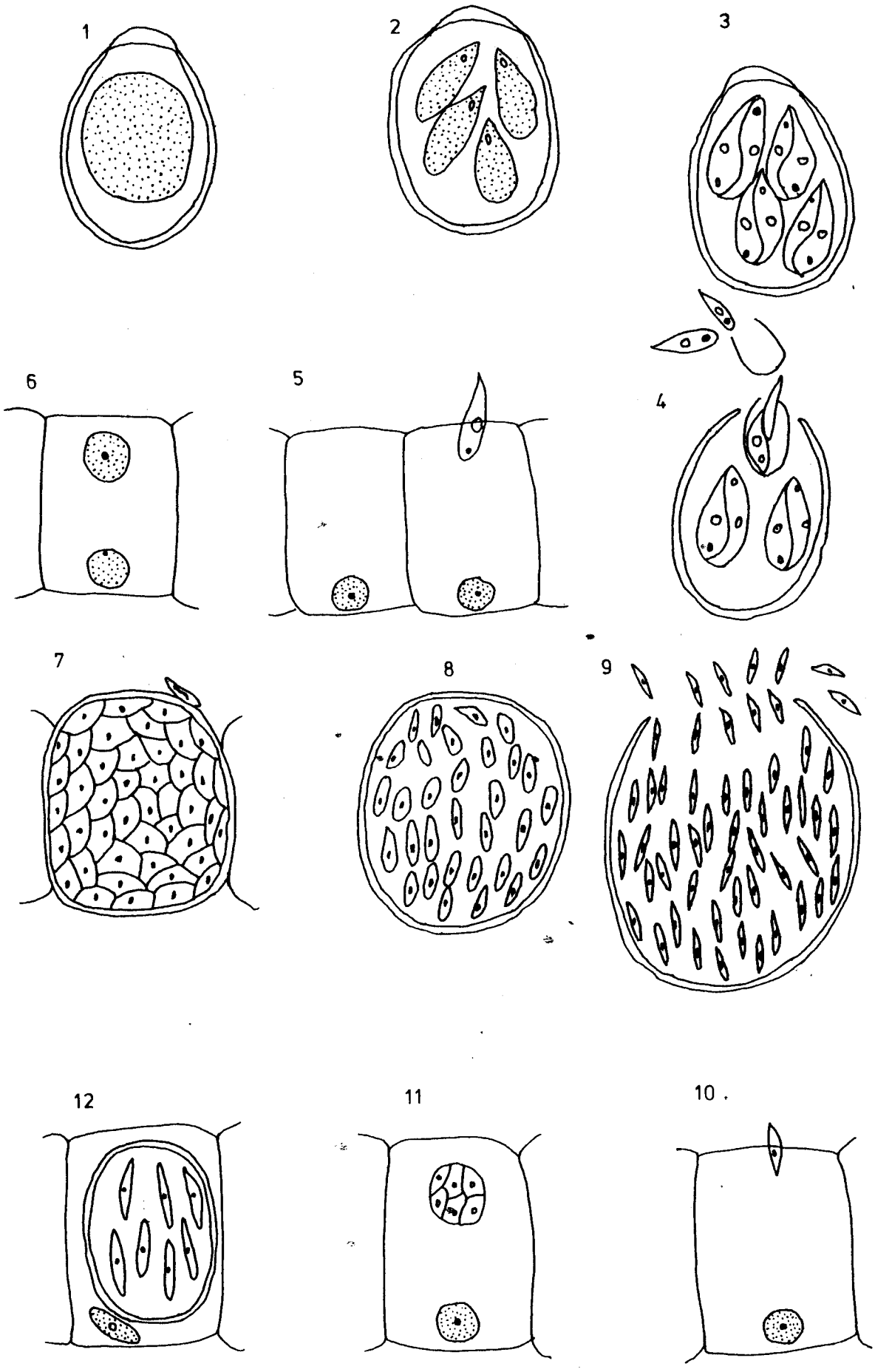
In goats the internal part of the life cycle takes between 2-4 weeks depending on the species of Eimeria.

It is reported that during the life cycle in the goat and sheep the most pathogenic stage is the sexual stage of the gametocytes whose development causes severe damage to the villi in mucosa lining and the crypts of Lieberkûhn (Gregory and Norton 1986).

General Life Cycle of Eimerian spp - (Fig. 1.2.1)

1. Mature oocyst
2. Sporulation taking place
3. Mature sporocysts with 8 sporozoites
4. Released sporozoites
5. Sporozoite penetrating host epithelial cell
6. Immature giant schizont being formed
7. Immature giant schizont
8. Immature giant schizont
9. Mature giant schizont releasing 1st generation merozoites.
10 Merozoites penetrating epithelial cells
11 Immature small schizont
12 Mature small schizont with 2nd generation merozoites
13 Ruptured small schizont with 2nd generation merozoites being released
14 Merozoites penetrating epithelial cells
15 Merozoite becomes a progamont
16 Progamont differentiates into a macrogametocyte
17 Progamont differentiates into a microgametocyte
18 Mature microgametocyte
19 Ruptured microgagetocyte releasing microgametes
20 Fertilisation of the mature macrogamete
21 Zygote formed
22 Oocyst released together with faeces

FIG. 1. 2. 1: GENERAL LIFE CYCLE OF EIMERIA SPECIES



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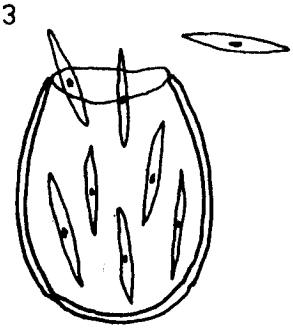

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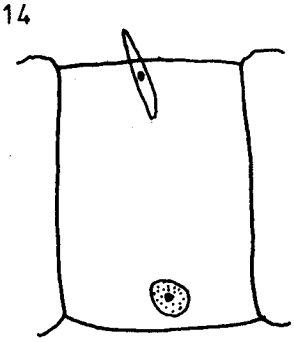

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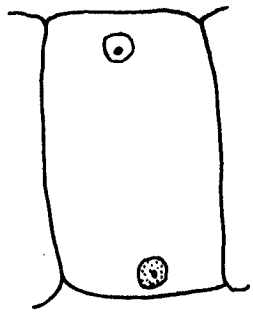

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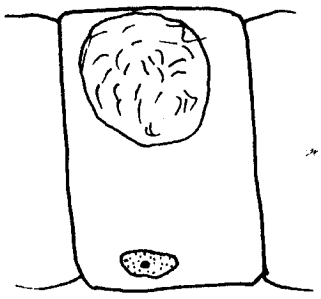

