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**COCCIDIA (PROTOZOA: APICOMPLEXA) OF THE DOMESTICATED
GOAT *CAPRA HIRCUS* IN NEW ZEALAND**

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF PHILOSOPHY IN VETERINARY SCIENCE AT MASSEY
UNIVERSITY

AYE KYAWT SOE
SEPTEMBER, 1989

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ABSTRACT

The literature on the history of the identification of *Eimeria* species infecting domesticated goats, and their morphological characteristics, the general life cycle of coccidia and epidemiology of infections, together with clinical signs, diagnosis and treatment is reviewed. In total 13 *Eimeria* species which are generally considered valid have been described from goats but relatively little has been published on their biology or significance.

Studies on the identification and seasonal prevalence of *Eimeria* species infecting domesticated goats were conducted on three farms in the Palmerston North area of New Zealand from August 1987 to August 1988. On one farm (Old West Road), 20 kids & 20 adult Saanen goats were sampled at 14 day intervals and monthly intervals, respectively; on a second farm (Ballantrae) 17 kids and 20 adult New Zealand 'feral' type were sampled at monthly intervals; on a third farm (Kimbolton) 23 Angora kids were sampled at 14 day intervals.

Faecal samples collected directly from the rectum were used for oocyst counts and to provide oocysts for sporulation for identification of species. More than 98% of the faecal samples from each group of kids and adults contained coccidial oocysts. Mixed infections were the rule, 59% of the faecal samples contained 6-8 species. The *Eimeria* species identified in this study were: ten previously described species - *E. christensenii*, *E. tunisiensis*, *E. jolchejevi* s.s., *E. arloingi*, *E. hirci*, *E. caprina*, *E. caprovina*, *E. apsheronica*, *E. ninakohlyakimovae* and *E. alijeji*; two others whose species status is uncertain - temporarily designated *E. jolchejevi* 'large form' and *E. hirci* 'small form'; three previously undescribed species - temporarily designated *E. n1*, *E. n2* and *E. n3*. The morphological characteristics of sporulated oocysts of the *Eimeria* species found in the present survey are described in detail and illustrated by microphotographs and schematic diagrams. Statistical analysis of oocyst and sporocyst dimensions of these species and, where necessary for differentiation of species, statistical comparisons are also given. In addition to these species, a single oocyst of *E. punctata* which was not recorded during the study period, was found later in a pooled sample kept as reference material.

E. jolchejevi 'large form' had many of the characteristics of *E. jolchejevi* as described in the literature but differed in size and shape from it. Comparisons of the large form with published data indicate that it represents a previously overlooked species infecting goats and that there may be an equivalent species in sheep. *E. hirci* oocysts observed did not differ from published descriptions but analysis showed that they were divisible into two distinct groups on the basis of size and shape. These may represent separate species but

further investigation is needed to verify this.

The species designated *E. n1*, *E. n2* and *E. n3* are shown to be clearly distinguishable from previously described species from goats. *E. n1* may represent the equivalent of *E. weybridgensis* from sheep but the other two species do not appear to have an ovine equivalent. Further work is needed to confirm their species status and investigate their biology.

Kids less than 1 year old had considerably higher oocyst counts than adults. Mean oocyst counts were at their highest from 2-6 months of age but they tended to decrease with time and from May on were at relatively low levels. The seasonal patterns of oocyst counts in the groups of kids differed between farms. This was associated with different management systems and breeds. The highest oocyst counts occurred in Saanen kids raised on milk-replacer in a heavily contaminated pen; the predominant species were *E. christenseni* and *E. arloingi*. Counts were lower in Angora kids reared on milk-replacer in pens that were cleaned daily and which opened onto a small paddock. Counts were lowest in 'feral' kids reared by natural suckling at pasture. In adults, on one farm there was no detectable seasonal trend in oocyst counts and on the other there was a tendency for mean counts to rise from December on. On both farms there were, in addition, some short-term fluctuations in mean counts with peaks associated with high counts in a few individuals.

Seasonal variations in the occurrence of individual *Eimeria* species were examined by considering the oocyst counts for each species, the percentage of the total oocyst counts represented and the prevalence of positive faecal samples. The mean percentages of total oocyst counts represented by each species over the whole year were compared. *E. arloingi* was found to be the most predominant species. Other species which were dominant in the coccidial population were *E. hirsi* and *E. n2*. The seasonal patterns differed between species although the patterns on the different farms were, on the whole, very similar. This indicates substantial differences between species in host-parasite relationships and it is suggested that these may chiefly involve the prolificacy and immunogenicity of the various species. Further work is needed to investigate this. All of the species recorded were found in all the groups of goats examined.

The sporulation of *E. christenseni* oocysts at various constant temperatures was examined. The log temperature:log development time relationship yielded a correlation coefficient of $r = -0.99$. The time required for 90 % of the oocysts to complete each development stage was taken as the endpoint. Sporulation was completed in 7 days at 27°C, in 10 days at 20°C, in 11 days at 15°C, 14 days at 10°C and in 32 days at 4°C.

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CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Coccidia are protozoa of the phylum Apicomplexa, members of which possess an apical complex (Fig.1.1) at some stage of their development. All members are parasitic. The phylum includes two classes, Sporozoasida and Perkinsorida. Sporozoasida produce spores or oocysts in their life cycle and are divided into two sub-classes, Gregarinasida and Coccidiasina. The former sub-class is chiefly parasitic in invertebrates and lower chordates. The latter are mostly intestinal parasites of vertebrates and are divided into three orders: Agamococcidiorida, Protococcidiorida and Eucoccidiorida. The first two orders contain a few parasites of marine annelids and the last contains four sub-orders: Adeleorina, Haemospororina, Piroplasmorina and Eimeriorina. The first three sub-orders are mostly blood parasites of vertebrates. In the sub-order Eimeriorina, the family Eimeriidae is represented by the two most common and important genera - *Eimeria* and *Isospora*, which are colloquially referred to as "coccidia".

The coccidia are generally highly host-specific and nearly all species develop in the intestinal tract in specific sites where they undergo asexual multiplication by shizogony before sexual reproduction. Sexual reproduction leads to oocyst production and sporogony mainly occurs outside the host. The sporulated oocyst is usually the infective stage and infection is acquired by ingestion. The oocyst is also of primary taxonomic importance as all species have been initially described on the basis of oocyst morphology. As will become apparent, this gave rise to considerable confusion over the identity of coccidia of sheep and goats. Nevertheless, oocyst morphology remains critical to the identification of species. Not only are shape and dimensions important but also a range of other characteristics. These are illustrated diagrammatically in Fig.1.2.

1.2 HISTORY

In 1901, coccidial oocysts were first described from sheep by Moussu & Marotel who subsequently named them *Coccidium faurei* (Moussu & Marotel, 1901). The oocysts lacked a polar cap and were 30-42 x 18-26 μm in size (cit. Lotze, 1953). Four years later, the first goat coccidian was found and named *Coccidium arloingi* by Marotel (1905) with the oocyst

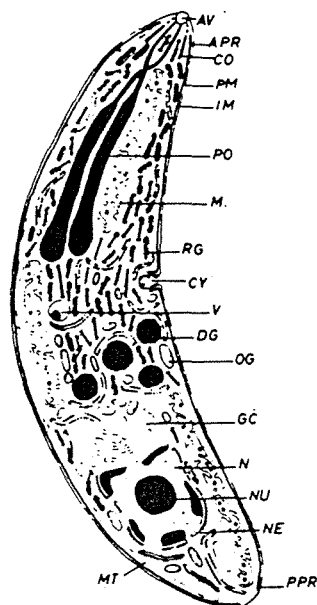


Fig. 1.1 A longitudinal section of coccidian merozoite showing the apical complex. APR = anterior polar ring, AV - anterior vesicle, CO = conoid, CY = micropore (cytosome), DG = dense granule, ER = endoplasmic reticulum, GC = Golgi complex, IM = inner membrane, M = mitochondrion, MT = subpellicular microtubule, N = nucleus, NE = nuclear envelope, NU = nucleolus, OG = ovoid granule, PM = plasma membrane, PO = rhoptry (paired organelle), PPR = posterior polar ring, RG = microneme (rod-shaped granule), V = vacuole. (From Andreassen and Behnke, 1968).

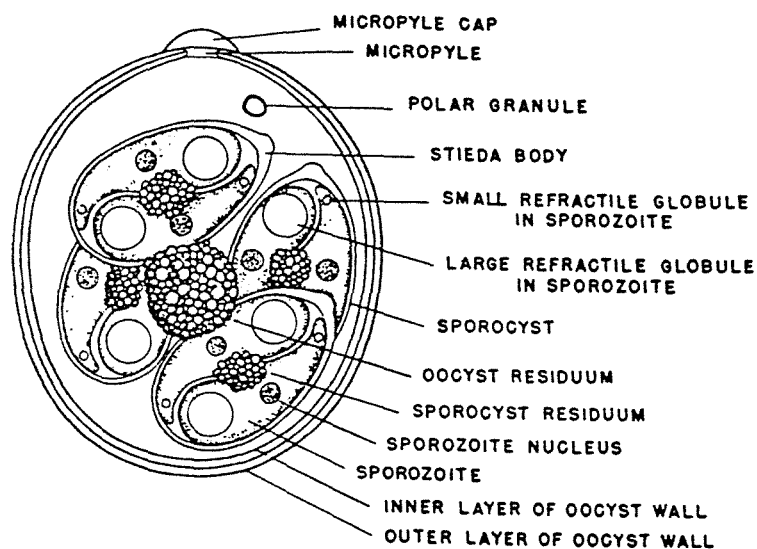


Fig. 1.2 A structure of sporulated *Eimeria* oocyst. (From Levine, 1986).

described as ellipsoidal, 25-27 x 16-18 μm and possessing a polar cap (cit. Lotze, 1953). Both names were amended to *Eimeria faurei* and *Eimeria arloingi* respectively in 1909 by Martin (cit. Norton, 1986). For many years thereafter, all coccidia of goats and sheep were referred to as *Eimeria arloingi* and *Eimeria faurei* respectively (Balozet, 1932).

The issue became somewhat confused when *E. arloingi*-like oocysts with a polar cap were recorded from sheep by several workers (Lerch, 1920 & 1921; Douwes 1920; Noller *et al.*, 1922; Muller, 1923 all cited by Lotze, 1953). Perhaps, they were dealing with the ovine counterpart of *E. arloingi*. However, they persisted with the previous practice and called it *E. faurei*. That there might be more than one species belonging to each of these hosts was not appreciated at the time.

About that time, Spiegl, 1925 (cit. Shah, 1963) described a large oocyst from domesticated sheep which he called *E. intricata*, with a polar cap, a size of 51 x 39 μm and a distinctive dark brown transversely striated wall (Shah, 1963). In 1929 Kotlan, Mocsy and Vajda found *E. parva* with an oocyst size of 11-14.3 x 9.5-11.8 μm , the smallest oocyst of any occurring in sheep (cit. Christensen, 1938).

Another species was reported from goats in 1930 by the Russian workers Yakimoff and Rastegaieff and named *E. ninakohlyakimovi* (amended to *E. ninakohlyakimovae* by Levine, 1961). They described their species' oocysts as ovoid, 18.9-25.4 x 14.4-21 μm , capless, usually with a micropyle and a distinctly double-contoured wall (cit. Christensen, 1938). Probably, the small size range and absence of polar cap were sufficient to convince them that it differed from *E. faurei* and *E. arloingi*, respectively.

Balozet (1932) examined the morphology of the coccidia of sheep and goats which had been described (*E. arloingi*, *E. faurei*, *E. intricata*, *E. parva*, *E. ninakohlyakimovae*) and found oocysts of all species in both hosts except for *E. intricata* which he found only in sheep. He concluded that *E. arloingi* and *E. faurei* are distinct species occurring in both hosts. Consequently, the earlier view which assumed that *E. faurei* is specific to the sheep and *E. arloingi* to the goat, was superseded by a widespread assumption that sheep and goats harbour the same species.

This view became widely accepted without proper verification. This was doubtless encouraged by the fact that many of the oocysts from sheep and goats are morphologically similar although there was no good evidence that the species with similar oocysts are identical. Little was known of the particularly strong host-specificity of *Eimeria* species, and few cross-transmission experiments had been carried out although as early as 1932,

Balozet attempted cross-transmission of infection with *E. ninakohlyakimovi* from a goat to a lamb. It failed and the reason given was that the lamb was too old (cit.Becker, 1934). Apparently he did not consider the possibility that sheep and goats could harbour different species with similar morphology.

Over the next few years, further species were described from sheep, *E. granulosa* with its characteristic pyriform oocysts and *E. pallida* with small ellipsoid oocysts which was differentiated from *E. parva* by small differences in oocyst size, shape and appearance (Christensen, 1938). In 1942, mainly on the basis of size, Honess separated a large form, 30-40x21-30 μm (mean 33x24 μm) and a small form 17-23x17-22 μm (mean 22x19) of *E.arloingi*-like oocysts from Rocky Mountain Bighorn sheep *Ovis canadensis* and also from domestic sheep *Ovis aries* and named them *E. ahsata* and *E. crandallis*, respectively.

The description of *E. arloingi* from sheep given by Christensen (1938) has an extraordinary wide range of oocyst sizes and also Honess (1942) said that *E. ahsata* resembled the larger forms of *E. arloingi* from domesticated sheep. The idea that *E. arloingi*, as then understood, could include more than one species was not immediately accepted. In part, the difficulties arose from the inadequate descriptions of the oocysts. For example, the characteristics of the sporocysts of *E. crandallis*, which clearly separate it from larger forms of '*E. arloingi*', were not mentioned by Honess. Because size was also the main criterion being used to distinguish these species, Lotze (1953) considered that it was necessary to re-examine their status. However, the problem was solved when Smith *et al.*, (1960) and Smith & Davies (1961) rediscovered *E. ahsata* and *E. crandallis* in sheep in Alabama and their identification was confirmed by Honess who had originally described both species, confirming that they were different from *E. arloingi* (now known as *E. bakuensis* in sheep).

Landers (1952) described a small and rare species, *E. honessi*, from sheep, with a multi-layered and a peculiar punctate wall. However, since this name was preoccupied by *E. honessi* Carvalho, 1943, Landers later (1955) renamed it *E. punctata*. Shah (1963), Joyner *et al.* (1966) and McKenna (1972) have all questioned its validity in sheep because of its scanty distribution. Chevalier (1966) first described this species in goats. Levine & Ivens (1970) stated that further study is needed to determine whether *E. punctata* occurs in sheep and goats.

In 1962, Levine *et al.* described from a kid *E. christenseni* oocysts which were comparable with those of *E. ahsata* from sheep. However, they justified the new name on the basis of the typical ovoid oocysts and broadly ovoid sporocysts of *E. christenseni* as distinct from the ellipsoidal oocysts and elongate sporocysts of *E. ahsata*. Two other species *E. arloingi* and

E. crandallis were recovered from the same goat. Levine *et al.* (1962) considered *E. crandallis* as described from sheep by Honess (1942) and Rysavy (1954) from unspecified hosts to be similar. Whereas Honess's photograph did not show the sporozoites' position in the sporocysts, Levine *et al.* (1962) and Shah & Joshi (1963) described them as lying transversely in the sporocyst. However, Rysavy illustrated them lying lengthwise. Levine *et al.* (1962) also said their form was similar to the sheep isolate described by Kamalapur, 1961 (cit. Levine *et al.* 1962). They stated that further study is needed to establish the morphology and identity of Rysavy's forms. Later, in 1966, Chevalier separated another new species *E. hirci* from *E. crandallis* from goats with the explanation that the sporocysts of *E. hirci* are wider and the sporocyst residuum larger than in *E. crandallis* as described by Honess (1942) from sheep.

In the 1960's, several cross-transmission experiments between goats and sheep were carried out. Various investigators (Krylov, 1961; Tsygankov *et al.*, 1963; Svanbaev, 1967 all cited by Levine & Ivens, 1970), Lotze *et al.*, 1961 and Spindler (1965) attempted to transmit *Eimeria* species of both origins between goats and sheep but none were successful.

Deiana *et al.*, 1953 (cit. Levine & Ivens, 1970) claimed they had transmitted *E. arloingi* from a goat to a lamb, but Levine & Ivens (1970) said that as it was an uncontrolled experiment, the result was doubtful. Fitzsimmons (1964) infected two coccidia-free kids (50-58 days old) with *E. ninakohlyakimovae* and *E. faurei*, reputedly from sheep, but the oocyst output was much lower than in a control lamb. However, the oocysts used were derived from sheep faeces collected from pasture lying under snow and the origin of the isolates is, therefore, uncertain. Another successful cross-transmission of *E. ninakohlyakimovae* and *E. faurei* from goats to sheep was claimed by Subramanian and Jha (1966). They fed 50,000 *E. ninakohlyakimovae* oocysts to one lamb and 160,000 *E. faurei* to another. The lambs were raised coccidia-free but the oocysts used were inadequately described, so these results are also questionable. Were it not for these two results, the clear-cut principle could be established that both goats and sheep harbour separate species, the oocysts of which are structurally similar. It is notable that McDougald (1979) using a pure isolate of *E. ninakohlyakimovae* from sheep failed to transmit it to parasite-free kids. He regarded the validity of previous reports of successful transmission with suspicion and made the comment that they were not working in strict isolation conditions and the results could have come from spurious exposure (personal communication). He also renamed the ovine equivalent of *E. ninakohlyakimovae*, *E. ovinoidalis*.

Whether or not some *Eimeria* species will infect both sheep and goats is not entirely clear as cross-transmission experiments have not been carried out with all species. But by the

end of 1960's it was becoming apparent that generally the *Eimeria* species infecting goats and sheep were distinct. By that time eight species had been recorded from goats, all with their counterparts in sheep and, at that stage, bearing identical names in the two hosts. Since then, further species have been described from both and many species renamed to distinguish between those from goats and sheep.

Because of negative cross-transmission results, Musaev (1970) proposed the names *E. apsheronica*, *E. alijevi*, *E. jolchejevi* and *E. korcharli* for caprine counterparts of *E. faurei*, *E. parva*, *E. granulosa*, *E. intricata*, respectively which were originally described from sheep. In addition, he renamed the ovine counterpart of *E. arloingi* as *E. bakuensis*. In the same year, Levine & Ivens (1970) renamed *E. arloingi* of sheep *E. ovina* but as the publication date for *E. bakuensis* was earlier it takes priority (Levine & Ivens, 1986).

In spite of the separation of what was originally described as "*E. arloingi*" in sheep, into three distinct species *E. ahsata*, *E. arloingi* and *E. crandallis* (Smith *et al.*, 1960 & 1961) careful examination of populations of these *E. arloingi*-type oocysts again revealed significant variations in size and shape which suggested that they might still include more than one species. Pout *et al.* (1973) recognised large and small forms of *E. arloingi* which they designated types A and B. Following further investigations of these types (Pout, 1974a & b; Pout & Catchpole, 1974), type B was isolated in pure form and named *E. weybridgensis* (Norton *et al.*, 1974).

In 1979, Lima described *E. caprina* with medium sized, capless, ellipsoidal or slightly ovoid oocysts from goats in U.S.A.. He reported the oocyst to be larger than those of other capless species from goats, *E. ninakohlyakimovae* and *E. alijevi*, and that it does not possess an internal plug as does *E. apsheronica* which also has ovoid oocysts (Lima, 1979a). In the next year, Lima (1980a) described another capless species in goats, *E. caprovina* which he differentiated from *E. caprina* by its more round shape and coloured inner layer of the oocyst wall.

When Chevalier (1966) described *E. hirci* from goats, he also recorded *E. ahsata*, *E. arloingi* and *E. crandallis*. At that time, the species in sheep and goats were still considered to be the same. Chevalier (1966) supported his separation of the last three species with a statistical analysis of oocyst dimensions but Levine & Ivens (1970) considered size alone an inadequate basis for separating species for the reasons put forward by Becker *et al.* (1956). Accordingly they considered the oocysts described by Chevalier (1966) to be all *E. arloingi*. At the same time Levine & Ivens (1970) were inclined to regard *E. hirci* as a synonym of *E. crandallis* though they offered no explanation for this. This view persists in later publications

(Levine & Ivens, 1986 & Levine, 1985) even though Chevalier (1966) considered the two clearly distinguishable. Subsequently, Musaev and Mamedova (1981) proposed the names *E. tunisiensis* and *E. africensis* for the species from goats resembling *E. ahsata* and *E. crandallis* respectively from sheep. Unfortunately their descriptions of *E. tunisiensis* and *E. africensis* are incomplete and details that might distinguish *E. africensis* from *E. hirci* or other capped species are not provided.

Levine & Ivens (1986) accepted *E. africensis* as a valid species but considered *E. tunisiensis* a synonym of *E. christenseni* but again no justification was given. Indeed, Levine & co-workers repeatedly make the assertion that records of *E. ahsata*-like oocysts from goats probably refer to *E. christenseni* even though in the original description of the latter several features distinguishing it from *E. ahsata* were given (Levine *et al.*, 1962).

Several investigators (Chevalier, 1966; Jha & Subramanian, 1966; Yvone *et al.*, 1980; Vercruyssen, 1982) have seen or experimented with oocysts with morphology similar to those of *E. ahsata* of sheep. It is clearly necessary to investigate properly whether or not a species equivalent to *E. ahsata* and distinct from *E. christenseni* occurs in goats.

The issue as to whether *E. crandallis* is a parasite of goats or not thus remains very confused. It seems possible that what has been described by various authors as *E. crandallis* from goats, does not comprise a single species but a mixture represented, wholly or in part, by *E. hirci* (as described by Chevalier, 1966) and *E. africensis* (as described by Musaev & Mamedova, 1981). Consequently, in this review *E. crandallis* and *E. africensis* are not listed as valid species infecting goats. The confusion will never be resolved because of the inadequacies of both the original descriptions and comparisons made by various authors.

So far then, thirteen species of coccidia have been described from goats (excluding *E. crandallis* and *E. africensis* because of the uncertainties surrounding them) and most have species with similar oocyst morphology occurring in sheep (Table 1.1). As yet *E. caprina* Lima, 1979a and *E. caprovina* Lima, 1980a have no ovine counterparts. Although Lima, 1980c (cit. Levine & Ivens, 1986) transmitted *E. caprovina* from a goat to a sheep and vice versa, it has not been found in naturally infected sheep. Oocysts of these two species are very similar and would be difficult to identify with certainty unless both were present in the same sample. Other ovine species, *E. gonzalezi* which resembles *E. granulosa*, described in Peru (Bazalar & Guerrero, 1970 cit. Levine & Ivens, 1986), the small capped oocysts discovered in Italy designated *E. marsica* (Restani, 1971) and also *E. weybridgei* (Norton, Joyner, & Catchpole, 1974) have no caprine counterparts yet.

There are still two species remaining which are considered to occur in both sheep and goats (Levine, 1985; Levine & Ivens, 1986) namely *E. pallida* and *E. punctata*. However, Pellerdy

Table 1.1. Names of *Eimeria* species of goat and their counterparts

Previous Name **	Present Name	Sheep Equivalent
<i>E. faurei</i> * Moussu & Marotel, 1901	<i>E. apsheronica</i> Musaev, 1970	<i>E. faurei</i>
<i>E. arloingi</i> Marotel, 1905	<i>E. arloingi</i>	<i>E. bakuensis</i> Musaev, 1970
<i>E. intricata</i> * Spiegl, 1925	<i>E. korcharli</i> Musaev, 1970	<i>E. intricata</i>
<i>E. parva</i> * Kotlan, Moscy & Vadja, 1929	<i>E. alijeви</i> Musaev, 1970	<i>E. parva</i>
<i>E. ninakohlyakimovae</i> Yakimoff & Rastegaieff 1930	<i>E. ninakohlyakimovae</i>	<i>E. ovinoidalis</i> McDougald, 1979
<i>E. granulosa</i> * Christensen, 1938	<i>E. jolchejevi</i> Musaev, 1970	<i>E. granulosa</i>
<i>E. pallida</i> * Christensen, 1938	<i>E. pallida</i>	<i>E. pallida</i>
<i>E. punctata</i> * Landers, 1952	<i>E. punctata</i>	<i>E. punctata</i>
<i>E. christenseni</i> Levine Ivens & Fritz, 1962	<i>E. christenseni</i>	<i>E. ahsata</i>
<i>E. hirci</i> Chevalier, 1966	<i>E. hirci</i>	<i>E. crandallis</i>
<i>E. caprina</i> Lima, 1979a	<i>E. caprina</i>	nil
<i>E. caprovina</i> Lima, 1980a	<i>E. caprovina</i>	nil
<i>E. tunisiensis</i> Musaev & Mamedova, 1981	<i>E. tunisiensis</i>	<i>E. ahsata</i> Honest, 1942

* Species originally described from sheep.

** Species are described in the order of the year discovered.

(1974) regarded *E. pallida* as a synonym of *E. parva* in sheep and *E. aljevi* in goats; *E. punctata* was described from sheep by Landers (1955) and from goats by Chevalier (1966) but whether these really represent a single species has not been investigated.

1.3 MORPHOLOGICAL CHARACTERISTICS OF NAMED *EIMERIA* SPECIES INFECTING DOMESTICATED GOATS

In the following descriptions, priority is given to the original author's description wherever possible. Also included is additional information from descriptions by others. Most of the information is summarised in Table 1.2. Species having a micropylar cap are considered first in descending order of oocyst size; those without a micropylar cap follow in the same order.

A Species with a micropylar cap

1.3.1 *Eimeria korcharli* Musaev, 1970

Synonym: E. intricata (sheep)

This is a comparatively uncommon species in goats. Vercruysse (1982) has given a brief description of the oocysts from goats (Table 1.2). Levine (1985) and Levine et al. (1986) have given more detailed descriptions but they are simply restatements of the description of *E. intricata* from sheep. This species has not been adequately described from goats.

1.3.2 *Eimeria christenseni* Levine, Ivens & Fritz, 1962

Synonym: E. ahsata (sheep)

From the original description the micropylar cap is prominent, colourless, and mound-shaped; one or more polar granules are present, sometimes shattered into many fine granules; an oocyst residuum is absent; sporozoites lie lengthwise, head to tail in the sporocysts; each contains one large and one small clear globule, one at each end of the sporozoite; the oocyst and sporocyst dimensions given by others (Shah & Joshi, 1963; Chevalier, 1966; Lima, 1980b) are consistent with the original data although the oocysts described by Vercruysse (1982) are relatively large. Chevalier (1966) described the sporocyst residuum as large though others have not mentioned its size.

Table 1.2 Morphological Characteristics of Named Eimeria Species from Domesticated Goats

Species	Authors	Oocyst Characteristics					Sporocyst Characteristics			
		[mean,range shape index (mean),shape]	N	Micro- pyle	Oocyst wall	Polar granules	[mean,range, shape index (mean),shape]	Stieda Body	Sporocyst residuum	Clear globules
With micropylar cap										
<u>E. korcharli</u>	Vercruysse 1982	45 x 37 41-50x34-37 NS ellipsoid	NS	+	thick and striated;	NS	NS NS NS NS	NS	NS	NS
<u>E. christenseni</u>	Levine, Ivens & Fritz, 1962	38 x 25 34-41x23-28 1.4-1.7(1.5) ovoid	51	+	2 layers;outer smooth, color- less to pale yellow;inner brownish-yellow;	1	15 x 10 14-16x8-10 1.3-1.8(1.6) ovoid	+	+	1 small 1 large
	Shah & Joshi, 1963	39 x 26 32-43x24-30 1.3-1.7(1.5) ovoid ellipsoid	28	+	2 layers;outer smooth yellow; inner brownish- yellow;	1	15 x 9 14-18x8-11 1.4-2.0(1.5) broadly ovoid	NS	+	1 small 1 large
	Chevalier, 1966	39 x 25 35-44x22-27 1.4-1.7(1.6) ellipsoid	110	+	2 layers;outer yellowish brown; inner greenish yellow;	+	16 x 9 15-18x9-11 1.5-1.8(1.7) broadly ellipsoid	NS	+	large
	Vercruysse, 1982	41 x 28 40-46x25-30 NS ovoid	NS	+	NS	NS	NS NS NS broadly ovoid	NS	NS	NS

	Lima, 1980b	38 x 25 31-44x 22-31 1.2-1.8(1.5) NS	NS	NS	NS	NS	15 x 9 12-17x8-10 1.3-2.0(1.6) NS	NS	NS	NS
<u>E. tunisiensis</u>	Chevalier 1966	33 x 22 30-36x19-25 NS ellipsoid	140	+	double layered yellowish brown;	NS	16 x 7 14-18x6-8 NS elongate	NS	+	large NS
	Musaev & Mamedova 1981	36 x 23 30-44x20-30 1.3-1.9(1.5) ovoid ellipsoid	5	+	yellowish brown;	+	13 x 10 10-22x6-12 NS ovoid	NS	+	NS
	Vercruysse, 1982	36 x 25 31-37x22-27 NS ellipsoid ovoid	NS	+	NS	NS	NS NS NS elongate	NS	+	prominent NS
<u>E. jolchejevi</u>	Shah & Joshi, 1963	32 x 23 30-34x20-26 1.3-1.5(1.4) ellipsoid pyriform	8	+	2 layers; outer pale yellow to yellowish brown; inner brownish yellow;	3	NS 13-15x8-9 1.4-1.8(1.8)(sic) elongate ovoid	+	+	1-2
	Lima, 1980b	31 x 22 26-37x18-26 1.2-1.7(1.4) NS	150	NS	NS	NS	15 x 8 12-18x6-10 1.4-2.2(1.8) NS	NS	NS	NS

	Musaev & Mamedova, 1981	33 x 20 30-36x18-22 1.2-1.7(1.5) egg shaped	32	+	2 layers;smooth, yellowish-brown;	3	11 x 9 8-14x6-10 NS bean or lemon	NS	+	1-2
<u>E. arloingi</u>	Marotel, 1905	NS 25-27x16-18 NS ellipsoid	NS	+	NS	NS	NS NS NS NS	NS	NS	NS
	Levine, Ivens & Fritz, 1962	28 x 20 22-31x17-22 1.3-1.6(1.4) ellipsoid slightly ovoid	52	+	2 layers;outer colorless;inner brownish yellow;	1	14 x 8 12-16x6-8 1.7-2.3(1.9) elongate ovoid	-	+	1 small 1 large
	Shah & Joshi, 1963	28 x 21 22-35x18-26 1.1-1.6(1.3) ellipsoid ovoid	60	+	2 layers;outer colorless;inner brownish yellow;	1	13 x 8 11-17x6-10 1.2-2.2(1.6) elongate ovoid	-	+	1 small 1 large
	Chevalier, 1966	28 x 19 26-31x16-22 1.5-1.8(1.6) ovoid and straight side	100	+	2 layers;outer yellow to brownish yellow;outer grey to yellowishgrey;	+	15 x 7 14-17x6-8 2.0-2.3(2.1) elongate	NS	+	NS
	Lima, 1980b	28 x 20 22-35x16-23 1.2-2.1(1.4) NS	700	NS	NS	NS	14 x 7 10-17x6-10 1.3-2.8(2.0) NS	NS	NS	NS
	Vercruysse 1982	30 x 21 27-35x17-24 NS ellipsoid, slightly ovoid	NS	NS	NS	NS	NS NS NS elongate	NS	NS	NS

<u>E. punctata</u>	Chevalier, 1966	26 x 20 21-31x15-23 1.2-1.7(1.3) ovoid, ellipsoid	100	+	pitted; greenish yellow	NS	NS NS NS NS	NS	NS	NS
<u>E. hirci</u>	Chevalier, 1966	21 x 16 18-23x14-19 1.2-1.4(1.3) spherical	130	+	1 layer;light yellow;oocyst inside light grey to colorless	+	10 x 7 8-11x5-8 1.4-1.7(1.5) round	NS	+	NS
	Levine, Ivens & Fritz, 1962	23 x 19 20-27x17-20 1.2-1.5(1.3) ellipsoid	52	+	2 layers;smooth outer colorless; inner light brownish-yellow;	1	11 x 7 10-12x6-8 1.3-1.6(1.5) broadly ovoid	+	+	1-2 tiny
	Shah & Joshi,1963	22 x 18 19-27x14-20 1.1-1.3(1.2) ellipsoid	45	+	2 layers;outer colorless;inner brownish yellow;	+	10 x 7 8-11x6-8 1.3-1.7(1.4) broadly ovoid	-	+	1-2
	Lima, 1980b	23 x 18 18-29x16-21 1.1-1.7(1.3) NS	300	NS	NS	NS	11 x 7 9-13x6-9 1.1-2.1(1.6) NS	NS	NS	NS
Without micropylar cap										
<u>E. caprina</u>	Lima,1979a	32 x 23 27-40x20-26 1.2-2.1(1.4) ellipsoid, slightly ovoid	100	+	2 layers;outer brownish yellow; inner colorless;	1	15 x 9 13-17x7-10 1.6-2.2(1.8) elongate ovoid	+	+	1 small 1 large

<u>E. caprovina</u>	Lima, 1980a	30 x 24 26-36x21-28 1.1-1.5(1.3) ellipsoid, sub- spherical, ovoid	100	+	2 layers; outer colorless; inner brownish yellow;	1	14 x 8 13-17x8-9 NS elongate ovoid	+	+	2 large
<u>E. apsheronica</u>	Shah & Joshi, 1963	29 x 22 27-33x19-25 1.2-1.6(1.2)(sic) ovoid	32	+	1 layer; colorless;	1	13 x 9 11-16x8-11 1.2-1.8(1.4) pear	-	+	2 large
	Lima, 1980b	31 x 23 24-27x18-26 1.1-1.6(1.4) NS	100	NS	NS	NS	16 x 9 12-18x7-10 1.4-2.2(1.8) NS	NS	NS	NS
	Musaev Mamedova, 1981	26 x 20 14-42x12-34 1.0-1.6(1.1) ovoid	104	+	1 layer; colorless;	+	12 x 9 8-18x6-12 NS oval	NS	+	+
	Vercruyse, 1982	33 x 24 25-37x21-26 NS ovoid	NS	+	a small nob associated with a micropyle on the inside of oocyst;	NS	NS NS NS NS	NS	NS	NS
<u>E. ninakoh- lyakimovae</u>	Yakimoff & Rastegaieff, 1930	NS 20-25x14-21 NS ovoid	-	-	double contoured wall;	NS	NS NS NS NS	NS	NS	NS
	Shah & Joshi, 1963	25 x 21 23-28x18-23 1.0-1.4(1.2) ovoid	60	+	2 layers; outer colorless to slightly yellowish; inner yellowish brown;	1	12 x 8 9-14x6-10 1.4-1.9(1.5) elongate ovoid	+	+	1-2

	Chevalier, 1966	24 x 18 20-27x15-21 1.2-1.4(1.3) ovoid ellipsoid	100	+	1 layer;brownish yellow;oocyst inside yellowish grey;	-	13 x 7 12-14x6-8 1.7-2.0(1.9) elongate ovoid	NS	+	NS
	Lima, 1980b	24 x 19 20-28x17-21 1.1-1.5(1.3) NS	100	NS	NS	NS	12 x 7 10-15x6-9 1.3-2.4(1.9) NS	NS	NS	NS
	Vercruysse 1982	25 x 20 22-27x16-24 NS ovoid; sub- spherical;	NS	+	NS	NS	NS NS NS NS	NS	NS	NS
<u>E. alijeви</u>	Shah & Joshi, 1963	20 x 19 16-23x14-22 1.1-1.2(1.1) subspherical ovoid, ellip- soid;	30	NS	2 layers;pale yellow to yell- owish-brown; inner dark and thin;	1	10 x 7 7-13x5-9 1.1-1.6(1.4) broadly ovoid	-	+	2
	Chevalier, 1966	17 x 15 15-19x13-16 1.2-1.3(1.2) spherical subspherical	80	NS	2 layers; bright yellowish brown;	-	9 x 6 8-10x5-6 NS NS	NS	+	NS
	Lima, 1980b	17 x 15 15-23x12-18 1.2-2.1(1.2) NS	100	NS	NS	NS	9 x 5 7-12x4-7 1.5-2.4(1.8) NS	NS	NS	NS

	Musaev & Mamedova, 1981	20 x 19 16-23x13-22 NS round oval	21	+	1 layes;smooth pale yellow or yellowish brown	+	8.55(width) 6-10 NS round	-	+	2
	Vercruysee, 1982	17 x 14 15-20x13-16 NS NS	NS	NS	Double contoured appearance;	NS	NS NS NS NS	NS	NS	NS
<u>E. pallida</u>	Shah & Joshi, 1963	16 x 12 13-18x10-14 1.2-1.6(1.3) ellipsoid, slightly ovoid	22	NS	2 layers;outer colourless to pale yellow;	1	7 x 5 6-9 x 4-5 1.5-2.3(1.4) elongate ovoid	-	+	1

N = number measured; NS = not stated.

1.3.3 *Eimeria tunisiensis* Musaev & Mamedova 1981

Synonym: E. ahsata (sheep)

Chevalier (1966) separated *E. tunisiensis* (then known as *E. ahsata*) from *E. arloingi* in goats by its larger oocysts with a double-layered wall and large sporocyst residuum. The large sporocyst residuum seems to be a prominent characteristic of this species. The average oocyst dimensions of *E. tunisiensis* given by different authors in Table 1.2 are not consistent, however, and inadequate descriptions of the oocysts measured makes their identity questionable.

1.3.4 *Eimeria jolchejevi* Musaev, 1970

Synonym: E. granulosa (sheep)

Shah & Joshi (1963) gave a complete description of the oocysts from goats (as *E. granulosa*) and found they were morphologically identical with sheep forms. They described the micropylar cap as prominent and truncated cone-shaped, measuring 8-10x2-3 μm (mean: 9x3 μm) which is in the same size range as that given by Musaev (1970); the micropylar cap was originally described as being at the broader end of pyriform oocysts though Musaev did not mention this; the elongate sporozoites lie head to tail in the sporocysts. The oocyst and sporocyst dimensions given by different authors (Table 1.2) are similar.

1.3.5 *Eimeria arloingi* Marotel, 1905 amend Martin, 1909

Synonym: E. bakuensis (sheep)

This species was first described from the goat by Marotel, 1905 (cited by Lotze, 1953). The range of oocyst sizes given by Marotel is smaller than those of others so may be derived from a small sample and, perhaps, from a single species rather than the mix of species which later workers included under the name of *E. arloingi*. The following description from Levine et al. (1962) agrees with those of other authors unless otherwise stated. The micropylar cap is prominent, colourless, mound-shaped, size range 4-9x0.4-2 μm (mean: 7x2 μm) which is close to the range 5-9x1-3 μm (mean: 6x2 μm) given by Shah & Joshi (1963); the sporozoites lie lengthwise, head to tail, in the sporocysts. Chevalier (1966) reported the oocyst wall to be single-layered.

1.3.6 *Eimeria punctata* Landers, 1955

Synonym: none

The same name is used for the goat and sheep species though no attempts at transmission between sheep and goats have been made. This is not a common species in either sheep or goats. Chevalier (1966) first described it from goats (Table 1.2.). However, the multilayered wall of the oocysts prevented him from describing the internal characteristics of the oocysts. However, this species is easily identified by its peculiar punctate wall.

1.3.7 *Eimeria hirci* Chevalier 1966

Synonym: E. crandallis (sheep)

Chevalier's oocyst dimensions are smaller than those given by others (Table 1.2). Chevalier observed that *E. hirci* has a single-layered oocyst wall but Levine et al. (1962), and Shah & Joshi (1963) described the wall as double-layered. They also reported that the sporozoites lie approximately transversely and completely fill each end of the sporocyst. Only Levine *et al.* (1962) recorded a tiny stieda body as occasionally present and an indistinct, sparse sporocyst residuum.

B Species without a micropylar cap

1.3.8 *Eimeria caprina* Lima, 1979a

Synonym: none

This is the largest of the capless species from goats (Table 1.2). From Lima's description, polar granules are present, sometimes shattered into fine particles or apparently, absent; an oocyst residuum is absent; the sporozoites lie lengthwise, head to tail in the sporocysts; each sporozoite contains one large clear globule at the large end and a small one at the small end. The morphological characteristics of this species have not been described by others.

1.3.9 *Eimeria caprovina* Lima, 1980a

Synonym: none

This is another large capless species found in goats. In many respects the description (Table 1.2), overlaps that of *E. caprina*, except that *E. caprovina* oocysts are rounder and their outer wall is colourless and inner brownish yellow which is the opposite to *E. caprina*. The morphological characteristics of this species have not been described by others.

1.3.10 *Eimeria apsheronica* Musaev, 1970

Synonym: E. faurei (sheep)

The oocyst and sporocyst dimensions given by authors in Table 1.2 are not very consistent. Musaev and Mamedovas' extreme figures for oocyst and sporocyst sizes are very doubtful and it is almost certain they were not dealing with a single species. The measurements of sporocysts given by Lima (1980b) also suggest that they differed in shape from those described originally (Shah & Joshi, 1963). According to Shah and Joshi, the oocysts are typically ovoid with a micropyle at the narrow end which has a small internal plug as mentioned by Vercruyssen (1982). The sporozoites lie lengthwise, head to tail in the sporocysts.