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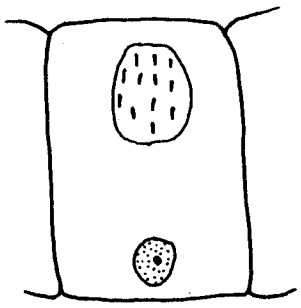

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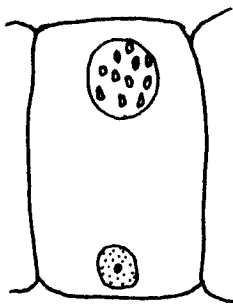

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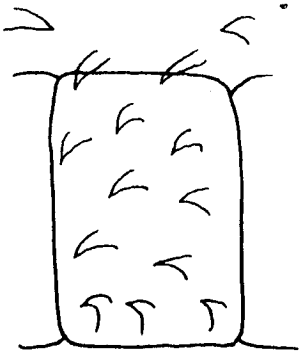

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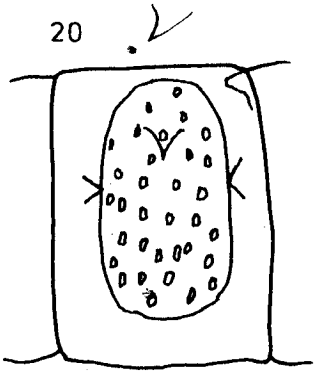

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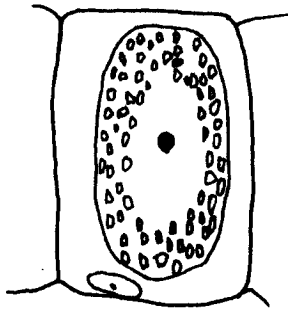

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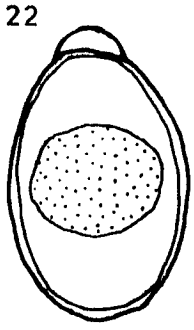


1.3 The Blood Constituents

Blood consists of a fluid medium with suspended individual cellular constituents. The circulating blood comes either directly or indirectly into contact with cells of the body. Its main functions are to supply oxygen, essential nutrients, enzymes, hormones, water, electrolytes and buffering systems to the tissue cells. It removes the metabolic waste products as well as exogenous poisons that may have been absorbed. The white cells provide a defence mechanism against pathogenic agents. Antibodies or the immunoglobulins of the blood circulate and protect the individual from further attack (Schalm, Jain & Carroll 1975).

The composition of the blood in normal animals is reasonably constant and falls within fairly narrow limits. The routine haematology is useful in the identification of animal patients with significant fluid balance disturbance through the peripheral blood parameters - packed cell volume, haemoglobin concentration, total blood cell count (Kelly 1984). This can provide significant information additional to that obtained from general clinical examination. The laboratory results are influenced by haemoconcentration and haemodilution.

1.3.1 Blood Cells and Functions

Erythrocytes: These are non-nucleated, non-motile cells. They appear as biconcave, circular discs varying in diameter and thickness according to species and nutritional status of the animal, but are capable of changing in shape while passing

through capillary beds. Erythrocytes of ruminants (goats and sheep etc) are discoid. They are manufactured in the bone marrow. Erythrocytes in adult animals contain 62-72% water and 35% solids; of the solids haemoglobin constitutes about 95%, and 5% are proteins in the stroma and cell membrane. The average mean diameter of a goat's red blood cell is 4 μm (Swenson 1977). The total number of red blood cells varies greatly among species and also within species because the cells are not uniformly distributed in the blood vascular system. In goats the cell count varies between 13-14 x 10^{6} /mm^{3}. The main function of the red blood cells is to transport oxygen, the exchange of which does not require energy.

Cells of the reticuloendothelial system destroy old erythrocytes. These cells are located in the spleen, liver, lymph nodes and bone marrow. These cells (histiocytes and macrophages) ingest particulate matter brought into contact with them.

Leukocytes: The white blood cells are much less numerous than erythrocytes in the circulating blood. There are approximately 1,300 erythrocytes to every leukocyte in the blood stream of goats. Some leukocytes are classified as granulocytes: these cells have specific granules in their cytoplasm, according to their staining reactions they are neutrophils, eosinophils or basophils. The agranulocytes are lymphocytes and monocytes.

Granulocytes, Neutrophils: These have abundant finely granular cytoplasm, and the granules stain with neutral dyes. The nucleus is divided into lobes connected by filaments. Neutrophils show ameboid activity and are active in phagocytosis to defend the body against infection or foreign matter. Neutrophils are formed in the bone marrow from extravascular neutrophilic myelocytes. They enter the circulatory system through their ameboid action.

Eosinophils: These are large cells containing numerous large cytoplasmic granules that stain with acid dyes. The nucleus is less lobulated than those of neutrophils. They are manufactured in the bone marrow and are very motile and slightly phagocytic. In allergic conditions, anaphylactic shock and certain parasitism they increase in number greatly. They participate in detoxification processes where histamine has been released. Where there is an antigen-antibody reaction they appear in large numbers around the area of infection.

Basophils: These have water-soluble cytoplasmic granules that stain with alkaline dyes. They originate in bone marrow. Basophils produce heparin to prevent coagulation of blood and histamine to attract the eosinophils. They occur in normal blood only in small quantities and phagocytic power is slight or absent.

Agranulocytes Lymphocytes: These are known to be the center of immune system. There are two classes: B lymphocytes (Bursa) and T lymphocytes (Thymus). The T cells are found in the peripheral blood and in the deep cortical areas and paracortical sinuses of lymph nodes. They are associated with cell-mediated immunity. The B cells were originally identified in the Bursa of Fabricius of the chicken. They are found in the peripheral blood and germinal center of lymph nodes. The B cells respond to antigenic stimuli, with the proliferation of plasma cells which produce specific antibody or immunoglobulin.

Monocytes: These are manufactured in the reticuloendothelial system in the spleen and bone marrow. They are relatively large with a single nucleus with faintly granular cytoplasm. They are motile and phagocytic. Monocytes have enzymes that are designed to digest tissue debris from chronic inflammatory reactions.

Platelets: These are small colourless rod or round shaped bodies. They are formed in bone marrow and they originate from megakaryocyte. They are numerous in the circulating blood. Their main function is in blood clotting or haemostasis. When a blood vessel has ruptured they accumulate at the site of injury. This aggregation process also requires calcium, fibrinogen and energy to form a haemostatic plug of platelets to seal the vessel.