1.3.11 *Eimeria ninakohlyakimovae* Yakimoff and Rastegaieff, 1930

Synonym: E. ninakohlyakimovi (sheep)

In the original description (which was relatively brief), the oocyst and sporocyst dimensions are smaller than those given by later authors. Chevalier (1966) considered the oocyst wall to be single-layered but others disagree (Yakimoff & Rastegaieff, 1930; Shah & Joshi, 1963). Shah & Joshi (1963) described the sporozoites as lying head to tail in the sporocysts.

1.3.12 *Eimeria alijevi* Musaev 1970

Synonym: E. parva

Shah & Joshi (1963) first described fully the morphological characteristics of this species from goats. It is easily identified as it is the only species of those infecting goats with such small and spherical oocysts. Shah & Joshi (1963) described the sporozoites as lying head to tail in the sporocysts. Except for the smaller oocyst dimensions given by Chevalier, the descriptions (Table 1.2) given by different authors are reasonably consistent.

1.3.13 *Eimeria pallida* Christensen, 1938

Synonym: none

This is not a common species in either sheep or goats. The same name is used for the goat and sheep species though no attempts at transmission between sheep and goats have been made. Shah & Joshi (1963) have given a complete description of isolates from goats and recorded the sporozoites as lying head to tail in the sporocyst, each sporozoite containing a single, clear globule.

1.4 LIFE CYCLE OF *EIMERIA* SPECIES

1.4.1 General Considerations

The life cycle of *Eimeria* is typically homoxynous and the endogenous stages are normally intrainestinal but may occasionally be extraintestinal, involving, for example, other organs such as the liver (Dubey, 1986) and mesenteric lymph nodes (Lotze *et al.*, 1964; Lima, 1979b). The life cycle can be divided into 3 stages: sporogony, schizogony (asexual development) and gametogony (sexual development). All stages of the organism are haploid except for the zygote (Hammond, 1973). Oocysts are passed in the faeces and fresh oocysts contain a single sporont which divides into 4 sporoblasts, each sporoblast becoming a sporocyst and then forming 2 sporozoites. When these are fully developed, the oocyst is infective. The sporulation is strictly aerobic (Hammond, 1973) and takes 1 or more days depending on the species and temperatures.

When the infective oocysts are ingested by ruminants, the sporozoites excyst from the oocyst after a primary stimulus provided by carbon dioxide in the rumen and a secondary stimulus provided by trypsin and bile in the small intestine (Jackson, 1962). The escaped sporozoites find the site of invasion appropriate for the species concerned and enter enterocytes. Each species has its own specific site of invasion and development. For example, the development of *E. arloingi* is confined to the small intestine (Sayin *et al.*, 1980) and *E. caprina* to the large intestine (Norton, 1986). Whether the entry of sporozoites into the enterocytes is by invagination or penetration of the host cell-membrane is still not clear (Wang, 1982).

Having entered a cell, the sporozoite rounds up to form a trophozoite. By multiple fission, a first generation schizont is formed and numerous, often hundreds of merozoites develop. When the schizont is mature, it ruptures and the released merozoites enter new epithelial cells and form second generation schizonts. The *Eimeria* species of ruminants which have been studied have two schizont stages, a giant first generation schizont and smaller second generation schizont. For example in *E. bovis* from cattle, there is a giant schizont (approximately 300 μ m diameter) which may contain 120,000 merozoites and a small schizont (approximately 10 μ m diameter) containing 36 merozoites (Hammond *et al.*, 1963). Some avian species such as *E. tenella*, *E. necatrix* and *E. mivati* undergo three or more asexual generations (Levine, 1985).

After a predetermined number of generations, the merozoites enter new cells and begin gametogony. Most merozoites develop into macrogamonts - 'female' gametes and some

into microgamonts - "male" gamonts (Levine, 1985). Macrogamonts have a large central nucleus with a prominent nucleolus and each gives rise to a single macrogamete which contains many eosinophilic granules in the cytoplasm; in each microgamont a large number of dark blue-staining peripherally arranged nuclei develop and these mature into hundreds of comma-shaped microgametes. After fertilization of the microgamete, the eosinophilic granules give rise to the oocyst wall which forms around the resultant zygote (Levine, 1985). The oocysts are discharged into the intestinal lumen and pass out in the faeces. The time from ingestion to the appearance of the first oocysts in the faeces varies from about 1-3 weeks depending upon the species of *Eimeria*.

1.4.2 Life Cycles of *Eimeria* Species of goats

As far as is known, the life cycles follow the typical pattern, but sites of predilection and utilization of host cells differ from one species to another. Until the 1980s, the endogenous stages of no species infecting goats had been adequately described. Before then, some workers who found endogenous stages in naturally infected goats, tried to relate them to the species of oocysts occurring in faeces or in the gut (Singh & Pande, 1967; Mugeru, 1968), or the most prevalent species in the vicinity (Sharma Deorani, 1968). Levine *et al.* (1962) tentatively extrapolated from studies on ovine species but admitted that relating observed endogenous stages to another species on this basis could only be speculative. Since the infections composed mixtures of species these observations are of little value.

So far the endogenous stages of the life cycles of only two species, *E. arloingi* (Sayin, *et al.*, 1980) and *E. christenseni* (Lima 1981) have been described fully. Some of the endogenous stages of *E. caprina* and *E. nina kohlyakimovae* have been briefly described by Norton (1986). There are some earlier descriptions relating to the latter species (Balozet, 1932 cited by Levine & Ivens, 1970) but it seems unlikely that these are correct since they described schizogony occurring 39 days after infection. Sayin (1964) described stages from a naturally infected goat which he claimed was only infected with the one species. Some stages of *E. alijevi* have also been described (Sayin, 1966 cit. Levine & Ivens, 1970)

(i) *Eimeria arloingi* Marotel, 1905 amend Martin, 1909

Sayin *et al.* (1980) infected coccidia-free kids with a pure infection. They observed mature first generation schizonts (mean 247x147 μ m) in the endothelial cells of the lacteals of the villi, and in Peyer's patches and mesenteric lymph nodes of the duodenum, jejunum and ileum 9 days after inoculation (DAI); mature second generation schizonts (mean 22x12 μ m) were found in the epithelial cells of small intestinal crypts and villi on 12 DAI; male and

female gamonts were first recorded in the same vicinity 11 and 12 DAI, respectively. The prepatent period was found to be 15-18 days and the patent period 14-15 days.

(ii) ***Eimeria christenseni* Levine, Ivens & Fritz, 1962**

Lima (1981) studied the life cycle in experimentally infected kids although it had been tentatively described from kids naturally infected with mixed species by Levine *et al.* (1962), Bhatia & Pande (1967) and Lima (1979b). Lima (1981) found two types of schizonts; mature giant schizonts (mean 184x100 μ m) were seen 14 DAI in the endothelial cells of lacteals of villi in the jejunum, ileum and in the mesenteric lymph nodes, and second generation schizonts (mean 14x10 μ m) were first seen in the epithelium of crypts and villi of the small intestine and sinuses of mesenteric lymph nodes 16 DAI. Mature gamonts were observed 16 DAI in the same vicinity. The prepatent period was 14-23 (mean 17) days and the patent period was 8 to >30 days.

(iii) ***Eimeria ninakohlyakimovae* Yakimoff & Rastegaief, 1930
emend. Levine, 1961**

Sayin (1964) described schizonts in the ileum, caecum and upper part of large intestine but he did not state how long the kids had been infected; Norton (1986) recorded schizonts in the caecum and colon of one kid with a pure infection 17 DAI. Both authors found gamonts and oocysts in the same location. Norton reported the appearance of the first oocysts at 17 DAI. Earlier stages have not been described and it is not known if there is a macroschizont stage as occurs in the equivalent species occurring in sheep (Lotze, 1953).

(iv) ***Eimeria caprina* Lima, 1979a**

Norton (1986) infected a 4 month old kid reared coccidia-free, with *E. caprina* and reported briefly that he did not see any parasites in the small intestine, which was normal in appearance throughout, and that smears from the mucosa of the caecum, lower colon and upper rectum showed gametocytes and oocysts. Twenty DAI, oocysts were found in the faeces together with mucus and blood. It is not stated how long after this the animal was killed.

(v) ***E. alijevi* Musaev, 1970**

In four experimentally infected kids which died 11-15 DAI, Sayin (1966, cited by Levine & Ivens, 1970) found giant schizonts (260x180 μ m) in the mid-small intestine and small ones

(15-18x9-12um) in the epithelial cells of the crypts of Lieberkuhn (in one kid), and gamonts and oocysts in mucosal cells of the posterior small intestine, caecum and colon. Oocysts were found in faeces 10-13 DAI.

1.5 EPIDEMIOLOGY

1.5.1 Prevalence of *Eimeria* species of domestic goats

The epidemiology of coccidial infections is complex and determined by the interaction of a variety of parasite, host and environmental factors. These are reviewed under a number of headings but it is to be understood that such a subdivision is arbitrary. *Eimeria* species are normally present in all ages of goats, and they are distributed worldwide (Table 1.3). The prevalence at any one time can be as high as 90-100% (Lloyd & Soulsby, 1978; Lima, 1980b; Norton, 1986) and 3-4 times higher in kids than adults (Mamedova, 1984). Mixed infections are usual (Norton, 1986; Kanyari, 1988a) and are especially common in kids (Kanyari, 1988a).

Information on the prevalence of individual *Eimeria* species in different countries is difficult to compare as the basis for the data is so variable both quantitatively and qualitatively. Commonly, the prevalence figures reported are point-prevalences based on a single sampling of a group of animals. However, the available information tends to indicate that *E. arloingi* is commonly the most prevalent as well as the predominant species (Sharma Deorani, 1966; Kshirsagar, 1980; Lima, 1980b; Norton, 1986; Gregory & Norton, 1986). *E. ninakohlyakimovae* seems to be the second most common species (Table 1.3). *E. alijevi*, *E. hirci*, *E. caprina* and *E. christenseni* have been found to be common in some places. In general, *E. apsheronica* and *E. jolchejevi* appear to be somewhat less common. *E. korcharli*, *E. caprovina*, *E. punctata* and *E. pallida* are relatively rare. *E. ahsata* and *E. crandallis* have been reported in some places but it is difficult to determine which of the currently recognised species these are equivalent to.

Published information on changes in prevalence with age (Lima, 1980b; Norton, 1986; O'Callaghan, 1989) shows uniformly that *E. christenseni* tends to be more prevalent in younger goats but the situation with other species varies from place to place. In addition, O'Callaghan (1989) has observed a variation of prevalence between breeds in that *E. caprina* and *E. jolchejevi* were found in domestic, but not in 'feral' goats. There is no published information on the identity or prevalence of goat coccidia in New Zealand.

Table 1.3 Percentage prevalence of *Eimeria* species in faecal samples of goats given by other authors

Authors	Location	Species*													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Kanyari, 1988	Australia	-	-	5	<1	18	-	3	-	-	-	8	56	9	<1
O'Callaghan, 1989	Australia	-	-	29	9	81	-	82	-	32	12	6	51	49	-
Shah & Joshi 1963	India	-	-	5	1	-	-	10	-	-	-	1	12	1	-
Jha & Subramanian, 1966	India	-	14	1	10	69	13	5	-	-	-	34	53	58	-
Chhabra et al., 1983	India	5	-	29	21	67	33	-	-	-	-	15	59	41	-
Krishnamurthy, 1986	India	-	-	-	10	71	-	-	-	-	-	-	15	27	-
Magi et al., 1987	Italy	-	-	-	-	70	-	36	-	40	-	-	68	-	-
Chevalier, 1966	Germany	-	39	9	-	14	15	8	1	-	-	-	12	2	-
Korkin et al., 1979	USSR	5	-	-	-	92	-	-	-	-	-	32	74	19	-
Vercruyssen, 1982	Senegal	7	35	5	-	64	67	-	-	-	-	15	56	9	-
Fernando, 1957	Sri Lanka	-	-	11	-	45	-	-	-	-	-	8	-	55	-
Sayin, 1966	Turkey	-	-	63	<1	77	-	11	<1	-	-	<1	25	26	<1
Sayin, 1986	Turkey	-	-	28	<1	31	-	-	-	-	-	28	22	<1	-
Norton, 1986	U.K.	-	-	64	8	94	-	69	-	55	4	23	48	42	-
Lima, 1980b	U.S.A.	-	-	58	35	99	-	93	-	50	2	15	33	-	-

Key: 1. *E. korcharli*; 2. *E. ahsata*; 3. *E. christenseni*; 4. *E. jolchejevi*; 5. *E. arloingi*; 6. *E. crandallis*; 7. *E. hirci*; 8. *E. punctata*; 9. *E. caprina*; 10. *E. caprovina*; 11. *E. apsheronica*; 12. *E. ninakohlyakimovae*; 13. *E. alijevi*; 14. *E. pallida*

1.5.2 Host Determinants

(i) Resistance

It was thought that coccidiosis was a disease of young animals because of "age resistance", but now it is known that this is, in fact, acquired resistance due to previous exposure (Todd & Ernst, 1973). This resistance is species-specific (Norton *et al.*, 1974). Generally kids produce tens of thousands of oocysts/g faeces in the first year of life (Lloyd & Soulsby, 1978; Lima, 1980b; Shelton *et al.*, 1982; Norton, 1986; Kanyari, 1988a) sometimes starting as early as at 2 weeks of age (Baxi *et al.*, 1973). Clinically affected kids may produce several million oocysts/g faeces (Lloyd & Soulsby, 1978; Yvone *et al.*, 1980). As immunity is established in the kids, oocyst counts fall and they continue to fall progressively in yearlings and adults (Lima, 1980b; Norton, 1986; Kanyari, 1988a) although adults may continue to produce 1000-2000 oocysts/g (Norton, 1986).

The duration of acquired resistance in the absence of reinfection is difficult to establish with certainty (Rose, 1987). Furthermore resistance is not necessarily solid in that, after reinfection, although the host is protected from significant pathological effects, a low-grade infection can develop and small numbers of oocysts be produced (Levine, 1985). Sayin, 1966 (cited by Pellerdy, 1974) found that no clinical signs and much lower oocyst counts developed in experimental goats challenged with a high dose of *E. alijevi* six weeks after a heavy initial infection with the same species. It is usual to find adults excreting small number of oocysts of a variety of species.

Nolan *et al.* (1986) demonstrated the transfer of maternal antibody against *Eimeria* via the colostrum to lambs; the antibody titre declined over about 6 weeks and then rose again as the lambs responded to infection. The relevance of antibodies resistance against infection is not known. From experiments with calves, it appears that immunity may be of shorter duration in very young animals (Niilo, 1969 cited by Rose, 1973). It has also been shown that repeated infection of sheep produced a more long-lasting immunity than a single dose (Joyner & Norton, 1973). No detailed work appears to have been done on the immunological aspects of coccidial infections of goats.

(ii) Animals at risk

As stated above, resistance to infection is induced by previous experience of infection so that animals which lack exposure have the potential to develop clinical disease. Naturally, young animals have had less chance of being exposed to infection than adults and,

therefore, kids are indeed most susceptible. Because of this, kids can produce millions of oocysts from a few oocysts ingested (Gregory & Norton, 1986; Lloyd, 1987) which can rapidly contaminate the environment. Since the development of disease is dose-dependent, if kids are kept in unhygienic and overcrowded conditions, their chances of exposure to large numbers of oocysts are high and they may develop severe disease and even die (Lloyd, 1987; Craig, 1986).

Older goats generally have developed acquired resistance which allows few parasites to complete their life cycle. However, this resistance can be depressed by malnutrition (Fitzgerald, 1980), physiological status such as pregnancy and "stressors" which may include extreme weather conditions, shipment, changes in feed and environment, and other diseases (Baxi *et al.*, 1973; Yvone *et al.*, 1982; Craig, 1986;). Baxi *et al.* (1973) postulated that the stress of pregnancy might have contributed to an outbreak of coccidiosis in pregnant goats 10-39 months of age. However, there is no evidence of effects of lactation or sex on susceptibility.

Not only age and physiological status of the host but also its genetic constitution (eg. breed) may affect susceptibility to infection. Saanen goats are reported to be less susceptible to coccidial infection than Angora and Feral goats (Howe, 1984 cited by Kanyari, 1988a) and than Anglo-nubian goats which is reflected in lower oocyst counts and higher post-infection antibody titres (Kanyari, 1988b) in Saanens.

1.5.3 Parasite Determinants

Eimeria species normally develop in the intestinal cells. Therefore, the physical damage done to the host is essentially that of cell disruption. In general, the pathogenicity of different species of coccidia depends on their reproductive potential and the site preferences of the species concerned particularly the depth in the mucosa where multiplication occurs.

Only some of the species infecting goats are thought to be pathogenic. However, the comparative pathogenicity of goat species has not been studied adequately and it is, therefore, difficult to decide which are the most pathogenic species. Some authors have reported experimental infections with single species. For example in controlled experiments, 6 week-old kids became clinically ill after infection with 1000 oocysts *E. arloingi* and died when infected with 10000 oocysts (Sayin *et al.*, 1980), but 100,000 oocysts *E. christensenii* caused only clinical illness in 6 week-old kids (Lima, 1981). Norton (1986) reported that two coccidia-free kids were inoculated with 5000 oocysts of *E. caprina*

or *E. ninakohlyakimovae*, and that the latter produced more severe haemorrhage in the intestine and bloody diarrhoea. Sayin (1966) reported that 25000-1,000,000 oocysts of *E. alijevi* caused the death of 4 kids. Gregory & Norton (1986) and Lloyd (1987) claimed that the most pathogenic species were *E. ninakohlyakimovae* and *E. caprina* which usually attack the crypt cells of large intestine and cause severe haemorrhages, though this is based on very little evidence.

Little is known about the immunological or pathophysiological effects resulting from *Eimeria* infections in goats. In general, the host immune system will be activated so that there will be an influx of inflammatory cells into the gut (Sayin *et al.*, 1980; Lima, 1981). Antibody responses may be associated with the release of vasoactive amines resulting in increased capillary permeability and increased plasma protein flow into the lamina propria. In the *Eimeria* life cycle, the host cells are ruptured when the parasites leave and the remaining mucosa may show complete loss of villar structure; villi are swollen and the lamina propria may be exposed to the lumen. Congestion of blood capillaries and haemorrhages also occur (Mugera, 1968; Sayin *et al.*, 1980; Lima, 1981). In chronic cases, hyperplasia of the mucosa develops (Sharma Deorani, 1965; Singh & Bagwan, 1973). Considerable enteric loss of protein will result from the leakage of plasma proteins through the damaged villi and capillaries, and increased cell production in the mucosa. Consequent changes in blood constituents include hypoproteinaemia (Fitzgerald, 1980) and anaemia.

Damaged mucosae will lose their normal physiological functions such as digestion and absorption. Impaired small intestinal digestion can lead to the accumulation of undigested food particles in the colon which is ideal for colon bacteria to produce low molecular weight, osmotically active metabolites; this results in hyperosmolarity within the lumen which draws water from the circulation and causes diarrhoea (Bywater, 1980). There will also be reduced absorption of water if the large intestine cells are destroyed.

Severe infections may depress the appetite of the kids (Prasad *et al.*, 1981; Norton, 1986). The cause of anorexia in parasitised animals is still not known (Vercruysse *et al.*, 1988) but abdominal pain may be responsible in this case. As a result of malnutrition and anorexia, growth is retarded (Kanyari, 1988a) and usually a longer period of time is needed for an animal to reach a specific weight and many believe that survivors of severe infections never become profitable (Fitzgerald, 1980).

1.5.4 Environmental Determinants

(i) Effects On Parasites

There is a little information on the effect of climate on the occurrence of coccidial infections in goats. It is one of many factors that might influence seasonal patterns. Its effects are also complex affecting not only the sporulation and survival of oocysts but, indirectly, animal breeding cycles and systems of management. In India infection tends to increase in the wet season (Baxi *et al.*, 1973; Krishramurthy & Kshirsagar, 1976; Chhabra *et al.*, 1983) probably because of more suitable conditions for oocyst sporulation.

Many oocysts are removed from the epidemiological cycle by environmental factors before they reach susceptible animals (Davies *et al.*, 1963). When the oocysts are discharged in faeces they require oxygen, moisture and an adequate temperature for sporulation which, at optimal temperatures of 20-25°C (Christensen, 1939) will take 1-6 days or more, depending on the species. If the oocysts remain trapped in faeces this may prevent sporulation; extreme temperatures (>39°C) and desiccation are lethal to oocysts in 4 days (Baker, 1975). Dry conditions greatly reduce oocyst survival and the chances of contamination of feed with resultant infection (Christensen, 1939).

Experiments with oocysts derived from sheep show that under a cover of snow, at soil temperatures of -5°C to -32°C, oocysts became deformed in 15 days; in manure >10 cm deep, oocysts perished in 2-3 days (Korkin *et al.*, 1979). At near-freezing temperatures, 20% of unsporulated oocysts were still viable after 10 months, the moisture content of the faecal pellet apparently keeping the oocysts alive so that they could become infective when appropriate conditions occurred (Christensen, 1939). This indicates that the overwintered oocysts might be an important source of infection for kids turned out on to pasture in spring.