1.3.2 The Biochemical Parameters

1.3.2.1 Proteins

Food proteins are hydrolysed to peptides and amino acids by rumen micro-organisms. Some of the amino acids are degraded further to organic acids, ammonia and carbon-dioxide. The ammonia produced together with small peptides and free amino acids is utilised by rumen organisms to synthesise microbial proteins. When the organisms are carried through to the abomasum and small intestine their cell proteins are digested and absorbed. Bacteria are capable of synthesising amino acids thus rendering the host independent of dietary supplies of amino acids.

The ammonia in rumen liquor is the key intermediate in the microbial degradation and synthesis of protein. If the diet is deficient in protein or if protein resists degradation, the concentration of rumen ammonia will be low and the growth of rumen organisms will be slow and hence the breakdown of carbohydrates will be retarded (McDonald et al 1988). If protein degradation proceeds more rapidly than synthesis, ammonia will accumulate in rumen liquor and the optimum concentration will be exceeded. When this happens ammonia will be absorbed into the blood, carried to the liver and converted to urea. Some of the urea may be returned to the rumen via the saliva and also through the rumen wall, but the greater part is excreted in the urine and is wasted.

The rumen microbes have a balancing effect on the protein supply of the ruminant. They supplement, both quantitatively and qualitatively, the protein of foods such as low-quality roughages but have a deleterious effect on protein-rich concentrates.

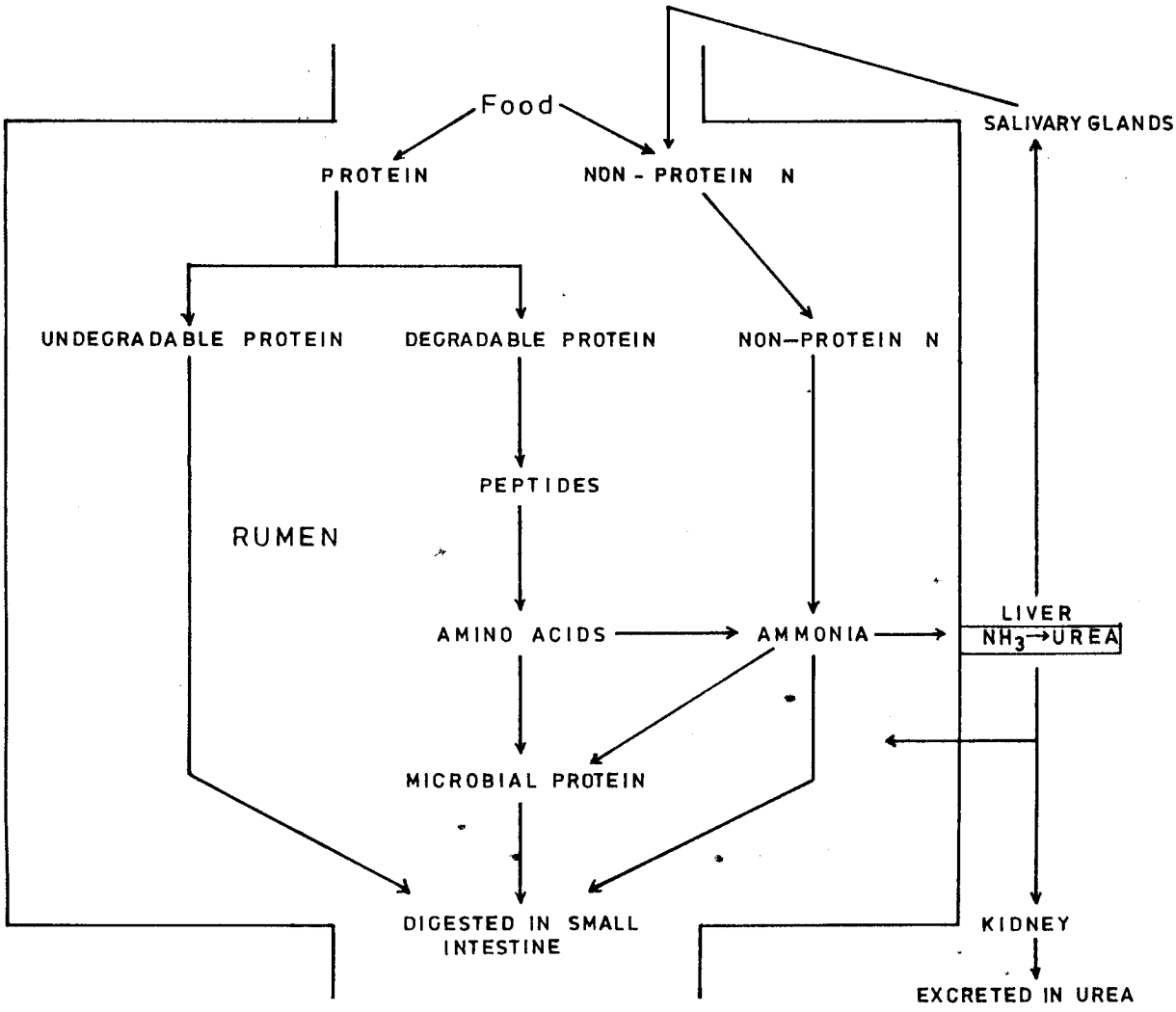
Non-protein nitrogen compounds in ruminant foods may be in the form of simple organic compounds such as amino acids, amides an amines or of inorganic compounds such as nitrates. Most of these are degraded in the rumen and their nitrogen enters the ammonia pool. The rumen micro-organisms are able to convert non-protein nitrogenous compounds to protein. Urea entering th rumen is hydrolysed to ammonia by bacterial urease. For this ammonia to be efficiently incorporated into microbial protein, the initial ammonia concentration must be below the optimum and the micro-organisms must have a readily available source of energy for protein synthesis.

1.3.2.1.1 Blood Proteins

Proteins constitute the major part of the dissolved solids of blood plasma and play a crucial role in all biological processe of the body. Their total concentration is between 65-75 g/l. They comprise a mixture of simple and conjugated proteins including lipo-proteins and gluco-proteins. The plasma protein are identified as albumin, fibrinogen and globulin fractions. When the blood is allowed to clot, several plasma proteins contribute in forming the matrix of the clot. The resulting solution is known as serum. The concentrations of some of thes blood proteins change markedly in some diseased states.

FIG. 1.3.2.1

DIGESTION AND METABOLISM OF NITROGENOUS COMPOUNDS IN THE RUMEN



A. Albumin: This is the most abundant protein in the plasma and is manufactured in the liver. Albumin is a globular protein consisting of a single polypeptide chain and has a molecular weight of about 66,300. The functions of albumin are to maintain the colloid osmotic pressure of the blood and to transport small molecules. Since proteins are colloidal and non-diffusable the osmotic pressure produced by albumins opposes the hydrostatic blood pressure in the capillaries and thus prevents excess passage of fluid into the tissues which may cause oedema. Osmotic pressure is the main force that draws interstitial fluid back into the capillary at its venous end. Albumin acts as a carrier molecule for bilirubin, fatty acids, trace elements, many drugs, hormones and vitamins. Albumin constitutes 55% of the total plasma protein.

B. Fibrinogen: This is the precursor of fibrin, the main substance of the blood clot. It is a highly elongated fibrous protein with a molecular weight of between 350,000 - 450,000. It constitutes 4-6% of the total proteins of the plasma. Fibrinogen is manufactured in the liver.

C. Globulins: Globulins are proteins that are insoluble in salt water. The serum globulins are manufactured in the liver except for a few like the gamma globulins which are formed extrahepatically in the lymph nodes and in other cells of the reticuloendothelial system.

Globulin fractions are divided electrophoretically into four components alpha $_1$, alpha $_2$, beta, and gamma globulins.

Alpha 1 Globulins are glyco-proteins and high density lipo-proteins. The main alpha 1-antitrypsin has a MW of 45,000. This protein inhibits the action of many proteolytic enzymes e.g. trypsin, chymotrypsin and plasmin. The lack of this trypsin-inhibiting activity allows the destruction of lung tissue because Alpha 1 antitrypsin inhibits the action of elastase and other lung proteases.

Alpha 2 Globulins: These include haptoglobin, which binds with any haemoglobin that has been released into the plasma after the red blood cells have been destroyed. It thus prevents the loss of haemoglobin and thus iron in the urine. Ceruloplasmin is the copper transport protein. Prothrombin is a proenzyme that is involved in blood coagulation. The molecular weight of alpha 2 globulins are around 20,000.

Beta Globulins: The major globulins are transferrin and low density lipo-proteins. Transferrin has a MW of 90,000 and is a protein responsible for transporting iron in the plasma. It has an iron-binding capacity of between 20-33% saturation with iron.

Gamma Globulins: These are made up of immuno-globulins or antibodies. The MW is between 53,000 - 75,000 and there are several fractions. IgG and IgA are found in larger quantities, IgM, IgD and IgE are present in smaller amounts. These five classes are heterogenous, each class is made up of hundreds or thousands of individual immunoglobulins. The synthesis of a specific immunoglubulin is stimulated by an antigen: a protein or a complex carbohydrate that is foreign to the species or

individual. The newly synthesised immunoglobulin has the property of recognising the antigen that stimulated its synthesis and combining with it. This antigen-antibody interaction occurs through specific non-covalent bonds. This mechanism is marked for elimination from the body using the protein complement which attaches to the Ig and is later recognised by phagocytes.

1.4 The Immunology of Coccidia Infection

Animals tend to develop immunity to coccidia after an initial infection. This immunity is specific for the species of coccidia to which the animal has been exposed (Ruff & Reid 1977). Coccidiosis is believed to be a disease of young animals due to their lower resistance to the parasite. With older animals which have built resistance due to early exposure to the parasite the disease is not as prevalent, and certainly not as serious. Rose and Co-workers (1984) determined that the induction of immunity to infection with coccidia was influenced by the parasite, the host, the method of administration of the parasite, and the differences in immunogenicity between different stages of the life cycle. The sexual stages are poorer in inducing immunity than the asexual stages which are the most effective (Rose, 1978).

Work done by Prowse and Pallister (1989) suggested that immunity to coccidiosis in chickens is dependent upon a T-cell response. Lymphocytes from chickens which were immune to infection with coccidia secreted interferon when appropriately infected with antigens from E. tenella. Recent experimental work by Lillehoj (1986) suggested that cell-mediated immunity is the predominant

mechanism involved in protecting the host against reinfection. The development of an antigen specific T-cell response elicited against the Eimerian parasites during the course of infection suggested that the T-lymphocytes released soluble substances which controlled the development of Eimeria spp.

Vaccination against coccidiosis in chickens is being studied as it provides a natural form of control with no drug residue problems, toxicity to the bird or consumer or build-up of resistance. The life cycle of Eimerian parasites is self limiting, that is, one oocyst can give rise to a number of progeny which can only develop further and reinfect the host if they undergo sporulation within the external environment and are ingested (Parry, 1989). Oral immunisation is the most effective method of stimulating protective immunity against enteric parasites, though a single immune mechanism would not provide total protection from the challenge. In order to provide total protection, application of live vaccines with proper methods is probably necessary. Joyner and Norton (1973) described the "Trickle Dosing of Oocyst," where chicks were given a few viable oocysts orally daily (5 oocysts per day per chick) for up to 28 days. This proved to give a bird solid immunity to challenge as long as the floor was cleaned daily.

Due to the complexity of the Eimerian life cycle it is desirable to provide vaccines and immunisation strategies to stimulate the protective response which has naturally evolved in the

host-parasite relationship; these are known to provide solid protection from challenge (Parry, 1989).

1.4.1 Anti-Parasitic Drugs

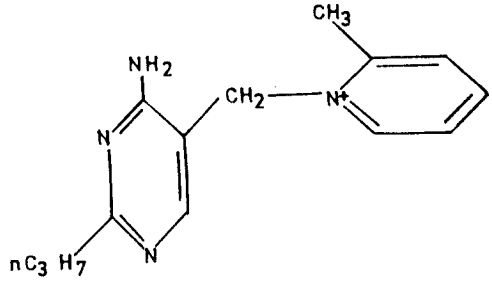
The treatment and control of coccidiosis is a major problem facing goat, sheep and chicken farmers. There are many anticoccidial drugs available but most of them are of limited value due to resistance developed by the parasite. Prevention of the disease requires that the drugs be given either continuously in the feed or water or in the very early stages of infection. This is because many of these drugs are effective against the early developmental stages of coccidia (Anon, 1988).

A lot of work is being carried out on the improvement of old drugs which have been on the market for a long time. New drugs are being tried as well. Researchers are looking for a drug that can prevent coccidiosis by inhibiting the biological cycle of coccidia without many side effects to the animal. Extensive study of biochemical modes of action of antimicrobial drugs has led to the identification of areas of metabolism as targets for drug action including: energy metabolism, membrane function, cofactor synthesis, nucleic acid synthesis, protein synthesis and cell wall synthesis (Gutteridge & Coombs, 1977).