(ii) Effects On Animals

Clinical coccidiosis can be a considerable hazard in the rearing of goats, particular under intensive systems (Fitzsimmons, 1967; Marlow, 1968; Shelton *et al.*, 1982). In such systems, kids are usually confined in pens where they can build up their own lethal dose of infection particularly when pens are unclean, badly drained and overcrowded (Lloyd, 1987).

If the pens are completely free of contamination, the kids will remain susceptible and when they are transferred to contaminated pastures or production pens with older goats, a high rate of infection or disease is almost certain to occur immediately (Baker, 1975). At this

point, the consumption of contaminated straw bedding due to malnutrition or undernutrition will exacerbate the above conditions (Lloyd & Soulsby, 1978; Baker, 1975).

Clinical coccidiosis under pasture conditions is comparatively, much less common. Despite overwintered or freshly discharged oocysts being present on the pasture, there are several factors that influence the chances of kids being exposed to heavy infections, such as stocking density, growth of grass and topography of the pastures. Low stocking densities will allow the grass to grow longer which will dilute the pasture contamination, so that animals acquire lower levels of infection and can develop resistance without disease developing. With high stocking densities, the animals are likely to overgraze so that more oocysts are ingested and this may lead to clinical coccidiosis (Lloyd, 1987). As the topography determines the amount of sunlight to which the pasture is exposed, it also partially determines the environmental temperature of the oocysts on pasture which affects their sporulation and survival.

1.6 CLINICAL SIGNS

Coccidiosis is not clinically recognizable until the tissue damage associated with the intrainstestinal development of coccidia occurs. Clinical signs do not develop uniformly in all infected animals because they depend upon the balance between the rate of development, the level of resistance and the level of infection (Vercruysse, 1982). The symptoms are predominantly enteric. The most common clinical signs associated with acute or chronic coccidiosis are diarrhoea with or without blood, colic, tenesmus, anorexia, dehydration, inability to stand or eat, emaciation and death (Sayin *et al.*, 1980; Foreyt *et al.*, 1986). The faeces of clinically affected animals do not always contain blood; this probably depends on the *Eimeria* species involved. Kids which were infected with *E. ninakohlyakimovae* and *E. caprina*, which usually invade the crypts of Lieberkuhn of the large intestine, developed mucous bloody diarrhoea (Norton, 1986). Marlow (1968) reported that 6-7 months old Angora bucks which developed clinical coccidiosis were in very poor condition and shed their hair, whereas in the treated group all were in good condition and the mohair appeared healthy and lustrous.

As the disease develops, malabsorption becomes pronounced. Eventually the combination of diarrhoea, anorexia, impaired digestive and absorptive functions leads to weight loss (Deb *et al.*, 1981; Shelton *et al.*, 1982; Kanyari, 1988a; Vercruysse *et al.*, 1988). In severely affected animals (chiefly young ones), clinical signs may last for a week or more (Lima, 1981) and death may occur subsequently (Vercruysse *et al.*, 1988). However, some may

die very rapidly without clinical signs being observed (Yvone *et al.*, 1980) probably where massive tissue damage and haemorrhage occurs, leading to shock. Death can also occur in chronic cases (Foreyt *et al.*, 1986). Reported mortalities in outbreaks involving kids vary from 20-25% (Shelton *et al.*, 1982; Foreyt *et al.*, 1986) to 69-80% (Korkin *et al.*, 1979; Tarlatzis *et al.*, 1955). Severe clinical coccidiosis and deaths are more likely to occur in kids than adults (Lloyd, 1987).

Even if the animals recover from severe coccidiosis, growth is usually retarded and a longer period of time is needed for them to reach a specific weight than for uninfected animals kept under the same conditions (Fitzgerald, 1980; Lima, 1981).

1.7 DIAGNOSIS

Diagnosis of clinical coccidiosis is often difficult. The presence of oocysts, even in large numbers, is not of itself diagnostic although clinically affected animals may produce tens of millions of oocysts/g faeces (Lloyd & Soulsby, 1978; Prasad *et al.*, 1980; Yvone *et al.*, 1980).

However, clinical signs or death may occur before oocyst discharge (Jegatheeswaran, 1967; Yvone *et al.*, 1980; Sayin *et al.*, 1980). On the other hand clinically normal animals may have also high oocyst counts (Horak *et al.*, 1969; Vercruyse, 1982). A further problem in the interpretation of oocyst counts is that species clearly differ in their pathogenicity although the pathogenicity of many species remains to be determined. Identification of the species of coccidia represented in an oocyst count may thus be of some value though it needs an expert to do it (Levine, 1985). The clinical signs of severe coccidiosis are strongly suggestive of the disease but in less severe cases are similar to those caused by a variety of other agents or conditions (Todd & Ernst, 1973; Levine, 1985). In living animals, diagnosis rests, therefore, on a consideration of the clinical signs, faeces examination and differential diagnosis of other causes of the signs. The only certain way to diagnose coccidiosis is by finding appropriate lesions and coccidia at necropsy (Levine, 1985).

1.8 TREATMENT

Few licensed drugs are available for the prevention or treatment of coccidiosis in goats and few workers have studied the effect of drugs against caprine coccidia. However, many drugs used in other animals, have been used empirically in goats. The sulphonamides were the first group of drugs to be used (Todd & Ernst, 1973) for treating coccidiosis in goats and

other species and are still being used in most places. Nitrofurazone (10 mg/kg) was claimed to be effective against goat coccidiosis (Tarlatis *et al.*, 1955). Amprolium has been evaluated in various doses and treatment schemes. Fitzsimmons (1967) gave doses of 25 or 50 mg/kg and found reduced oocyst output in naturally infected goats. Marlow (1968) treated naturally infected goats with single doses of 33 or 66 mg/kg of amprolium, with good results and body weight increases in treated goats. Horak *et al.* (1969) suggested that amprolium be given in daily doses of 100 mg/kg for four days for the treatment of coccidiosis.

A combination of monensin and sulphamethazine has been found to be of value in preventing coccidiosis in Angora goats but monensin appeared to have an additional advantage of promoting increased efficiency of feed utilization (Shelton *et al.*, 1982). Monensin was recently approved for use in Angora goats in the United States as a coccidiostat (Craig, 1986). Foreyt *et al.* (1986) obtained decreased oocyst counts and increased weight gains by treating kids with coccidiosis with decoquinate; no signs of toxicity were observed. McKenna (1988) reported that a single dose of toltrazuril (20 mg/kg live weight) resulted in rapid and significant reductions in oocyst counts in adults and kids with no adverse reactions.

CHAPTER TWO

MATERIALS AND METHODS

2.1 SOURCES OF SAMPLES

A total of 1342 faecal samples were collected from 40 does aged 2-8 years and 60 kids belonging to three different breeds (Saanen; Angora; Feral) on three different farms in the Palmerston North area (Table 2.1). The survey commenced in August 1987 and ended in September 1988. Kids from the Old West Road farm and from the Kimbolton farm were sampled fortnightly. Ballantrae does and kids, and does from the Old West Road farm were sampled monthly. The total numbers of adult and kid samples collected from each farm are given in Table 2.1.

The three farms were chosen for the study because they provided an opportunity to make interesting comparisons between them. Not only did they involve different breeds of goat, but very different systems of management, particularly in relation to the rearing of the kids, and different stocking densities. As the occurrence of heavy coccidial infections and clinical coccidiosis are greatly influenced by factors affecting the levels of oocysts available to young animals, one would expect significant differences between the three farms to emerge.

2.1.1 Old West Road Farm

This is a small (4.2 hectare) heavily stocked, private, dairy goat farm, adjacent to Massey University. Twenty Saanen does and twenty kids were selected randomly from the 60 adults and 35 kids on the farm. The kids were born in the last week of August and were sampled from approximately 6 weeks of age. Kids were separated from their does when they were 5-10 days old and then housed in a small pen (2x4 metres) with a dirt floor and fed reconstituted powdered milk *ad lib.* and concentrates for 3 months. The floor was poorly drained and so, became wet and muddy; also the pen received little sunlight.

Sampling of the kids started in the last week of October. At this time half of the faecal samples were not pelleted. By the beginning of November, two-thirds of the samples were not pelleted and six of the smallest kids (not in the sample group) had developed a watery diarrhoea. As the oocyst counts of sampled animals were high, the owner was informed that this was probably a manifestation of clinical coccidiosis but no treatment was given as the owner considered the cause to be nutritional. The six worst affected were later treated

by the author with Scourban Plus^R at the standard dose-rate (approx. 4-8ml/8-10kg). The initial dose was double, thereafter treated 24 hour intervals for twice. But over the next three weeks, three of the six died and one was killed for necropsy. The pathology report indicated severe chronic intestinal lesions attributable to coccidiosis. Consequently, at the end of November, all of the remaining kids including the sample group were treated by the owner with Neostriacin^R. Following treatment the kids showed normal appetites but their faeces remained soft.

In the last week of November, the kids were weaned onto a paddock which had not been grazed by the other stock for 2 months. Hay and concentrates were also given but the latter was stopped at the end of December. From mid-March, kids and does grazed together except for three buck kids which were separated from the main group at weaning and placed in a separate paddock together with adult bucks. After weaning, kids were drenched monthly for nematode parasites with Ivomec^R at the manufacturer's recommended dose-rate, and does when mean faecal nematode egg counts exceeded 1000 eggs/g.

2.1.2 Ballantrae Farm

This is a research farm owned by the Department of Scientific and Industrial Research, and located 35 km North East of Massey University in the county of Woodville. Pastures are well developed. Cattle, sheep and "Feral" goat are run on various parts of the farm for experimental purposes. The goats used in this study were not grazed with other species.

The sample groups comprised 20 does and 17 kids run with varying numbers of other goats over an area of approximately 16 hectares. The overall stocking rate varied with the requirements of other experiments in progress but was in the region of 12-15 does and 12-15 kids/ha for most of the year. This is regarded as a relatively high stocking rate for the type of country (Betteridge pers. com.). At the start of the sampling period, the does were 2 years of age and were being used as a control group for another experiment. Kids were born in the last week of September and remained with the does until they were

R Neostriacin (Pitman-Moore)
Containing sulphadiazine, sulphamerazine, sulphapyridine, neomycin sulphate and kaolin.

Scourban Plus (Vetco)
Containing sulphaguanidine, sulphadimidine, sulphadiazine, neomycin, streptomycin, hyoscine hydrobromide, electrolytes, glycine and pectin.

Ivomec Liquid (Merck Sharp & Dohme)
Containing ivermectin.

weaned in February. No hay or concentrates were given and grazing was on a very slow rotational basis. Seventeen kids (10 females, 7 castrated males) were randomly selected from 30 of the earliest born. Kids were sampled from approximately 4 weeks of age. Because the anal sphincters of the kids were too small for the conventional method of faecal collection, the technique described in 2.2 was used.

Table 2.1 **Source of Faecal Samples**

Place Samples	Kids			Adults	
	Breed	No. used	Total Samples	No. used	Total
Old West Road Farm Palmerston North	Saanen	20	404	20	185
Ballantrae Farm Woodville	Feral	17	176	20	227
Kimbolton Farm Kiwitea	Angora	23	350	nil	-
Total Samples		930		412	

In March, about 50 kids on the farm died of clinical coccidiosis and yersiniosis; the rest were treated with Scourban Plus^R at the manufacturer's recommended dose-rate. Some of the kids from the present study group were dosed accidentally, but it is not known which. Throughout the study period, none of the experimental group showed signs of coccidiosis and their faeces remained pelleted. From June, the kids were again grazed together with does. Does and kids were drenched monthly for nematode parasites with Ivomec^R at the manufacturer's recommended dose-rate.

2.1.3 Kimbolton Farm

This farm is run privately and is located 50 km North of Massey University in the county of Kiwitea. The owner specialises in sheep breeding but also rears Angora goats and cattle. Kids were born from the last week of August and 23 kids (7 females, 16 males) were randomly selected from 100 for the study. The kids were separated from their mothers by

3-5 days after birth and housed in a spacious pen with a slatted floor. The floor was cleaned daily by hosing. The kids were given reconstituted powdered milk *ad lib.* and from 3 weeks of age, access to grazing (approx. 0.4 ha/40 kids). The kids were weaned in December and given hay and concentrates for one month.

Kids were sampled from approximately 4 weeks of age, initially using the technique described in 2.2. In the last week of February, none of the faecal samples were pelleted. In the first week of March, all kids were given Coxiprol^R at standard dose-rate (approx. Amprolium 20mg/kg) daily by mouth for 3 consecutive days by the owner who thought the +animals were suffering from coccidiosis. Treatment was given without my knowledge. The females, including those in the sample group, were sold in May and some of the males were sold subsequently so that by September only 8 remained. Kids were drenched monthly with Ivomec^R at the manufacturer's recommended dose-rate for nematode parasites.

2.2 COLLECTION OF FAECAL SAMPLES FROM KIDS AGED <2 MONTHS

The anal size of kids age <2 months was too small for the conventional method of faecal collection. A glass test tube 10x75 mm with a smooth edge was therefore used as an extractor. The tube top was dipped in lubricant or liquid soap. The tube was half-filled with tap water and inserted into the rectum. Faecal pellets were expelled into the tube together with water. This procedure was repeated until the required amount of faeces was obtained. This method was not suitable for non-pelleted samples.

2.3 EXAMINATION OF INDIVIDUAL SAMPLES

A total oocyst count was carried out on a 2 g subsample of each sample collected. In addition, oocysts were recovered from a further subsample and sporulated, and the species present identified in a random sample of 100 oocysts. The details of the procedures used follow.

2.3.1. Oocyst counting

All the samples were collected directly from the rectum and stored at 4°C until used. Oocysts were counted using a modified McMaster technique (Soulsby, 1984) as follows:

1. Two grams faeces were weighed and 58 ml saturated NaCl solution (specific gravity 1.2)

R Coxiprol (Technik)
Containing amprolium.

measured out. The faeces were homogenised in some of the salt solution in a bowl. The suspension was then poured through a 500 µm aperture sieve and the remaining salt solution was used to wash the bowl and the material retained on the sieve. If the oocyst count was >100,000 oocysts/g, a second sample was processed using a total of 118 ml salt solution. This was to facilitate counting.

2. Samples were withdrawn using a Pasteur pipette and run into two counting chambers. The total number of oocysts counted, multiplied by 100 (or 200 if the larger volume was used), represented the number of oocysts contained in one gram of faeces (OPG).

2.3.2 Separation of oocysts for sporulation for samples with >500 OPG

Oocysts were separated from faecal debris by flotation with salt solution. Detailed procedures are as follows:

1. 5 g faeces from each sample were homogenised with 50 ml tap water and filtered through a 500 µm aperture sieve followed by 105 µm sieve; the residue in each sieve was washed with a jet of tap water from a wash-bottle.
2. Approximately 50 mls filtrate were centrifuged at 800 g for 6 minutes.
3. Two-thirds of the supernatant was discarded. The sediment was resuspended in tap water and recentrifuged as in step 2.
4. The resultant sediment was resuspended in NaCl salt solution (specific gravity 1.2) and allow to stand for 10 minutes to allow coarse material to sink with little chance of trapping oocysts. The suspension was then centrifuged at 400 g for 6 minutes.
5. The tube was removed gently from the centrifuge and allowed to stand for a further 10 minutes in order to compensate for any disturbance of the oocyst band at the top.
6. Approximately 5 mls was sucked from the top using a "U" tip pipette attached to a suction pump and collected in a 50 ml centrifuge tube.
7. The oocysts were washed free of salt solution by suspension and centrifugation in distilled water twice.

8. The washed sediment was transferred to a 15 ml graduated conical centrifuge tube and centrifuged at 150 g for 6 minutes.
9. The supernatant (approx: 12 mls) was discarded and the sediment resuspended in 2.5% potassium dichromate solution. The total volume was not >5 ml. The suspension was then placed in a 35 mm petri dish.
10. The petri dish was placed in a 27^oC room for 14 days. A few drops of potassium dichromate solution were added on the 7th day to protect the oocysts from desiccation. The recovery of oocysts for examination was carried out as in 2.3.4.

2.3.3 Separation of oocysts for sporulation from samples with <500 OPG

Oocysts were recovered from faecal debris as in 2.4 steps 1 to 4 but the procedure was carried out at room temperature. The collected oocysts were then processed as in 2.3.2 steps 7 to 10 above.

2.3.4 Recovery of sporulated oocysts

Petri dishes were removed from the 27^oC room and the oocysts were recovered as follows:

- 1 The oocyst suspension was stirred thoroughly in order to free the oocysts which usually adhere to the bottom.
2. The suspension was washed into a 15 ml tube; sucrose solution (specific gravity 1.2) was added carefully to form a convex meniscus and a coverslip placed over it.
3. After 10 minutes the coverslip was removed, placed on a microscope slide and the sporulated oocysts examined at 500x magnification.

2.3.5 Method of Identification of species

Most samples contained mixed infections. Identification of unsporulated oocysts is difficult. Although there are a few species such as *E. christenseni*, *E. apsheronica*, and *E. alijevi* which can be recognized by their typical oocyst size and shape without sporulation, many species have specific structural characteristics which can only be seen clearly in fully sporulated oocysts. In the present study, therefore, species were only identified after sporulation. The number of layers and the colour of oocyst walls were confirmed by

crushing individual oocysts with gentle coverslip pressure.

Species were identified according to descriptions given by the following authors:- Levine *et al.* (1962); Shah & Joshi (1963); Chevalier (1966); Levine & Ivens (1970); Lima (1979a) & (1980a&b); Musaev & Mamedova (1981) and Vercruysse (1982); Levine (1985); Levine & Ivens (1986).

Species were identified on characteristics considered in the following order:

1. Presence and absence of micropylar cap and its characteristics.
2. The oocyst size and shape.
3. Characteristics of the micropyle if present (distinct or indistinct).
4. Number of polar granules.
5. Size and shape of sporocysts.
6. Presence or absence of sporocyst residuum and its characteristics, if present.
7. Presence or absence of a stieda body.
8. Position of sporozoites in the sporocyst.
9. Number and size of refractile globules in each sporozoite.

To determine oocyst and sporocyst dimensions for comparative purposes and statistical analysis, 100 oocysts of each species were measured using an Olympus BH2 microscope with apochromatic objectives and a digital micrometer. The oocysts measured for each species were from samples collected from different farms on different days. No more than five oocysts of a given species were measured from any one sample.

2.4 EXPERIMENT FOR THE DETERMINATION OF SPORULATION TIMES OF *E. CHRISTENSENI* OOCYSTS AT DIFFERENT TEMPERATURES

As large numbers of *E. christensenii* oocysts were available, it was decided to determine the sporulation times at 4°C, 10°C, 15°C, 20°C, 27°C and 37°C. A preliminary experiment indicated the stages of sporulation and that sporulation was complete in approximately 1 week or less at 27°C. Oocysts were considered to be fully sporulated when the morphological features evident in oocysts sporulated for 14 days at 27°C were present.

The experiment was conducted as follows:

Faeces were collected from 4 kids with very high *E. christensenii* oocyst counts. Samples were collected per rectum into plastic containers which were immediately placed in a

polystyrene "chilly bin" half-filled with ice. Processing was completed within 24 hrs of collection and at 4°C. For the recovery and cleaning of oocysts, tap water, distilled water, NaCl solution and two 150 mm petri dishes were kept at 4°C; bottles of potassium dichromate solution were kept at each of the different experimental temperatures; 35 mm petri dishes for cultures were also kept at the appropriate experimental temperatures prior to use.

The procedure for oocyst recovery was as follows:

1. A faecal homogenate was prepared as in 2.3.2. steps 1 & 2, but the volume increased to 100 ml. This was then divided between two 50 ml centrifuge tubes and centrifuged at 800 g for 6 minutes.
2. The sediment was resuspended in approximately 200 mls saturated NaCl solution and poured into a 150 mm petri dish lid. The base portion of the petri dish was floated on the surface of the salt solution for 30 minutes (Fig 2.1). This was carried out at 4°C in a refrigerator.

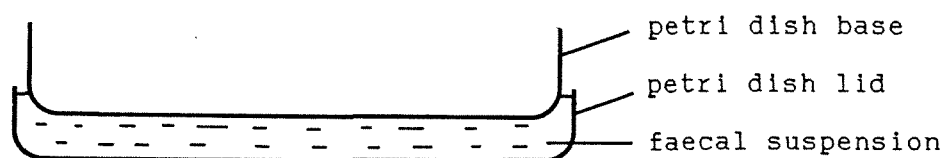


Fig. 2.1 Flotation and recovery of coccidial oocysts using a petri dish lid.