Amprolium

It is suggested that amprolium is a drug which interferes with cofactor uptake. The drug is a structural analogue of the vitamin thiamine.

AMPROLIUM



THIAMINE

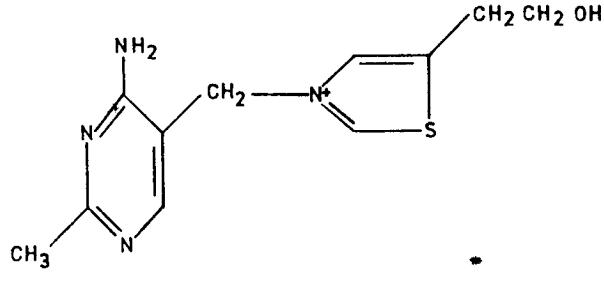


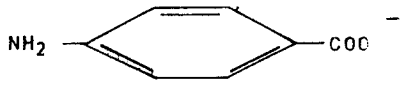
FIG.1.4.1.1 Chemical similarity of the vitamin thiamine to the anticoccidial drug amprolium.

Thiamine pyrophosphate is a cofactor of a number of decarboxylase enzymes and plays a vital role in intermediary metabolism. Amprolium lacks the hydroxyethyl group so it cannot be pyrophosphorylated and thus it is assumed that it does not inhibit at the coenzyme level. It acts by competitively inhibiting the uptake of thiamine by the parasite.

Sulphonamides

It is known that these act by blocking the synthesis of tetrahydrofolate, (fig. 1.4.2.2b) a cofactor required for many cellular methylation reactions. Inhibition is thought to occur at the dihydropteroate synthetase reaction during which a pteridine moiety and p-aminobenzoic acid are conjugated.

p-Aminobenzoic Acid

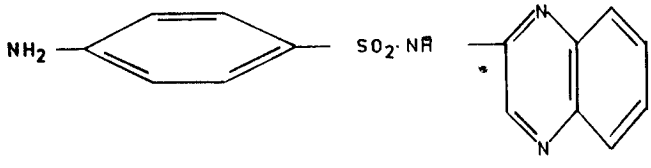


p-Aminobenzoic acid

Sulphanilamide

Sulphanilamide

Sulphaquinoxaline



Ethopabate

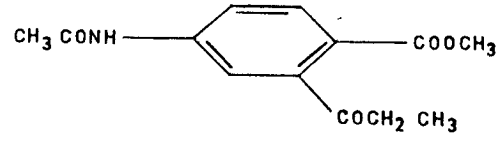
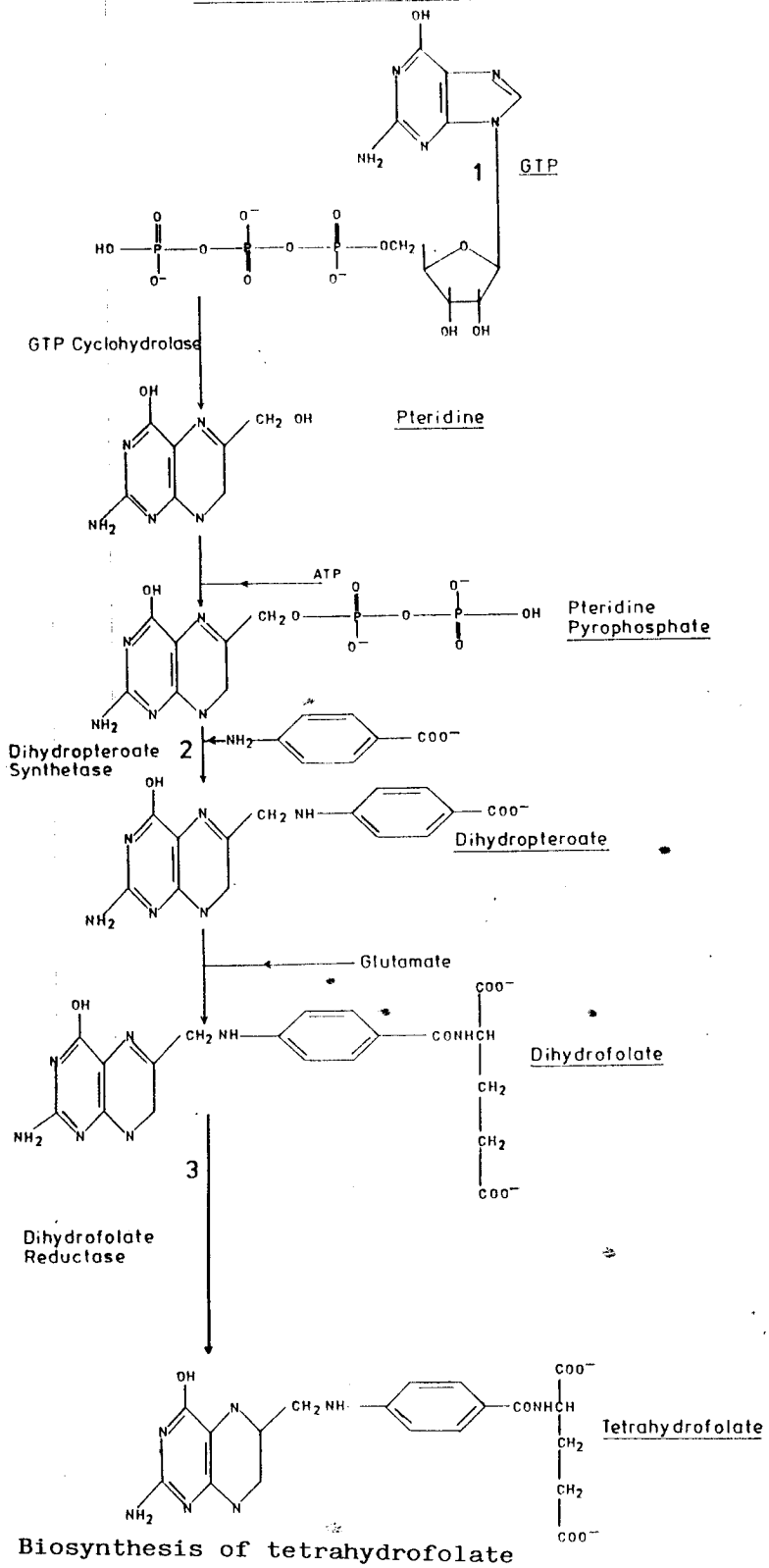


FIG.1.4.2.2 Chemical similarity of the growth factor P-aminobenzoic acid to sulphonamides.

The sulphonamides are not only used in the chemotherapy of the protozoan diseases coccidiosis, toxoplasmosis and malaria but also in the chemotherapy of bacterial diseases.

FIG.1.4.1.2b BIOSYNTHESIS OF TETRAHYDROFOLATE



Biosynthesis of tetrahydrofolate

Enzymes 1, GTP cydohydrolase
2, Dihydropteroate synthetase
3, Dihydrofolate reductase

Polyether Ionophores

This group include drugs such as monensin, salinomycin, lasalocid which have been used for several years. The drugs are fermentation products of Streptomyces species and their effect is on stimulating energy linked transport in mitochondria of the parasite in Eimeria sporozoites and a more static effect on schizont replication (Chappel, 1979). It has also been shown that monensin can interact with alkaline metal cations and specifically inhibit the transport of K+ into liver mitochondria (Gutteridge and Coombs 1977).

Monensin

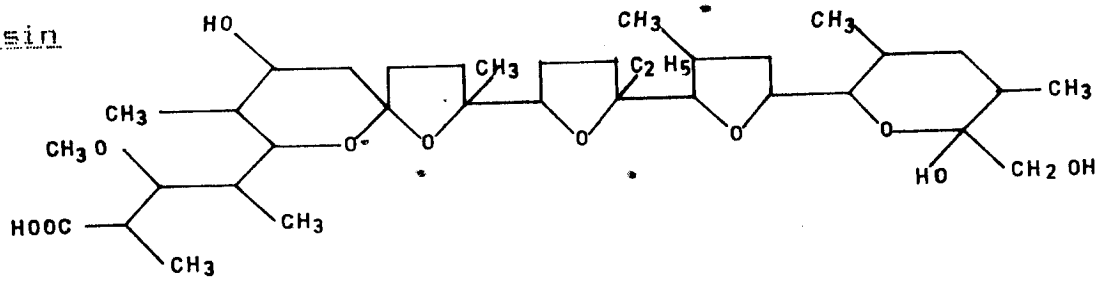


FIG.1.4.1.3 Chemical structure of monensin.

Many of the drugs mentioned above have their own disadvantages. Sulpha drugs only act against certain stages of the parasite and are not always highly effective. Overdosing of dehydrated kids with sulpha drugs can cause kidney failure (Anon, 1988). Amprolium if used for a long time can predispose to polioencephalomalacia. On-going field coccidia isolate sensitivity testing by Wilson et al (1988) and McDougald (1988) has shown significant reduction in the efficacy of monensin and salinomycin.

Many reports have been given of clinical and subclinical coccidiosis occurring as a result of insufficiently effective medication with conventional anticoccidials (Chapman, 1987). Thus effective therapeutic agents from new classes of chemicals compounds are recommended as supplements to current treatments or as alternatives to them (Mundt & Haberkorn, 1989). Such new drugs must fulfill the following criteria: high level of efficacy against all Eimeria spp, a coccidiocidal effect on as many stages of development cycle as possible, and an unimpeded immune response in the host after treatment of the coccidial infection with therapeutic dosages.

New drugs are being introduced on the market such as Toltrazuril It is a chemically synthesised substance from the symmetrical triazintrione class of compounds. Its mode of action is coccidiocidal against all intracellular stages of development of the Eimeria species (Braunius, 1987) (Haberkorn 1984, 1989). This drug has been tried on many animal species including poultry and small ruminants.

Toltrazuril

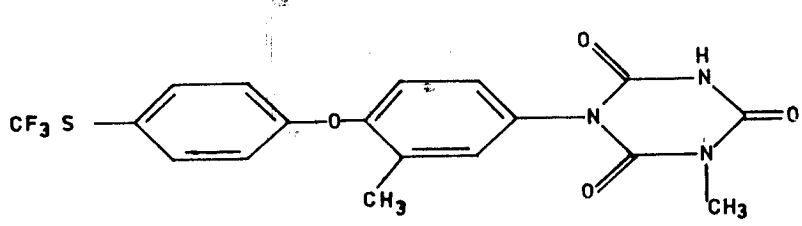


FIG.1.4.1.4 A new anticoccidial drug

Decoquinate is a quinoline derivative. It was brought onto the market in 1987 as a prophylaxis in calves and lambs by Rhone Poulene and May and Baker. The drug has a coccidiostatic effect and not a coccidiocidal mode of action, this means a permanent longterm administration is essential for it to be effective. Just like any other quinoline drug it is believed to block the respiratory chain of coccidia at a point near to cytochrome b. Gutteridge and Coombs (1977) suggest that the drug may block the development of the parasite at schizogony. This point can be clarified if preparations of progamonts and schizonts are isolated and studied biochemically.

Decoquinate

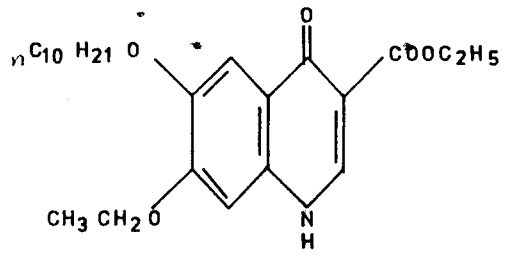


FIG.1.4.1.5 Chemical structure of anticoccidial quinolone

A study on the prophylactic efficacy was assessed by Spelman et al (1989) in U.K. under differing natural challenge conditions, from extensive late-lambing ewes at pasture to intensively housed early-lambing flocks. The results showed that faecal oocyst output from decoquinate medicated lambs was rapidly and significantly reduced to very low numbers in all of the trials and in 5 trials, live-weight gains were improved compared with the controls.

Another new drug is diclazuril which is believed to be a broad spectrum anticoccidial belonging to the benzeneacetonitrile group. Stenorol (halofuginone hydrobromide) is a chemical which was introduced to the United States broiler industry in 1985 (Kling et al 1989). It has been used as a shuttling chemical with ionophore coccidiostats to provide optimum protection against coccidiosis.

Research work on preyention and therapy of coccidiosis on all livestock is still on going.

1.5 The Objectives of the Coccidia Study

Zambia is a developing country with limited resources for the improvement of agriculture. A study was carried out on coccidiosis in Zambian goats with the aims of:

1. To study coccidia in Zambian goats, especially the incidence and the life cycle of Eimeria species.
2. To study the progress of the disease of coccidiosis in goat kids, looking at the clinical symptoms, biochemical and haematological parameters and faecal oocyst counts.
3. To observe the effect of the curative drug amprolium on the life cycle of the parasite.

CHAPTER TWO

MATERIAL AND METHODS

CHAPTER II

MATERIALS AND METHODS

2.1 SAMPLING AND METHODOLOGY

2.1.1 Preliminary Sampling

Feacal samples were collected from goats herded at the School of Agriculture, kept in closed animal accommodation in the School of Veterinary Medicine and kept at free range in a village in Chongwe 60 km East of Lusaka.