3. The base was lifted gently and the surface washed into another petri dish to collect adherent oocysts.
4. The faecal suspension was stirred and the base washed and dried before a further collection was made.

5. The collected oocysts were washed and concentrated by centrifugation as in 2.3.2 steps 7 & 8.
6. The supernatant was discarded. The sediment was resuspended and made up to 60 ml with distilled water. The suspension was kept on ice in a 'chilly bin' until samples were taken. Using a micropipette, 0.25 ml aliquots of thoroughly mixed oocyst suspension were put into the petri dishes kept at the various temperatures and 5 ml temperature-conditioned potassium dichromate solution were added to each. All cultures were set up on the same day.
7. Samples of oocysts were examined on alternate days from two culture dishes kept at 4^oC (as development was expected to be slow), daily for those at 10^oC, 15^oC, 20^oC and 27^oC and 12 hourly for those at 37^oC. The recovery of oocysts were carried out as in 2.3.4. One hundred oocysts from each dish were classified according to their development stage. The time required for completion of each development stage was taken as that needed for 90% of oocysts to reach that stage.

2.5 DATA ANALYSIS AND GRAPH PRODUCTION

All data were analysed using Statistix PC DOS Version 2.0, NH Analytical Software, USA and the graphs were produced using SlideWrite Plus, Advanced Graphic Software Inc., USA.

CHAPTER THREE

EIMERIA SPECIES IDENTIFIED

3.1 RESULTS

During the examination of the 1342 faecal samples from adult and kid goats, ten previously named species, two others whose species status is uncertain and three previously undescribed species (Table 3.1) were identified. *E. korcharli*, *E. punctata*[@] and *E. pallida* were not identified.

The species identified were as follows:-

Named *Eimeria* species**With micropylar cap**

1. *E. christenseni*
2. *E. tunisiensis* (*E. ahsata*)
3. *E. jolchejevi*^{*}
4. *E. arloingi*
5. *E. hirci*^{*}

Without micropylar cap

6. *E. caprina*
7. *E. caprovina*
8. *E. apsheronica*
9. *E. ninakohlyakimovae*
10. *E. alijevi*

* These species were identified as having two distinct size-populations of oocyst as described below.

Previously undescribed species

As an interim measure, these have simply been given code names as follows:

With micropylar cap

1. *E. n1*
2. *E. n2*

Without micropylar cap

1. *E. n3*

[@] After the study was completed, during re-examination of some oocysts in a pooled sample kept as reference material, a single oocyst of *E. punctata* was found (see Appendix 7).

The morphology of the various oocysts identified is shown in Figs. 3.1-3.4 and the morphological characteristics, based on detailed examination and measurement of 100 oocysts of each species from different sources are given in Table 3.1. The descriptive statistics of each species are recorded in Appendix 1. As the typical characteristics are given in the tables and figures, in the following descriptions only those which were different from, or additional to, those previously published (Table 1.2) are given. Detailed descriptions are given for previously undescribed species.

General remarks

In the descriptions of the oocyst walls of various species, the number of layers and colours of the layers recorded, vary between different authors (Table 1.2). To clarify this matter, oocysts of each identified species were examined after sporulation in distilled water, rather than potassium dichromate, and the oocysts broken by pressure to separate the layers of the wall. This revealed that all the oocysts possessed a smooth oocyst wall with 2 layers, including those of *E. apsheronica* which was described as having a single-layered oocyst wall (Shah & Joshi, 1963; Musaev & Mamedova, 1981). In all species the outer layer accounted for almost the whole thickness of the wall; in intact oocysts, the inner layer appeared simply as a dark line on the inner surface of the wall. In all the oocysts, the outer layer was a yellowish brown color and the inner was colourless. The colour of the outer layer could be separated into 3 different tones depending on the species concerned. Deep colouring was observed in *E. christenseni*, *E. tunisiensis*, *E. jolchejevi* 'large form' and *E. caprina*; intermediate colouring in *E. jolchejevi* s.s., *E. arloingi*, *E. n2*, *E. hirci* 'large form', *E. caprovina*, *E. apsheronica* and *E. ninakohlyakimovae*; pale colouring in *E. n1*, *E. hirci*, *E. n3*, and *E. alijevei*. The colours were deeper in oocysts which were sporulated in 2.5% potassium dichromate solution. The oocyst layers appeared thicker and the colours more intense in oocysts which were prepared in sucrose flotation medium rather than in NaCl solution, presumably because of the different refractive indices of the two solutions. For these reasons, the colour of oocysts is of limited value in their identification and should be used with caution.

An oocyst residuum was not seen in any of the oocysts examined. All the oocysts possessed a micropyle which varied in the degree of distinction. *E. caprina*, *E. caprovina* and *E. apsheronica* have a much more distinct micropyle than other species. The number of polar granules present in each oocyst was not consistent even within the same species and they were sometimes absent. However, fragmentation of the polar granules into fine granules was seen in a high proportion of certain species such as *E. tunisiensis*, *E. n1*, *E. caprina* and *E. ninakohlyakimovae*.

A variety of terms have been used to describe the shape of oocysts and sporocysts. To avoid confusion, the terms used in descriptions in this study are defined as follows:

ellipsoid/ellipsoidal:	symmetrically elliptical in shape
ovoid:	egg-shaped with the broader end remote from the micropyle/polar cap
urn-shaped:	egg-shaped with the broader end towards the micropyle/polar cap

3.1.1 Morphological characteristics of named species with a micropylar cap

(i) *Eimeria christenseni* Levine, Ivens & Fritz, 1962 (Figs. 3.1a, 3.2a)

Synonym: none

No features differing from or additional to those previously published were observed.

(ii) *Eimeria tunisiensis* Musaev & Mamedova, 1981 (Figs. 3.1d, 3.2d)

Synonym: *E. ahsata*

Morphological characteristics: The oocyst is large, ellipsoidal with a prominent flattened dome-shaped micropylar cap and a micropyle. The oocyst wall is composed of 2 layers, the outer one smooth, dark yellowish brown and the inner colourless. In 87% of the oocysts measured, the polar granules were shattered into fine granules; in the others approximately 10-12 polar granules could be seen. The dimensions of the oocysts and sporocysts are given in Table 3.1. The sporocysts are elongate ovoid with a large, coarse sporocyst residuum. A stieda body is present. The sporozoites lie lengthwise, head to tail in the sporocyst. Each contains one small and one large refractile globule, one at each end.

Remarks: Before the publication of Honess' (1942) paper, the ovine species *E. ahsata* was included under the name *E. arloingi* (now known as *E. bakuensis*). Levine & Ivens (1970) considered Chevalier's (1966) finding of *E. ahsata* in the goat as a variant of *E. arloingi* and they still maintain that *E. ahsata* (or its equivalent) does not occur in goats (Levine & Ivens, 1986). However, various other workers have recorded *E. ahsata*-like oocysts from goats (see Table 1.3) and Musaev & Mamedova (1981) have renamed these as *E. tunisiensis*. It seems that *E. ahsata* and *E. bakuensis*, and *E. tunisiensis* and *E. arloingi* share many features although they differ in size.

Table 3.1 Morphological Characteristics of Eimeria Species of goats found in New Zealand

Species	Oocyst Characteristics				Sporocyst Characteristics			
	size(um)*; mean(SE); range; shape index(mean) shape;	Micro- pyle	Oocyst wall colour	Polar granules	Size(um)*; mean(SE); range; shape index(mean) shape;	Stieda body	Sporocyst residium	Refractile globules
With a micropylar cap								
<u>E. christenseni</u>	39(0.19)x25(0.14) 35-44x22-30 1.4-1.8(1.5) ovoid;	+	dark	+	15(0.08)x9(0.06) 15-18x7-10 1.4-1.8(1.6) broadly ovoid	+	+ large coarse	1 small & 1 large
<u>E. tunisiensis</u>	34(0.16)x24(0.09) 30-38x21-26 1.3-1.6(1.4) ellipsoid;	+	dark	+ & S	16(0.07)x8(0.04) 14-17x7-10 1.6-2.3(1.9) elongate ovoid	+	+ large coarse	1 small & 1 large
<u>E. jolchejevi s.s.</u>	31(0.13)x23(0.08) 28-34x20-24 1.1-1.5(1.4) ellipsoid; occ. urn-shaped;	+	medium	+	15(0.08)x8(0.04) 12-17x7-10 1.5-2.3(1.9) elongate ovoid	+	+ clustered	1 small 1 large
<u>E. jolchejevi</u> <u>large form'</u>	37(0.23)x26(0.11) 32-41x24-29 1.2-1.6(1.4) broadly ellipsoid; oblong; urn-shaped;	+	dark	+	16(0.10)x9(0.05) 15-18x7-10 1.6-2.0(1.8) elongate ovoid	+	+	1 small & 1 large or 2 large
<u>E. arloingi</u>	29(0.13)x19(0.12) 26-32x16-23 1.2-1.7(1.5) ellipsoid/ovoid;	+	medium	+	14(0.09)x7(0.04) 13-17x6-8 1.7-2.4(2.0) elongate ovoid	+	+	1 small & 1 large

<u>E. n2</u>	29(0.16)x20(0.16) 25-34x18-25 1.3-1.7(1.5) ellipsoid;	+	medium	+	13(0.06)x8(0.04) 11-14x7-9 1.4-1.9(1.7) broad ovoid	+	+	sparse	1 small & 1 large
<u>E. hirci</u> <u>small form'</u>	20(0.12)x17(0.11) 17-22x15-20 1.0-1.3(1.2) broadly ellipsoid; subspherical;	+	light	+	10(0.07)x7(0.05) 8-13x5-8 1.0-1.3(1.2) broad ellipsoid	+	+	few ring form	1 small & 1 large
<u>E. hirci</u> <u>large form'</u>	26(0.17)x19(0.11) 23-31x16-23 1.1-1.7(1.4) ellipsoid; occ. ovoid;	+	medium	+	11(0.08)x8(0.04) 10-13x6-8 1.3-2.0(1.5) broad ellipsoid;	+	+	few ring form	1 small & 1 large
<u>E. n1</u>	22(0.16)x17(0.10) 18-26x15-20 1.0-1.5(1.3) broadly ellipsoid; broadly ovoid;	+	light	+ & S	12(0.08)x6(0.05) 10-14x5-7 1.7-2.6(2.0) elongate ovoid	+	+		1 small & 1 large
Without a micropylar cap									
<u>E. caprina</u>	33(0.12)x23(0.08) 29-36x21-25 1.3-1.6(1.4) ovoid/ellipsoid;	+ & D	dark	+ & S	15(0.06)x8(0.04) 14-17x7-9 1.6-2.2(1.8) elongate ovoid	+	+	coarse	1 small & 1 large
<u>E. caprovina</u>	28(0.18)x22(0.15) 22-32x18-25 1.0-1.4(1.3) broadly ovoid; subspherical	+ & D	medium	+	14(0.08)x8(0.05) 12-15x6-9 1.5-2.2(1.8) elongate ovoid	+	+		1 small & 1 large
<u>E. apsheronica</u>	30(0.23)x22(0.13) 25-36x19-25 1.2-1.6(1.3) broadly ovoid	+ & D	medium	+	15(0.10)x8(0.01) 12-17x7-9 1.6-2.0(1.8) elongate ellipsoid	+	+	coarse	1 small & 1 large

<u>E. ninakohlyakimovae</u>	24(0.13)x19(0.09) 21-27x16-21 1.1-1.5(1.3) broadly ellipsoid broadly ovoid	+	medium	+ & S	13(0.07)x7(0.05) 11-14x5-8 1.6-2.3(1.9) elongate ovoid	+	+	clustered	1 small & 1 large
<u>E. n3</u>	23(0.10)x17(0.05) 20-26x16-19 1.1-1.5(1.3) broadly ellipsoid	+	light thin	+	13(0.05)x6(0.03) 11-14x5-7 1.7-2.5(2.0) elongate ellipsoid elongate ovoid	+	NV		1 small & 1 large or 1 large
<u>E. alijeви</u>	17(0.11)x14(0.06) 15-20x13-15 1.1-1.4(1.2) ovoid subspherical	+	light	+	9(0.06)x5(0.03) 8-11x4-6 1.5-2.1(1.9) elongate ovoid	NV	+	clustered	1 small & 1 large

* The figures are rounded up to the nearest figures for the convenience of presentation (the actual data are presented in the Appendix 1). NV = Not visible. S = Polar granules are shattered into fine granules. D = Distinct.

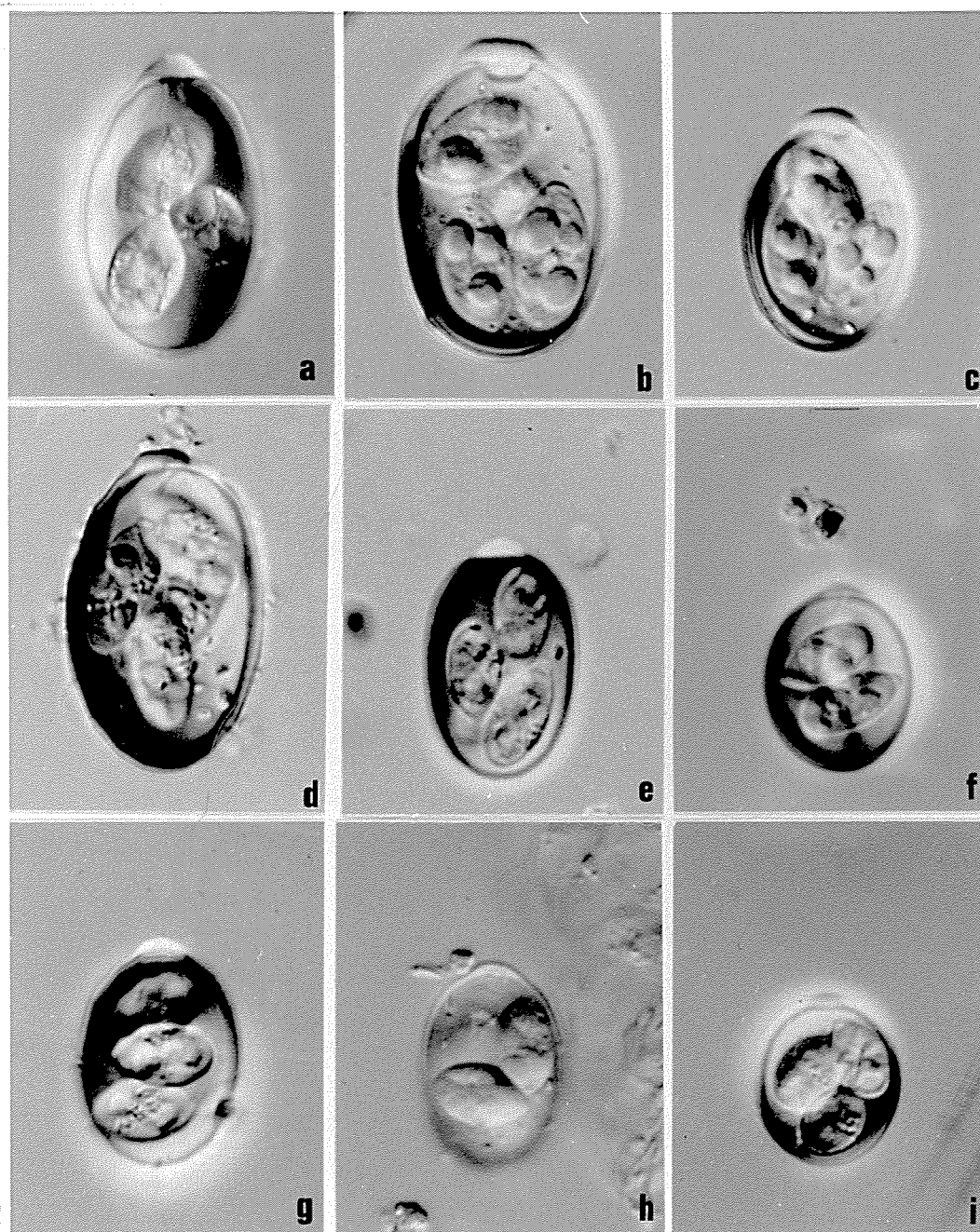


Fig. 3.1 Photomicrographs of the sporulated oocysts of *Eimeria* species (with a micropylar cap) identified in goat faeces. Differential interference contrast x1122. (a) *E. christenseni*; (b) *E. jolchejevi* 'large form'; (c) *E. jolchejevi* s.s.; (d) *E. tunisiensis*; (e) *E. arloingi*; (f) *E. n1*; (g) *E. n2*; (h) *E. hirci* 'large form'; (i) *E. hirci* 'small form';

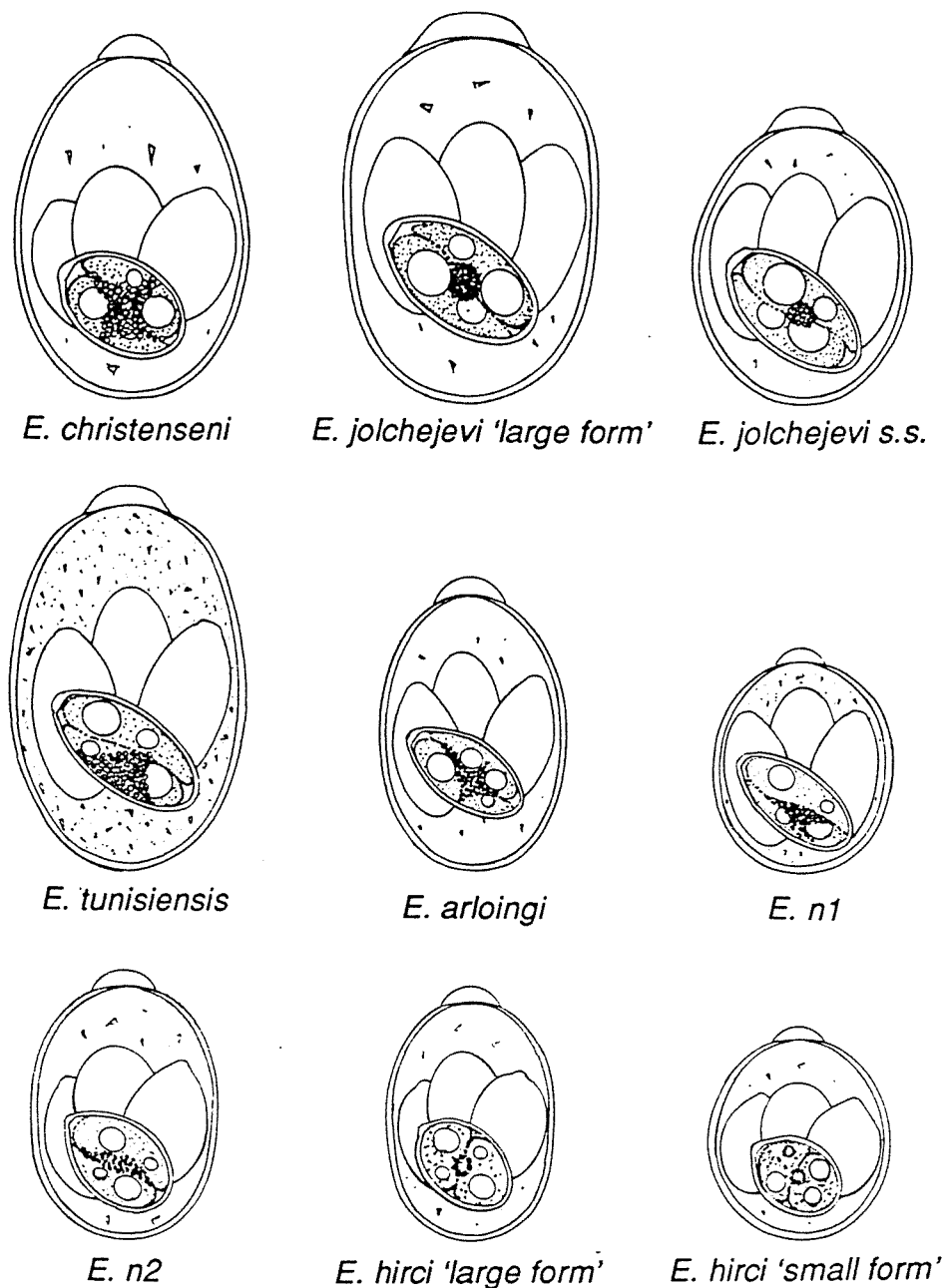


Fig. 3.2 Schematic diagrams of the sporulated oocysts of *Eimeria* species (with micropylar cap) identified in goat faeces. X 11 22

It is accepted that for most *Eimeria* species infecting sheep there is an equivalent species with oocysts of similar morphology infecting goats. It was not surprising, therefore, to find the features of the *E. ahsata*-like oocysts from the present study to be virtually identical to those described from sheep (Smith *et al.*, 1961, Gregory *et al.*, 1987 and Hidalgo-Arguello & Cordero-Del-Campillo, 1988) although the oocysts of the sheep species are larger. The

dimensions of the oocysts identified in the present study as *E. tunisiensis* (Table 3.1) are consistent with the published data from goats (Table 1.2) although Musaev and Mamedova (1981), who only measured 5 oocysts, give a wider range than anyone else. The oocysts in the present study were also larger than those identified as *E. arloingi*, as has been recorded by others (Table 1.2), and formed a discrete size group.