2.1.2 Isolation of Ten Kids

In October 1987, a set of five healthy goat kids varying from 12-20 weeks old, were weaned and confined in a ventilated room which had been previously sterilised. The goats had been naturally infected from their mothers and the infection was maintained by not cleaning the room regularly (i.e. once a week only) to promote development of the clinical disease, (fig 2.1.1). Initially, faecal and blood sampling, temperature, weight and clinical examinations were done weekly and data recorded. In the final stages when the animals' condition worsened due to disease (i.e. 4 days before the animal was sacrificed) temperature, weight and clinical observation were done daily.

In January 1988 another set of five healthy goat kids varying from 5.5 to 16 weeks were weaned from their mothers. They were placed in the same room which had been previously sterilised. One goat, number 1, from the previous set was used as a source of infection to the other kids. The conditions were the same as above. These were very young goats compared to the first set because the experiment needed goats with little resistance in order to see the complete life cycle.

Fig 2.1.1 Goat kids under confinement.

In this experiment it was not possible to isolate all goats of the same age at the same time, so the sets carried goats of different ages. When symptoms of coccidiosis developed, five of the total of ten goats were treated with amprolium and the rest were not.

2.1.3 Survey

A small survey was carried out on three farms each with different types of management. Two were commercial farms and the other was under village management.

Grasmere is a commercial farm with high management, i.e. breeding is not indiscriminate, tests are done on the male sperm count, size of scrotum etc, and the female is checked to determine whether it is healthy and for its past performance, cross breeding too is practised. Animals are dewormed regularly, supplementary feed is also given.

Dans Farm has middle management, i.e. breeding is indiscriminate, (i.e. no cross-breeding,) supplementary feed would be given if the need arose, that is during winter and also if there is drought. Animals are treated when sick, and dewormed regularly.

Luangwa district has a large number of goats under village management where the animals are not under proper supervision; they free-graze, rarely have any medical treatment and indiscriminate breeding is allowed.

Faecal samples were collected once from twenty goats in each farm during the dry season and once during the wet season; Luangwa in December, Dans and Glasmere Farms in February. This was done to check the prevalence of coccodia.

2.2 MANAGEMENT

(a) Experimental

The ten goat kids were kept in a room in the Ruminant Animal Accommodation Area. Bedding, made of straw, was provided and changed every other day. The room was cleaned once a week. The goats were fed twice daily on dairy meal and hay. Dairy meal was composed of 5% crude oil, 16% crude protein, 8.7% crude fibre with the rest starch, and was bought from the National Milling Company, Lusaka. The meal was put in an open trough for the goats to feed from. Drinking water was provided in an open container. For identification purposes the goats were ear marked 1, 2, 3, 4, 5, 6, 7, 8, 9, 10. Every morning, including week-ends, the goats were checked for any clinical signs associated with coccidiosis.

(b) Preliminary Survey

The goats in the School of Agriculture were kept in paddocks and were left to graze in the School grounds. In the commercial farms, e.g. Grasmere farm, goats were kept in three different pens, the kids in one, adults in the second and those still suckling in the third with their mothers. They were let out of the pens to the paddocks early in the mornings (7:00 hours) and they were brought back at midday to be fed on mollasses, husks and dry stocks of maize. This supplementary feed was provided during dry season, otherwise animals would be brought back from the paddock at 18:00 hours. Under village management there was no supplementary feed, the goats were let out of the pens at

sunrise into open land to graze till sunset. Goats slept in thatched huts with wooden walls and raised slatted floors, which allowed the faeces to drop on the ground for easy cleaning.

2.2.1 Clinical Observations

The general clinical signs were observed such as eating habits, the colour and state of stool, the condition and colour of the coat and the state of the genital parts.

2.2.2 Temperature

The determination of temperature was done using a short blunt bulb clinical thermometer. After shaking the mercury the bulb end of the thermometer was lubricated with water and gently inserted with a rotary action through the anal sphincter and left for 3 minutes before reading. The thermometer was later rinsed in water and wiped with cotton wool.

2.2.3 Weight

The animals were weighed weekly on a FHK 30-3 platform scale for animals. When the disease got worse, weight was recorded daily until the animal was sacrificed. The attendant was weighed with the animal and the weight recorded. Later the weight of the attendant was recorded. The weight of the animal was calculated by subtracting the weight of the individual from the weight of individual and goat.

2.3 Parasitology

2.3.1 Collection of Faecal Samples

Faecal collection was done in the morning soon after the goats were fed. The plastic bag had to be wetted to reduce friction during entry into the rectum. One finger was used for penetration since the size of the anus was still small. The plastic bag was very thin and was worn as a glove. One finger was inserted into the rectum to collect the droppings. After collection the gloye was turned inside out and then served as a receiver. It was carefully tied and labelled (date, goat number) and the samples were taken to the laboratory for further analysis.

2.3.2 Treatment of the Faecal Samples

The Flotation Method (HMSO, 1984, Soulsby 1982) was used, which is based on the theory that oocysts and eggs will float in certain solutions in which a preparation of faecal debris will not. The higher the specific gravity of the solution the more oocysts will be recovered; though a higher tonicity will have a damaging effect on the oocysts.

Solutions were used at saturation point. Sodium chloride can be used but has a disadvantage in that it forms crystals as it dries and it has not got the sticking effect. Its specific gravity is normally 1.2 but varies with temperature. In this case sucrose was used.

2.3.3 Preparation of Sheathers Solution

Sucrose has a specific gravity of between 1.117 - 1.300. 430 g of sucrose was dissolved in 400 ml of distilled water using a magnetic stirrer and phenol (6.6 ml) was added. The solution was cooled and labelled.

2.3.4 Flotation Method

Faecal sample was weighed as a 1.0 g portion and 12 ml of water was added and mixed thoroughly using a pestle to break up the faeces. The debris was strained with a tea strainer and the oocysts and eggs allowed to pass through with the filtrate. The filtrate was divided into two 15 ml centrifuge tubes and centrifuged at 1200 rpm for 10 minutes. The supernatant fluid was discarded. 2.0 ml of Sheathers solution was added to all tubes and using an electric stirrer the deposites were loosened and thoroughly mixed. 10.0 ml of Sheathers solution was added to fill the tubes and centifugation repeated at 1200 rpm for 10 minutes. Cover-slips of size 18 x 18 mm were placed on top of the centrifuge tubes and left to stand for 1 - 2 hours. The oocysts attaching to the cover slip were examined under the microscope. Oocysts were counted using a hand counter and identification of species was attempted to see which species occurred more frequently than others. Identification of species was attempted using size and appearance of oocyst (Soulsby, 1982, Norton, 1986).