Furthermore, the *E. ahsata*-like oocysts found in the present study were morphologically different from other valid capped species from goats. The ellipsoid shape of the oocyst and the elongate-ovoid sporocysts of this species distinguish it from *E. christenseni* with its ovoid oocyst and broadly ovoid sporocysts. In the *E. ahsata*-like oocysts the refractile globules in the sporozoites are much less prominent than in *E. jolchejevi*. In addition to these oocysts being larger than *E. arloingi*, the sporocyst residuum is coarsely granular whereas in *E. arloingi* the granules are fine.

E. tunisiensis is not only morphologically distinguishable but is also statistically significantly different in all oocyst and sporocyst dimensions from *E. arloingi* ($p < 0.001$) (Appendix 2, Table 1), *E. christenseni* ($p < 0.001$) (Appendix 2, Table 9), *E. jolchejevi* 'large form' ($p < 0.05$ - 0.001) (Appendix 2, Table 10) and from *E. jolchejevi* s.s. ($p < 0.001$) except for sporocyst length ($p > 0.05$) (Appendix 2, Table 11). For these reasons, the *E. ahsata*-like oocysts observed are considered to be those of *E. tunisiensis* and the above morphological data as confirmation of this as a separate species infecting goats, equivalent to *E. ahsata* of sheep.

(iii) ***Eimeria jolchejevi*** Musaev, 1970 (Figs. 3.1b,c, 3.2b,c)

Synonym: none

As well as oocyst size and shape (variously described as urn-shaped or ellipsoid Table 1.2), the diagnostic features of *E. jolchejevi* include a prominent truncated-cone shaped micropylar cap and extremely prominent globules in the sporozoites. The latter are depicted in O'Callaghan's (1989) paper and are also shown in photographs of the equivalent species (*E. granulosa*) from sheep (Gregory *et al.*, 1987; O'Callaghan, 1988) but have not been commented on by any author.

In the examination of oocysts with the characteristics of those of *E. jolchejevi*, the range of oocyst sizes observed was much wider than reported for this species. These oocysts appeared to fall into two populations in terms of size (Figs. 3.3a, b) and shape. The smaller ones (Figs. 3.1c, 3.2c) were generally ellipsoidal, occasionally slightly urn-shaped and ranged in size from 28.0-34.4 x 20.4-24.5; the larger ones (Figs. 3.1b, 3.2b) were more broadly ellipsoidal or oblong, often somewhat broader towards the micropylar end (ie. urn-

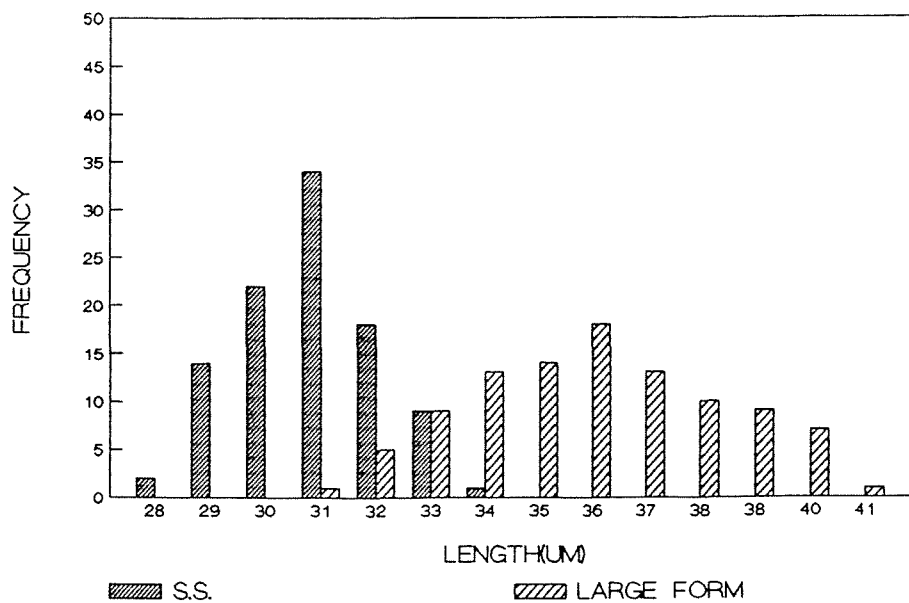


Fig. 3.3a Frequency distribution of oocyst length of *E. jolchejevi* s.s. and *E. jolchejevi* 'large form'

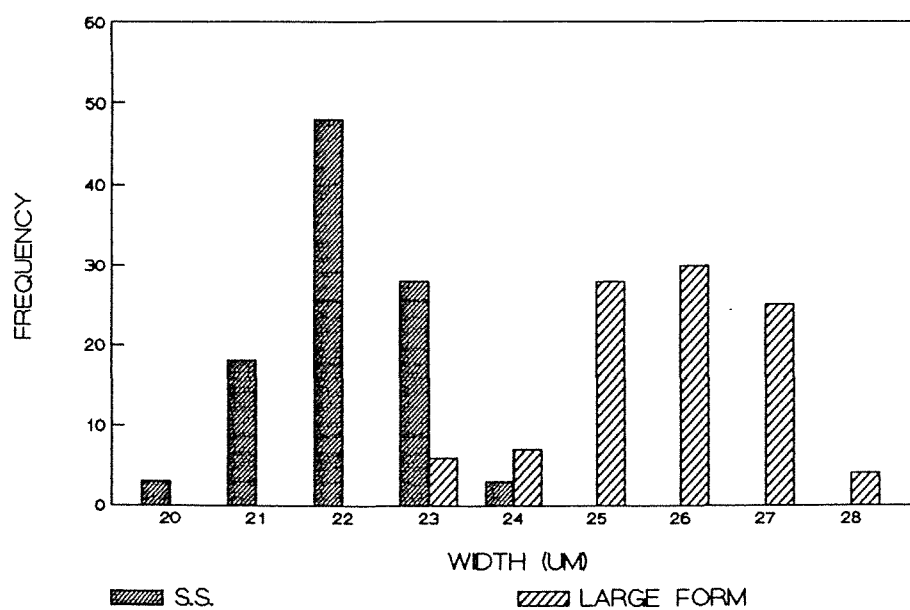


Fig. 3.3b Frequency distribution of oocyst width of *E. jolchejevi* s.s. and *E. jolchejevi* 'large form'

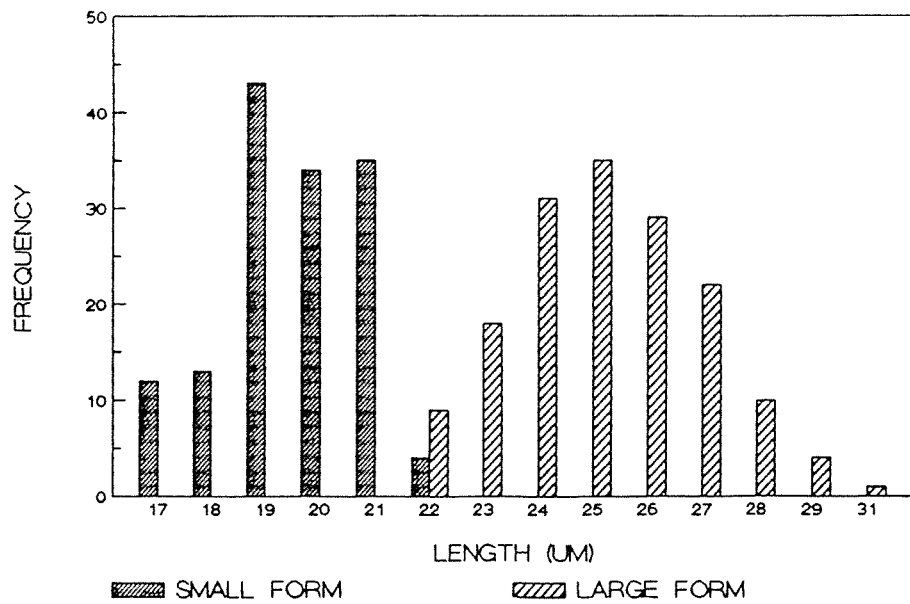


Fig. 3.4a Frequency distribution of oocyst length of *E. hirici* small form and *E. hirici* 'large form'

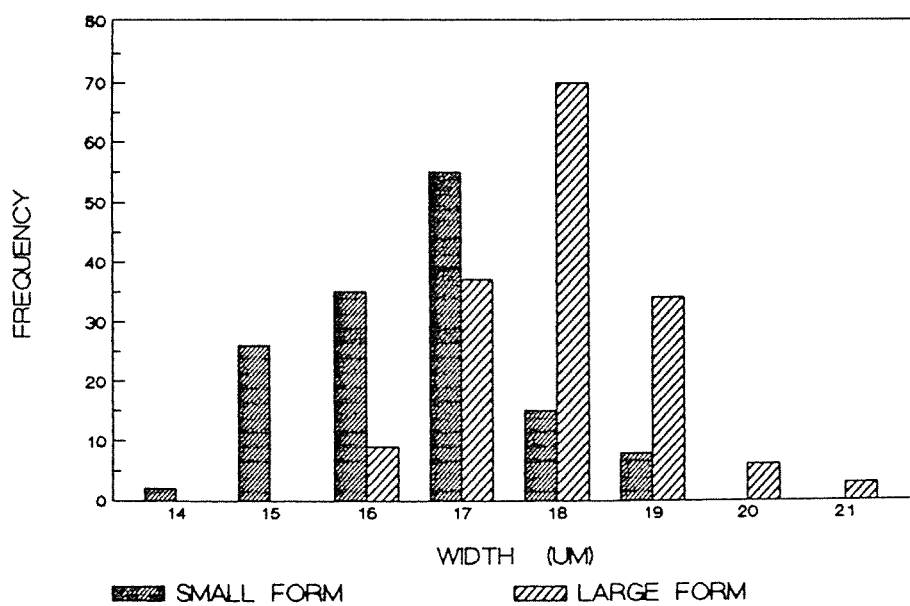


Fig. 3.4b Frequency distribution of oocyst width of *E. hirici* 'small form' and *E. hirici* 'large form'

shaped), and ranging from 32.0-41.4 x 23.6-29.0 μm in length. The oocyst and sporocyst dimensions and length-width ratios of the two forms (Appendix 1A) were compared by two sample t-tests and all were significantly different ($p < 0.001$) (Appendix 2, Table 2). The dimensions of the smaller form correspond with the published data for *E. jolchejevi* (Table 1.2) and this form has, therefore, been designated *E. jolchejevi sensu strictu (s.s)*. The data for the larger oocysts overlap to a small extent both the published ranges for *E. jolchejevi* and those of the smaller form described here, but are clearly distinct. Oocysts in this category have been designated *E. jolchejevi 'large form'* as an interim measure. In both forms, the sporocysts are elongate ovoid, the sporozoites lie lengthwise, head to tail in the sporocysts; each contains one large and one small, prominent refractile globule. Sporocyst residuums were mostly aggregated rather than diffuse. Polar granules were present.

(iv) *Eimeria arloingi* Marotel, 1905 (Figs. 3.1e, 3.2e)

Synonym: E. ovinoideal

No features differing from those previously published were observed. In addition, most oocysts had a tendency towards relatively straight sides.

(v) *Eimeria hirci* Chevalier, 1966 (Figs. 3.1h,i, 3.2 h,i)

Synonym: E. crandallis

In the examination of oocysts corresponding morphologically with *E. hirci*, it became clear that again there was more than one size category of oocyst involved. Initially, it was considered there might be three size populations and oocysts were arbitrarily allocated to one of three size-groups. One hundred oocysts of each of these size-classifications were carefully measured. Subsequently, analysis of the data showed that, in fact, there were two size categories (Fig.3.4 a, b), oocysts in the intermediate group falling into 2 groups indistinguishable from the larger and smaller categories. As the published dimensions of *E. hirci* oocysts encompassed the range of both size categories, in the following description these species have been classified as *E. hirci 'small form'* and *E. hirci 'large form'*.

The results of two sample t-tests showed that except for the sporocyst shape index, all of the other measurements of oocysts and sporocysts were significantly different ($p < 0.001$) (Appendix 2, Table 3). As indicated by the oocyst shape indices, the small form oocysts are more broadly ellipsoid than the large. In both forms, however, the sporozoites and their refractile globules were usually difficult to see but, where visible, the sporozoites were found at either end of the sporocyst, lying transversely rather than lengthwise; this agrees with descriptions by Levine, Ivens & Fritz (1962) and Shah & Joshi (1963). In addition to the size

differences, the oocyst wall colours also differed being pale in *E. hirci* 'small form' and medium yellowish brown in *E. hirci* 'large form'.

3.1.2 Morphological characteristics of named species without a micropylar cap

(i) *Eimeria apsheronica* Musaev, 1970 (Figs. 3.5a; 3.6)

Synonym: E. faurei Moussu & Marotel, 1901

The observed oocysts closely resemble the descriptions given by others (Table 1.2) except for that of Musaev & Mamedova (1981), whose size-ranges are very wide and probably do not represent the true range for this species. In contrast to published information, the oocyst walls are double-layered, the outer one intermediate yellowish brown and the inner colourless. A distinct stieda body is also present.

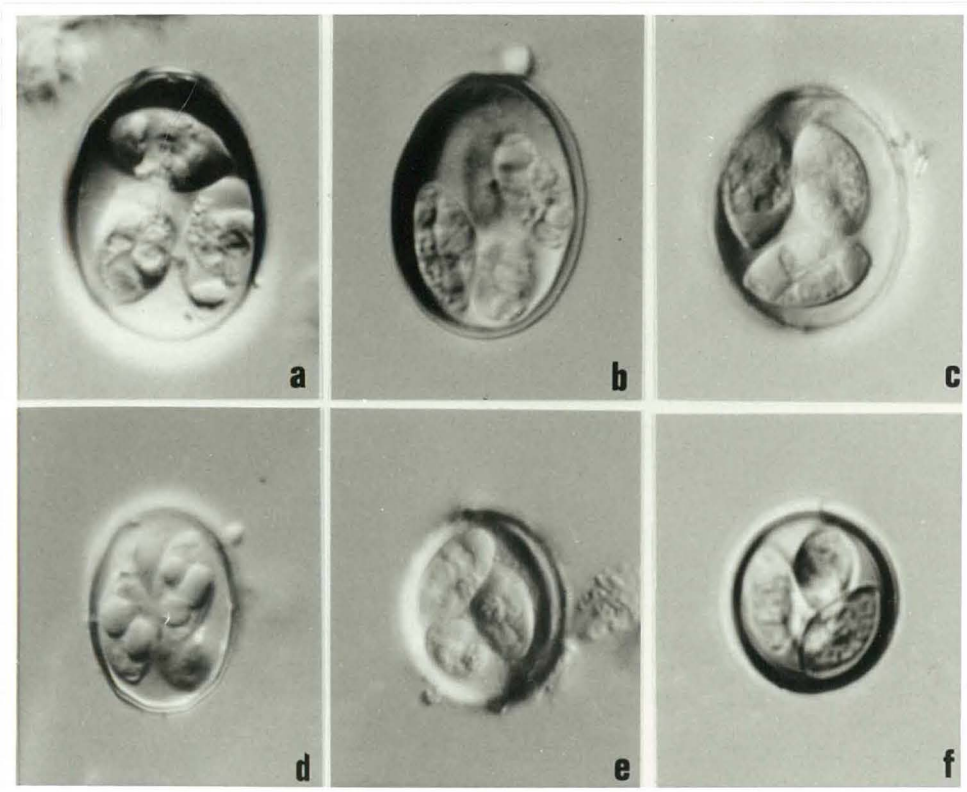


Fig. 3.5 Photomicrographs of the sporulated oocysts of *Eimeria* species (without a micropylar cap) identified in goat faeces. Differential interference contrast x1122.

(a) *E. apsheronica*; (b) *E. caprina*; (c) *E. caprovina*; (d) *E. n3*; (e) *E. ninakohlyakimovae*; (f) *E. aljevi*

(ii) *Elmeria caprina* Lima, 1979a (Figs. 3.5b; 3.6)

Synonym: none

No features differing from or additional to those previously published were observed.

(iii) *Elmeria caprovina* Lima, 1980a (Figs. 3.5c; 3.6)

Synonym: none

E. caprovina was identified by oocyst shape, the presence of a distinct micropyle, the sporocyst characteristics and the majority of oocysts having 1-5 polar granules, all of which are as described by Lima (1980a) (Table 1.2). However, the oocyst size was somewhat smaller than recorded by Lima (1980a) who also stated that the inner oocyst wall was coloured. This latter feature, referred to earlier, was not seen in any oocyst. This species has only been described once previously and because of the observed differences from the description by Lima (1980a), its identification in the present study must be regarded as tentative.

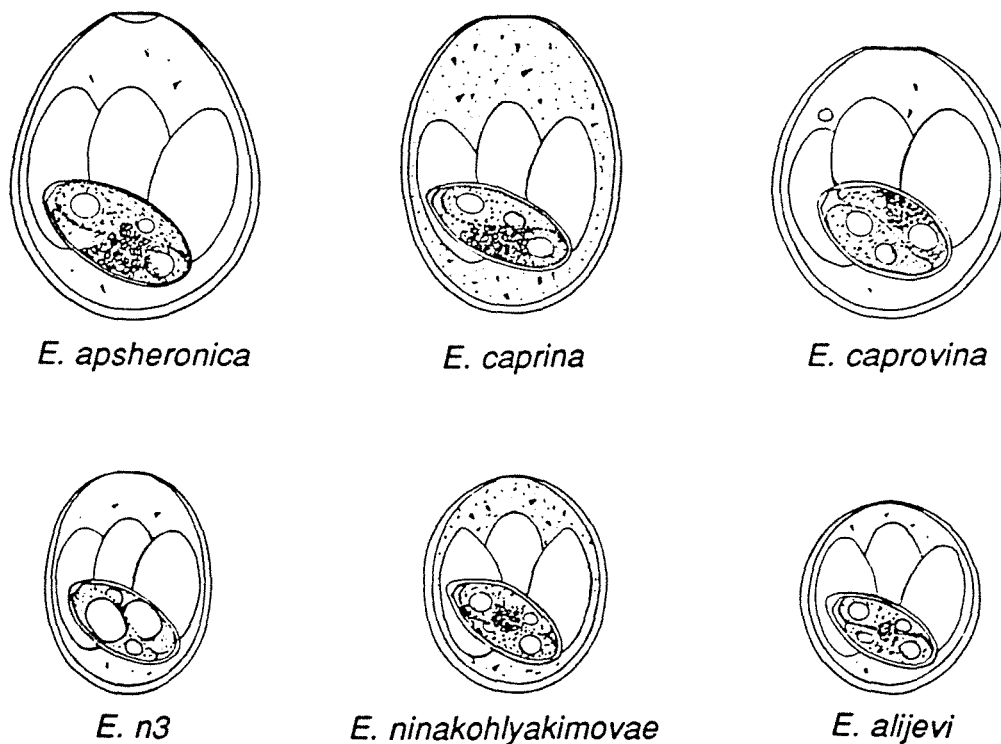


Fig. 3.6 Schematic diagrams of the sporulated oocysts of *Eimeria* species (without a micropylar cap) identified in goat faeces. X1122

- (iv) *Eimeria ninakohlyakimovae* Yakimoff & Rastegaieff, 1930 (Figs. 3.5; 3.6)

Synonym: Eimeria ovinoidalis

No features differing from those previously published were observed. In addition, the sporocyst residuums were mostly aggregated in between the two sporozoites.

- (v) *Eimeria alijevi* Musaev 1970 (Figs. 3.5f; 3.6)

Synonym: E. parva Kotlan, Mocsy and Vadja, 1929

The oocyst and sporocyst sizes are most similar to those given by Chevalier (1966); others have recorded a wider range of sizes (Table 1.2) and it is possible that they included oocysts of other similar species in their measurements. The sporocyst residuum is not visible. The sporozoites lie lengthwise, head to tail in the sporocysts; each contains one large and one small refractile globule.

3.1.3 Morphological characteristics of undescribed species with a micropylar cap

- (i) *Eimeria n1* (Figs. 3.1f; 3.2)

Synonym: none

Morphological characteristics: This is temporarily designated as *E. n1* (Table 3.1). It is a small, broadly ellipsoidal or broadly ovoid oocyst with a distinct dome-shaped micropylar cap. The oocyst wall is smooth, composed of 2 layers, the outer one pale yellowish brown and the inner colourless. A micropyle is present. Polar granules are shattered into fine granules in the majority of oocysts (72%), the rest containing 1-5 granules. The measurements of the oocysts and sporocysts are recorded in Table 3.1. The sporocysts are elongate ovoid with an indistinct stieda body. A sporocyst residuum composed of many scattered granules is present. The sporozoites are elongate and lie lengthwise, head to tail in the sporocyst. Each sporozoite usually contains one small and one large refractile globule, one at each end.

Remarks: Comparing the oocyst dimensions of this species with other capped-species described previously and in the present study, *E. hirci* 'small form' and *E. hirci* 'large form' (Tables 1.2 and 3.1) are the only species whose dimensions are sufficiently similar that the oocysts might be confused in the unsporulated state. However, statistical comparisons of oocyst and sporocyst dimensions with these species showed that they were significantly different ($p < 0.05-0.001$) (Appendix 2, Table 4 & 5).

When sporulated, the oocysts of *E. n1* are easily distinguished from those of *E. hirci* on the basis of the shape of the sporocysts, which are elongate in *E.n1* and broadly ellipsoid in *E. hirci*, and by the alignment of the sporozoites. Statistical comparison of the sporocyst shape indices of these two species from the present study show them to be significantly different also ($p < 0.001$) (Appendix 2, Table 4 & 5).

The characteristic features of this species by which it may be readily identified are its small size combined with elongate, ellipsoid sporocysts and sporozoites lying lengthwise in the sporocyst. On the basis of these observations *E. n1* was recorded separately in the present study and is considered a separate species.

(ii) *Eimeria n2* (Fig. 3.1g, Fig.3.2)

Synonym: none

Morphological characteristics: This species is temporarily designated as *E. n2* (Table 3.1). It is a medium-sized, ellipsoidal oocyst with a prominent dome-shaped micropylar cap and a micropyle. The oocyst wall is composed of 2 layers, the outer one smooth, intermediate yellowish brown and the inner colourless. Polar granules usually number up to 10. The dimensions of oocysts and sporocysts are given in Table 3.1. The sporocysts are broadly ovoid with a distinct stieda body. A sporocyst residuum composed of many scattered, coarse granules is present. The sporozoites lie lengthwise head to tail in the sporocyst. Each sporozoite contains one small and one large refractile globule, one at each end.

Remarks: Although the oocyst size-range overlaps those of *E. arloingi* and *E. hirci* 'large form', except for the length of *E. arloingi* oocysts, the differences between them are highly significant ($p < 0.01$) (Appendix 2, Table 7 & 8). In addition, there are other distinguishing features. The sporocysts of *E. n2* are broadly ovoid rather than elongate ovoid as in *E. arloingi* or broadly ellipsoidal or round as in *E. hirci*; the difference from *E. arloingi* is reflected in the significant difference in sporocyst shape index ($p < 0.001$) (Appendix 2, Table 7). This is also the case with *E. hirci* 'large form' although the widths of the sporocysts are not significantly different (Appendix 2, Table 8). The sporozoites and sporocyst residuum are different from those of *E. hirci*. The sporozoites of *E. n2* are clearly visible and have distinct clear refractile globules and there is a coarse sporocyst residuum. This contrasts with the indistinct sporozoites and refractile globules sporozoites and the tiny aggregated sporocyst residuum of *E. hirci*. Given its distinctive characteristics, *E. n2* is considered a separate species.

3.1.4 Morphological characteristics of undescribed species without a micropylar cap

(i) *Eimeria n3* (Figs. 3.5d, 3.6)

Synonym: none

Morphological characteristics: This is a broadly ellipsoid oocyst with an indistinct micropyle. There is no micropylar cap. Polar granules usually number up to 5. The oocyst wall is thin and composed of 2 layers, the outer one smooth, pale yellowish brown and the inner wall, colourless. The sporocysts are elongate ellipsoid, with a stieda body which is flattened and broad which gives the narrow end of the sporocyst a truncated appearance. The measurements of oocysts and sporocysts are given in Table 3.1. No sporocyst residuum is visible. The sporozoites are elongate and lie lengthwise, head to tail in the sporocysts. Very prominent refractile globules are present in the sporozoites, either one large and one small globule or one large, centrally placed in each. Forty large globules measured $4.33 \pm 0.48SD$ (range 3.60-5.28) in diameter.

Remarks: Of all the species without a micropylar cap, only the oocysts of *E. ninakohlyakimovae* overlap in size those of *E. n3*. They are, however, readily distinguishable when sporulated. *E. ninakohlyakimovae* (Figs. 3.5e, 3.6) has an aggregated sporocyst residuum, a stieda body that is narrow, forming a pointed end to the sporocyst and its sporozoites do not possess prominent refractile globules as in *E. n3*. On the basis of these differences, *E. n3* is considered to be a separate species.

3.2 DISCUSSION

Apparently no attention has been given to the *Eimeria* species infecting goats in New Zealand and this is the first study on the identification of them in this country. Of the 13 *Eimeria* species (Table 1.2) whose validity is generally accepted, ten (Table 3.1) were found which fitted published descriptions satisfactorily although some minor differences were observed.

It was not entirely surprising to find *E.korcharli*, *E. punctata* and *E. pallida* absent from the samples examined although as noted earlier, a single *E. punctata* oocyst was found subsequently. These species have been found to be of very low prevalence by others (see Table 1.4).

In spite of Levine & Ivens (1986) reluctance to admit its existence, there can be no doubt

from this study that an equivalent of *E. ahsata* (*E. tunisiensis*) occurs in goats. Not only was it readily identifiable but, as will be shown in the next Chapter, found to be common in all the groups of kids and adults sampled.

The discovery of two sizes of oocysts with *E. jolchejevi*-like characteristics was unexpected. However, they also differed in shape in that the smaller form was predominantly ellipsoidal, the larger predominantly broadly ovoid or urn-shaped. The smaller form, whose dimensions fit those published for the species, almost certainly corresponds with *E. jolchejevi* s.s. as described by others. So what, then, is the identity of the large form? *E. granulosa* oocysts with similar dimensions were reported from Rocky Mountain Bighorn sheep by Honess (1942) and these were substantially larger than other authors have reported for this species. Also the microphotographs of *E. jolchejevi* from goats published by O'Callaghan (1989) appear similar in size and morphology to the large form described here. It seems likely that the large form of *E. jolchejevi* oocysts observed represent a previously undescribed species that may have an equivalent in sheep.

The observed range of sizes of *E. hirci* oocysts agrees with published data for this species but the possibility that this range is subdivisible into discrete subpopulations appears not to have been investigated previously. There were no discernible differences other than size and shape between the two size-categories found. The question arises as to whether these represent separate species or different morphs of the same species. Perhaps the development of this size difference is the result of polyploidy. It has been suggested that because of the high frequency of multiplication within a short time in *Eimeria* species, genetic variation is likely to occur (Wang, 1982). Determining whether or not the 'large' and 'small' forms of *E. hirci* are separate species will necessitate the establishment of pure infections and, perhaps, chromosome or DNA analysis.

The three previously undescribed species designated *E. n1*, *E. n2* and *E. n3* have been shown to be clearly distinguishable from described species from goats and, for this reason, are considered to be separate species and not variants of others.

E. n3 is quite unlike any described species lacking a polar cap, the extremely prominent refractile globules being diagnostic. When this oocyst was first discovered in a sample it was recorded as an abnormal oocyst because such very prominent refractile globules have not been recorded in any capless species of the size! Subsequently, however, oocysts of this type were found in large numbers of samples from all three farms providing a sound basis for concluding that it is a valid species. It is proposed that this species be named *E. charlestoni* in honour of Dr. W.A.G. Charleston, Reader in Parasitology, Faculty of

Veterinary Science, Massey University, Palmerston North, New Zealand.

It is of interest to consider if these species have counterparts infecting sheep. Of the sheep species with a micropylar cap, only *E. marsica* (Restani, 1971) and *E. weybridgensis* (Norton *et al.*, 1974) are comparable with *E. n1*. But *E. marsica* oocysts are elongate elliptical (length-width ratio approx. 1.5) (Restani, 1971; Norton & Catchpole, 1976) whereas those of *E. n1* and *E. weybridgensis* tend to be broadly ellipsoidal (length-width ratio approx. 1.3 & 1.4 respectively). Furthermore the range of oocyst widths of *E. marsica* (Restani, 1971; Norton & Catchpole, 1976) do not overlap the range of *E. n1* although that of *E. weybridgensis* does (Norton *et al.*, 1974). It is possible, therefore that *E. weybridgensis* is the ovine counterpart of *E. n1*.

There appears to be no equivalent of *E. n2* or *E. n3* described from sheep.

The identification of *Eimeria* species in goats (and sheep) presents several problems. In the past, most authors proposing new species have provided inadequate descriptions of their material and have failed to provide clear differentiation from comparable species or statistical comparisons. In many cases also, descriptions have been based on very small numbers of oocysts. There is no standard procedure or detailed set of criteria for establishing new species of coccidia. Inevitably this results in confusion of nomenclature and identities. It is not a problem to identify species whose characteristics are clearly diagnostic, but with those sharing many characteristics with other species it can be difficult. The importance of using fully sporulated oocysts and attention to detail cannot be overemphasized.

In the final analysis, proof of the validity of species requires more than a description of oocysts in most cases. There are several ways of approaching this but producing pure isolates of the species of interest in parasite-free goats and establishing the biological parameters properly is of particular importance. Having established pure isolates, species-specific antibodies could be looked for, as has been done with avian species (Shirley, 1975)). The detection of species-specific enzyme variations in different avian *Eimeria* species is now well documented (Long, 1982) although very little has been done with other species. A further possibility that could be explored is DNA analysis using restriction endonucleases, so-called "DNA-fingerprinting". To date, few *Eimeria* species of goats and other ruminants have been studied as pure isolates and biochemical procedures of the type mentioned have scarcely been used at all.

CHAPTER FOUR

SEASONAL PATTERNS OF INFECTION

A. TOTAL OOCYST COUNTS

4.1 RESULTS

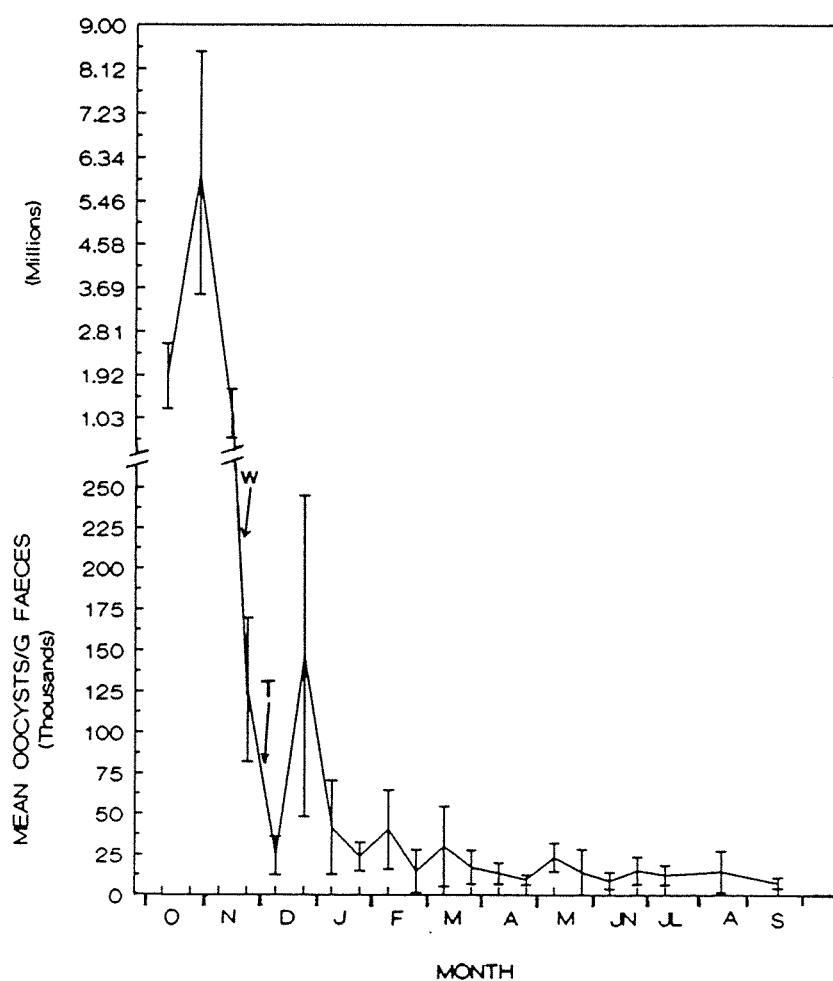
The mean total oocyst counts of each group were calculated from the individual oocyst counts on each sampling day. All the means referred to in this section are arithmetic means; 95% confidence intervals of each mean value were also calculated. The mean total oocyst counts of kids from individual farms and a comparison of seasonal patterns between farms are shown in Figs 4.1-4.3, and 4.4 respectively; the mean counts from the adults from two farms are also shown separately (Figs 4.5-4.6) and comparatively (Fig. 4.7). The relevant data are presented in Appendix 3.

Kid Samples

Early in the season, the Saanen kids from the Old West Road farm produced the highest total oocyst counts among the three groups (Fig. 4.6). The initial mean oocysts per gram faeces (opg) was 1.8 million in mid-October at an age of approximately 6 weeks, rising to a peak of approximately 6 million two weeks later (Fig. 4.1). On that occasion, counts of 17 million opg were obtained in two samples. Two weeks later the mean count was approximately 1 million opg and several kids had watery diarrhoea. Treatment was recommended but this was not done until 2 weeks later. Although no treatment was given, the initial mean count fell sharply to 125,000 opg by three days after weaning in late November. At this time, most faecal samples from the experimental group were of soft, unpeletted consistency. Because some of the other kids still had diarrhoea, and all kids were treated for coccidiosis by the owner. Following treatment the mean oocyst count fell to 24000 opg but rose again to form a secondary peak in December at age 15 weeks. The count had fallen substantially by January and then continued to decline gradually until sampling ended in September.

On the Kimbolton farm (Fig. 4.2) the mean oocyst count was 112,000 opg at the start of sampling in October. It gradually declined to 57,000 in December but then rose suddenly to form a peak (mean 232,000 opg) just after weaning. A second, lower peak occurred in February. At this time most faecal samples were soft and the kids were treated for

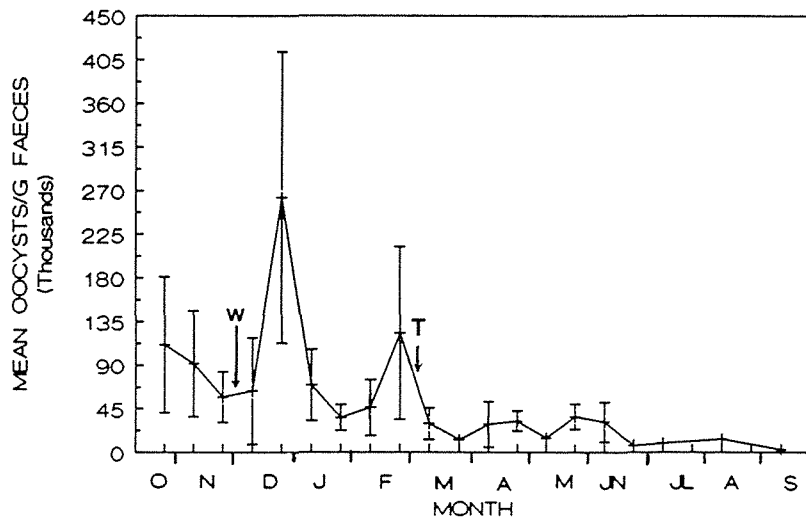
coccidiosis by the owner. After that the mean count fell steadily with time. Compared with other groups, the 'feral' goat kids from the Ballantrae farm produced the lowest oocyst counts (Fig. 4.3). The mean count never exceeded 80,000 opg although approximately 5% of individual faecal samples yielded counts of 100-700 thousand opg. There was a small peak in mean count in January and a further small peak immediately after weaning in February. In March, all the kids on the farm except the study group, developed severe diarrhoea; most of the affected kids were unable to stand and some died. All were treated



— 95 % CONFIDENCE LIMITS W - WEANING T - TREATMENT

TO MAKE COMPARISONS POSSIBLE, SAMPLE DATES HAVE BEEN ADJUSTED SO THAT THEY COINCIDE.

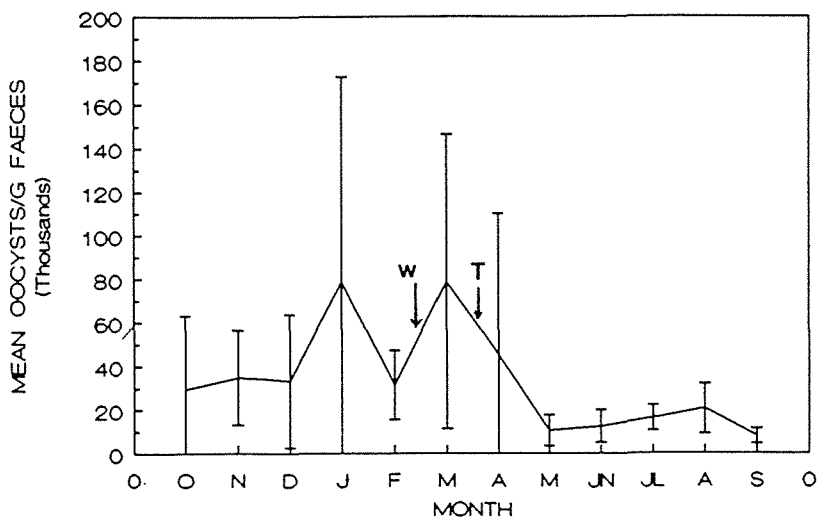
Fig. 4.1 Seasonal pattern of oocyst output of kids from Old West Road Farm.



— 95 % CONFIDENCE LIMITS W - WEANING T - TREATMENT

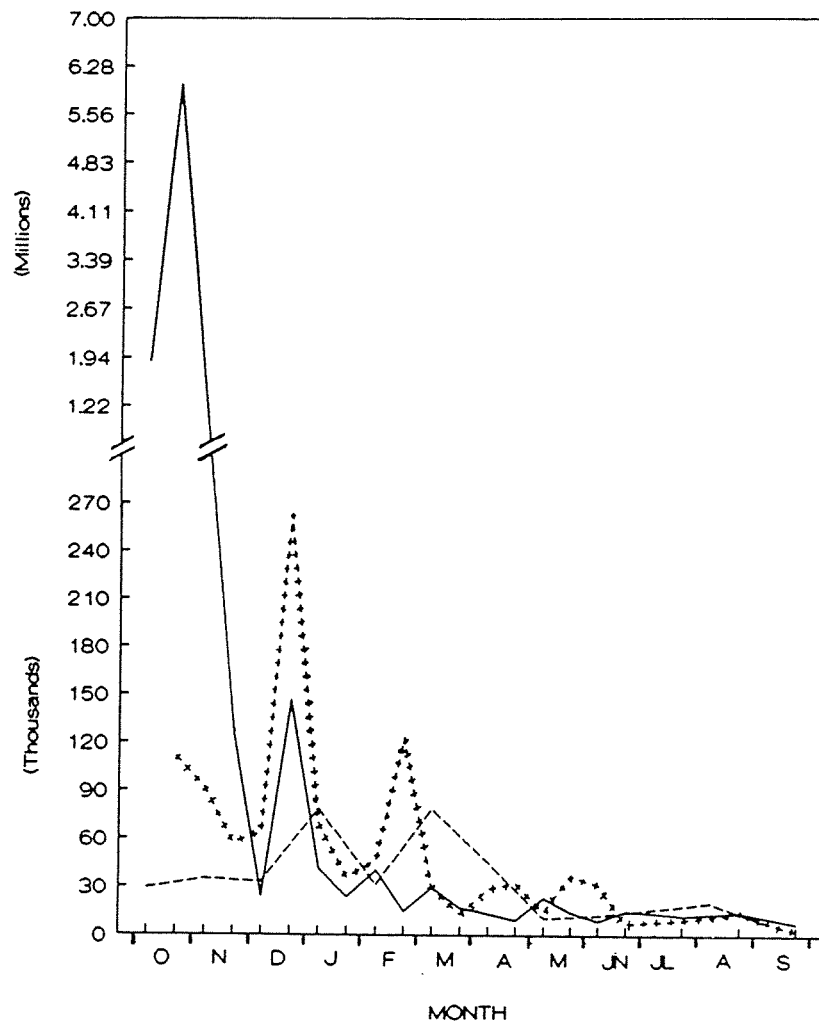
TO MAKE COMPARISONS POSSIBLE, SAMPLE DATES HAVE BEEN ADJUSTED SO THAT THEY COINCIDE.