2.3.5 Sacrificing the Animal and Autopsy

Death of the animal was produced by bleeding (exsanguination) through the left common carotid artery (Kelly, 1984). The artery was first identified then tied at two points about

2 cm between the knots. Using a scapel blade, a small opening was cut and a catheter tube of 2 meters long was inserted through the cut. The knots were tied and the animal was allowe to bleed to death.

Dissection through the abdomen was done exposing the intestines The intestines were taken out of the abdomen and were cut according to their anatomical structures. The duodenum, which was 1 meter long was cut, beginning at the pylorus and terminating at the duodenojejunal flexure (Habel 1975). The jejunum formed numerous close coils arranged like a chain makin a curve around the border of the mesentery. Just before it joined the ileum, it is prolonged by a U-shaped series of loops on an extension of the mesentery. The ileum is the terminal part of the small intestine. Its anterior part is adhered to the caecum and colon obliquely on the surface. Using naked eyes, or at times a hand lens, portions were examined for coccidial lesions. The lesions were identified as haemorrhagic foldings, rosettes, nodules and white spots. These were designed as A, B, C, D, E respectively. For further identification of these lesions a Schott stereo microscope (model No KL 1500) was used.

The sections were immediately fixed in 10% formal-saline. Bottles were labelled accordingly (i.e. type of lesions, the number of the goat, date and whether the tissues were for makin smears or for histopathology.

2.4 Histology

Smears Three methods were used to make smears for observations under the microscope.

Wet Smears A slide and cover slip were thoroughly cleaned to make sure that they were grease-free. A drop of water was placed on the centre of the slide. Using a scapel blade, surface scrapings were obtained from the infected area of the intestine. This was transferred to a clean slide with a drop of water and gently mixed to form a smear. To achieve the correct thickness of the smear more water may be added as it would be difficult to distinguish the gametocytes if the smear was too thick. The slide was either left to dry for staining or examined under a phase contrast microscope (Olympus Research Microscope Model No. BH2).

Pressed Tissue A piece of infected intestine epithelia tissue was scraped off the intestine onto a clean slide and pressed between two slides for examination.

Washed Parasites The tissue sections were swirled in normal saline for 10 minutes to dislodge any parasites that may be on the walls of the intestines. The suspension collected was made into thin films and examined under a light microscope (Nikon SE Model No. 123 MB - 1).

2.4.1 Preparation of Histological Sections

2.4.1.1 Fixation

The sections were fixed for two weeks in 10% formal-saline. These sections were trimmed into smaller sizes and were placed in tissue cassettes according to the type of lesion. The cassettes were also labelled and left in the fixative in readiness for tissue processing (Archenhold et al, 1978).

2.4.1.2 Tissue Processing

The tissues were removed from the fixative and placed into a perforated basket that was mounted on the hood for processing. A Sakura Finetechnica tissue processor was used (Model No. RH12-EP). It prepares tissue specimens in a preserved state for cutting, staining and microscopic study. The movable hood rises, moves sideways, transports and lowers the basket through a series of ten glass beakers containing alcohol at 50%, 70%, 90% and 100% twice each. The alcohol is used to dehydrate the tissue. Then the tissue goes through xylene twice for clearing (dealcoholism) and finally twice through wax jars with a thermostat on them to keep the temperature of wax at 60 ^{o}C. The wax is for impreganation into the tissue (to make the tissue firm). The basket is left in each jar for 2 hours and the total time is 20 hours (Archenhold et al, 1978)..

2.4.1.3 Embedding

The tissue was further trimmed for embedding. The lid of the cassette was removed to expose the processed and trimmed tissue and a base mould taken of the right size. Paraffin wax at

60 °C was added to the base mould and placed over a warming area. The specimen was oriented in such a way that the side to be cut faced down. More paraffin wax was added and the cassette base was placed on top of the base mould dispensing additional paraffin. This was later placed in a refrigerator at 4 °C to cool. After 10 minutes, the embedded block was easily released from the base mould (Archenhold et al, 1978).

2.4.1.4 Cutting

The base of the cassette with the embedded tissue was placed on a Yamato Koki rotary microtome (Cat No. 840706). Sections were 0.04 cm^3 thick. The cut sections, were placed in a Electrothermal Paraffin Section Mounting Bath (model No. MH8514/B) at 30 °C, then later transferred onto a slide. The slides were placed in a slide rack to allow the water to drip off. These were then placed in a Sakura Paraffin Oven (Model No. PM-400) for 45-60 minutes for dewaxing.

2.4.1.5 Staining

The cut sections were stained in Mayer's Haemotoxylin and Eosin Stain. Haemotoxylin is an aqueous nuclear stain. It was prepared by dissolving 1.0 g of haemotoxylin in 1 litre distilled water. Then 50.0 g potassium aluminium (VI) sulphate 12H 0, 0.2 g sodium iodate (v), 1.0 g citric acid and 50.0g $_2$ chloral hydrate were added, and these were dissolved by stirring and warming. The solution was cooled and filtered (Gurr, 1979).

Eosin was used as a counterstain to stain the cytoplasm. 1.0 g of Eosin Y was dissolved in 100 ml of distilled water and a

crystal of thymol was added to inhibit the growth of fungi. Acetic acid ($0.5\ cm^3$) was added to sharpen the staining. The sections were stained using the following procedure (Archenhold et al 1978):

Placed in Xylene I for 2 minutes

Placed in Xylene II for 2 minutes

Placed in absolute alcohol I for 2 minutes

Placed in absolute alcohol II for 2 minutes

Placed in 90% alcohol for 2 minutes

Placed in 80% alcohol for 2 minutes

Placed in 70% alcohol for 2 minutes

Rinsed in tap water for 30 seconds to remove excess alcohol

Placed in Mayer's Haematoxylin for 5-8 minutes for progressive staining

Placed in 1% acid alcohol for 5-10 seconds - differentiation of tissue (1% HCl and 70% alcohol). Washed in tap water for 5 minutes to deepen the blue colour.

Placed in Eosin for 3 minutes - counterstain

Placed in 70% alcohol 30 seconds

Placed in 80% alcohol 30 minutes

Placed in 90% alcohol 30 seconds - dehydration

Placed in absolute alcohol I 1 minute

Placed in absolute alcohol II 1 minute

Placed in Xylene I for 2 minutes

Placed in xylene II for 2 minutes