Fig. 4.2 Seasonal pattern of oocyst output of kids from Kimbolton farm.



— 95 % CONFIDENCE LIMITS W - WEANING T - TREATMENT

Fig. 4.3 Seasonal Pattern of oocyst output of kids from Ballantrae farm



— OLD WEST ROAD FARM - - BALLANTRAE FARM xxx KIMBOLTON FARM

TO MAKE COMPARISONS POSSIBLE, SAMPLE DATES HAVE BEEN ADJUSTED SO THAT THEY COINCIDE.

Fig. 4.4 Comparison of seasonal pattern of oocyst output of kids

for both coccidiosis and yersiniosis. Some kids from the study group were also treated unintentionally by the farm staff. The mean count fell to a low level by May and remained low for the rest of the observation period.

On the Old West Road and Kimbolton farms, the highest oocyst output of kids was observed

up to about 2-4 months of age in the spring and early summer with a general tendency for oocyst production to decrease with time (Fig. 4.4). It is notable that the confidence limits also tended to become narrower with time. On the Ballantrae farm, on which oocyst counts were much lower, peak levels occurred somewhat later. Most peaks occurring were associated with an increase in oocyst count in 60-90% samples. However, particularly on the Kimbolton and Ballantrae farms, the peaks were also greatly influenced by a few extremely high counts with a corresponding increase in confidence limits. It is interesting to note that on all farms mean oocyst counts increased temporarily after weaning (Figs. 4.1-4.3). Oocyst counts of female and male kids from Kimbolton and Ballantrae farms were recorded separately but two-sample t-tests showed that there were no significant differences in oocyst production between male and female kids (Appendix 4A, B).

Adult samples

Adult goats consistently had lower oocyst counts than the kids (Figs.4.5, 4.6). The Ballantrae adults had higher mean oocyst counts than the Old West Road adults (Fig. 4.7). In Ballantrae adults the level of mean oocyst output tended to rise gradually from December from approximately 4500 to 20000 opg with peaks in January and April associated with

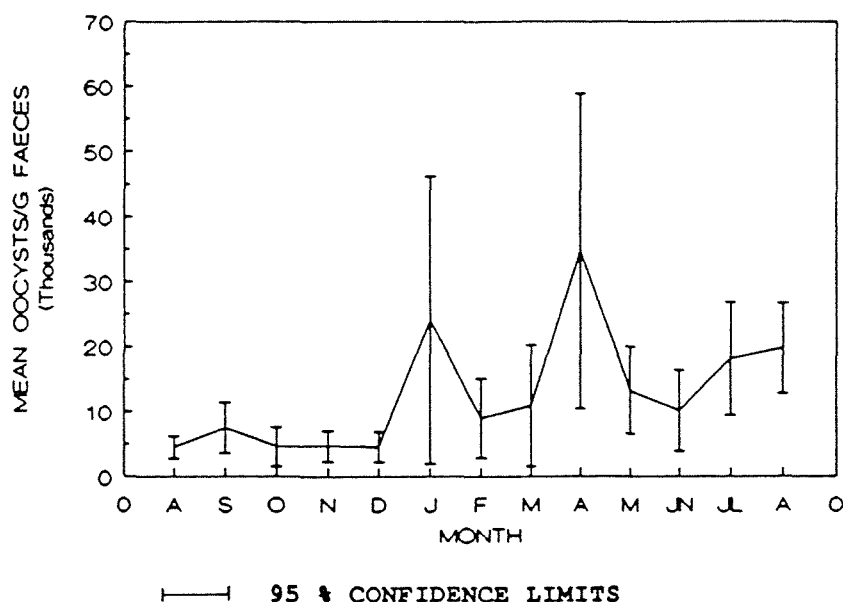


Fig. 4.5 Seasonal pattern of oocyst output of adults from Ballantrae farm

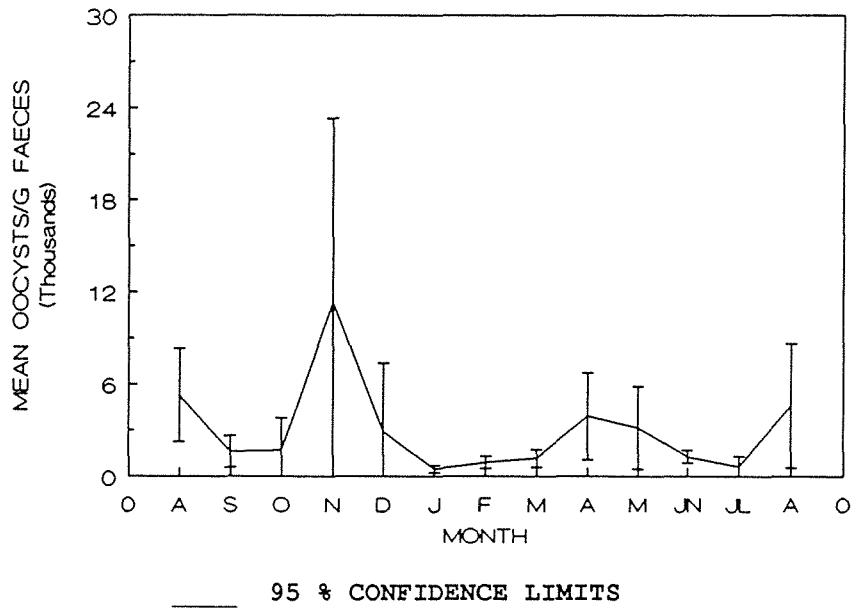


Fig. 4.6 Seasonal pattern of oocyst output of adults from Old West Road farm

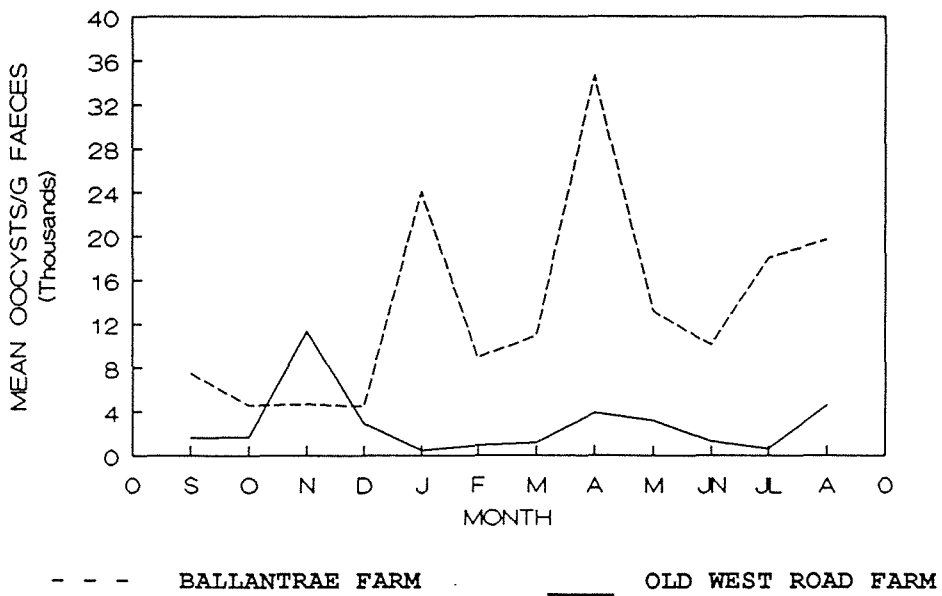


Fig. 4.7 Comparison of seasonal pattern of oocyst output of adults

raised counts in 90% and 60% of animals, respectively. In a few animals, counts reached over 100,000 opg and this influences the confidence intervals on those occasions. The mean counts for Old West Road adults varied between 400 and 12000 opg over the year with a low peak in November associated with relatively high counts in three goats. Apart from this, the mean counts from adults on this farm showed no seasonal trend.

4.2 DISCUSSION

A high prevalence of coccidial oocysts was found in samples from both adults and kids (see section 4.3). Kids 1-12 months of age produced much higher counts than adults as has been reported by many authors (Lloyd & Soulsby, 1978; Lima, 1980b; Shelton *et al*, 1982; Norton, 1986; Kanyari, 1988a). Although, in general, the mean opg of kids tended to decrease with time, it did not always decrease progressively as stated by Norton (1986). In all kid groups, major fluctuations in mean oocyst counts occurred within the first six-months in response to various conditions indicating that at this age acquired resistance was not fully established. Thereafter the mean counts declined steadily and on the Ballantrae farm paralleled the adult counts.

The seasonal patterns of oocyst counts of the three kid groups were found to be quite different. As the epidemiology of coccidial infection and coccidiosis is multifactorial, various factors could have been responsible for the different patterns of oocyst production including: the size of the primary infection; the presence and number of prolific species in the primary infection and environmental conditions affecting the buildup of levels of infection.

Firstly, kids from Kimbolton and Ballantrae were positive for coccidial infection as early as 4 weeks. The prepatent period of most *Eimeria* species infecting goats is approximately 2-3 weeks (Levine & Ivens, 1986). This means that these kids had been infected as early as 1-2 weeks of age. Due to a lack of acquired immunity, at an early age faecal oocyst counts reflect the level of oocyst intake and development of the parasite without interference by immune mechanisms. The primary infection of kids can derive from the contaminated udder of the doe or contaminated surroundings arising from the previous or present occupation of them by older stock. Although the total oocyst counts of the two adult groups were not much different at kidding, the Old West Road adults were excreting a higher proportion of *E. christenseni* than were Ballantrae does in which *E. arloingi* was predominant. This reflected the species composition of the oocyst output of the kids at that time. Although no does were sampled on the Kimbolton farm, in the kid samples *E. arloingi* was more predominant than *E. christenseni*.

From published information (Sharma Deorani, 1966; Hshirsagar, 1980; Lima, 1980b; Norton, 1986; Gregory & Norton, 1986), *E. arloingi* is regarded as the most prevalent and predominant species of goat coccidia and *E. christenseni* as only moderately prevalent. However, in the case of the Old West Road kid samples, *E. christenseni* was mainly responsible for the extremely high counts at 2-4 months of age. In absolute and relative terms counts of this species fell steeply to low levels at 6 months of age. It can be reasonably concluded that the sharp downward trend of *E. christenseni* was largely attributable to increasing host resistance resulting from repeated infections which indicates that *E. christenseni* is not only a species of high biotic potential but also highly immunogenic. However, as will be seen later, this is by no means the same for all species.

Secondly, there are several factors which would affect the levels of contamination and the mass of infection to which the kids were exposed. Kids were reared in three different management systems. The kids on the Old West road farm were removed from the does at approximately 5 days of age and confined in a small pen which was overcrowded and rarely cleaned. Under these conditions the kids would have been exposed to large numbers of coccidial oocysts derived from themselves and probably subject to stressors which could impair host resistance to coccidial infection. On the other two farms, the kids had access to grazing after birth and it would be expected, therefore, that the subsequent contamination levels would be lower. The Kimbolton kids were reared on their own after separation from the does at 3-5 days of age but the Ballantrae kids were run with the does until 6 months old. It is interesting to note that up to March the mean oocyst counts of Ballantrae kids were considerably lower than those of the Kimbolton kids on most occasions. Before weaning, the Kimbolton kids only had access to limited grazing and were heavily stocked (approx: 90/ha) compared with approximately 12-15/ha at Ballantrae so that higher counts in the Kimbolton kids might be expected. After weaning however, the Kimbolton kids were run at approximately 6/ha and separately from other goats and one might have anticipated that their oocyst counts would then be lower than those of the Ballantrae kids. The fact that they were not, is probably attributable to the does grazing with the kids on the Ballantrae farm until weaning in February. Being substantially resistant to infection, the does would have removed more oocysts than they contributed over this period effectively decreasing the levels of infection to which the Ballantrae kids were exposed. From April on, the counts in the two groups were very similar.

Genetic constitution is another factor which can affect susceptibility to coccidiosis and it has been claimed that Saanen goats are less susceptible to coccidiosis than Angora and 'Feral' (Howe, 1984 cited by Kanyari, 1988a) or Anglo-nubian goats (Kanyari, 1988b). However, in

the present study the three breeds were reared under different management systems so that it is impossible to decide if breed played any role in the observed differences between them.

A post-weaning rise in mean oocyst counts occurred on all farms although on the Old West Road farm its true relationship to weaning (if any) was obscured by treatment for coccidiosis. On the Kimbolton and Ballantrae farms the increase was probably due to a combination of increased oocyst intake associated with increased grass intake and perhaps a temporary decrease in host resistance associated with the stress of weaning.

After 6 months of age, the Old West Road kids had the lowest oocyst counts of all farms although initially they had the highest. This is most probably the result of higher levels of immunity being established following the extremely high level of exposure to infection before weaning. In this connection, it is notable that the counts in the Ballantrae kids, which had the lowest initial level of output, took until May to reach levels similar to those in Old West Road kids. Kimbolton kids were roughly intermediate between the two (see Fig. 4.4).

Not only did the kids on the three farms have different patterns of oocyst output but so did the adults. Although adults acquire a high level of resistance to infection, it is not solid and adults still excrete oocysts in varying numbers though much fewer than kids. The Old West Road adults had consistently lower mean counts than Ballantrae adults. Old West Road kids were kept separate from does until they were mixed with them in June when they were 10 months old. Consequently, the Old West Road farm does were not exposed to pasture contaminated by the kids when their oocyst counts were highest. On the other hand, on Ballantrae, the does grazed with the kids until February and were probably exposed to pasture contaminated by the kids in the post-weaning period. However, whether this explains why the Ballantrae does' counts tended to increase from December on or peaked in January and April is not known.

B. INDIVIDUAL *EIMERIA* SPECIES

4.3 RESULTS

More than 98% of the faecal samples from each group of kids and adults contained coccidial oocysts (Table 4.1). Mixed infections were usual and approximately 65% of the samples examined contained 6-8 species, except that 51% of the samples from Ballantrae kids contained only 4-6 species (Table 4.2). The mean percentage of the total year's oocyst

Table 4.1 Mean percentage of total year's oocyst counts and overall prevalence of individual species in kids and adults

Species*	Farm				
	Old West Road		Kimbolton	Ballantrae	
	Kids	Adults	Kids	Kids	Adults
<u>E. arloingi</u>	24.97 (100)	23.82 (100)	27.17 (100)	28.03 (100)	35.52 (100)
<u>E. hirci</u>	16.92 (100)	29.38 (100)	16.69 (100)	17.81 (100)	17.30 (100)
<u>E. n2</u>	19.41 (100)	12.66 (100)	16.05 (100)	15.23 (100)	18.94 (100)
<u>E. christenseni</u>	12.00 (100)	9.19 (100)	11.93 (100)	6.57 (100)	4.30 (100)
<u>E. jolchejevi</u>	9.42 (100)	5.55 (100)	5.91 (100)	5.65 (100)	5.39 (100)
<u>E. n1</u>	8.21 (100)	4.20 (100)	5.70 (100)	4.97 (100)	3.82 (100)
<u>E. alijeji</u>	5.41 (100)	3.17 (100)	4.31 (100)	5.28 (100)	3.36 (100)
<u>E. tunisiensis</u>	1.08 (100)	5.43 (100)	5.88 (100)	3.62 (100)	4.90 (100)
<u>E. caprovina</u>	0.60 (100)	2.85 (95)	1.39 (88)	5.81 (95)	2.50 (100)
<u>E. caprina</u>	0.98 (100)	1.91 (85)	1.46 (100)	2.09 (100)	2.19 (83)
<u>E. ninakohlyakimovae</u>	0.78 (100)	0.91 (90)	1.77 (76)	1.64 (95)	1.18 (100)
<u>E. apsheronica</u>	0.13 (100)	0.71 (90)	0.88 (88)	0.61 (100)	0.60 (100)
<u>E. n3</u>	1.60 (100)	0.25 (45)	0.04 (53)	0.46 (15)	0.01 (60)
Total samples examined	404	185	176	227	350
Positive samples[%]	402[99.5]	184[99.5]	173[98.2]	227[100]	349[99.7]

* Species are listed in descending order of the percentage of total oocyst count.

The figures in parentheses indicate the overall percentage of animals which excreted the particular species at some time during the sampling period.

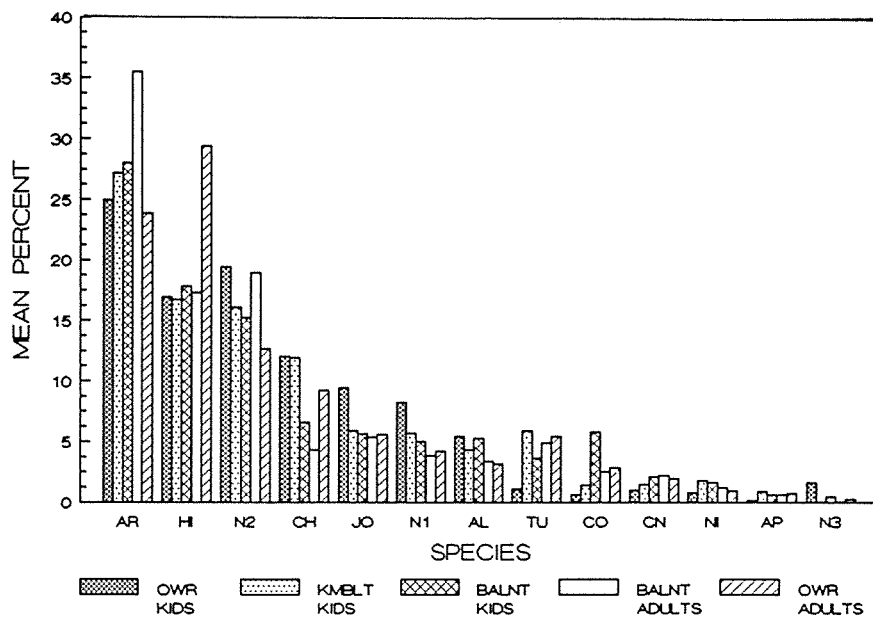
Table 4.2. Percentage of faecal samples containing different number of species

Farm	Number of Species													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Old West Road														
Kids	0.5	0.0	2.0	3.0	8.9	12.4	19.6	20.5	19.3	8.7	3.0	2.0	0.2	0.0
Adults	0.5	0.0	0.5	1.1	4.3	9.8	19.0	25.0	22.8	13.6	2.7	0.5	0.0	0.0
Ballantrae														
Kids	1.7	2.8	5.7	11.9	17.0	17.0	17.0	9.1	11.3	3.4	2.8	0.0	0.0	0.0
Adults	0.0	0.0	0.0	1.1	4.3	9.1	21.5	24.2	18.8	9.7	8.6	2.2	0.0	0.0
Kimbolton														
Kids	0.3	0.3	0.6	0.3	4.3	12.3	23.4	24.0	18.9	10.9	3.7	1.1	0.0	0.0

count by species and the overall prevalence of different species are recorded in Table 4.1 and illustrated in Fig. 4.8.

Seasonal variations of oocyst counts, percentages of total oocyst counts and prevalence of positive counts for each species are shown for kids (Figs 4.9-4.21 & Appendix 5, Tables 1-13) and for adults (Figs 4.22-4.33 & Appendix 6, Tables 1-13). There were considerable differences in the relative preponderance of the 13 species of *Eimeria* recorded. For convenience of presentation, the species are categorised into 3 groups according to their mean percentages in oocyst counts throughout the year (Table 4.1). It should be noted that the small and large forms of *E. jolchejevi* and *E. hirci* are combined in this section because they were not recorded separately at the beginning of the sampling period.

Group I Generally, *E. arloingi*, *E. hirci* and *E. n2* were found to dominate the coccidial population (Fig. 4.8 & Table 4.1). For most of the year, their prevalences were 80-100% in the examined samples (kids: Figs. 4.12, 4.13, 4.20; Appendix 5, Tables 4, 5, 12 and adults:



AR - *E. arloingi*; HI - *E. hirci*; N2 - *E. n2*; CH - *E. christensenii*; JO - *E. jolchejevi*; N1 - *E. n1* AL - *E. alijeivi*;
 TU - *E. tunisiensis*; CO - *E. caprovina*; CN - *E. caprina*; NI - *E. ninakohlyakimovae*; AP - *E. apsheronica*; N3 - *E. n3*

Fig. 4.8 Percentage of total year's oocyst count by species of kids and adults

Figs. 4.25, 4.26, 4.32; Appendix 6, Tables 4, 5, 12). Among these species *E. arloingi* was the most predominant species maintaining a very high prevalence throughout the year in kids and adults. In kids the highest numbers of *E. arloingi* oocysts were found at 2-4 months of age. The counts dropped sharply from very high levels between October and December in Old West Road kids but more gradually on other farms; thereafter the rate of decline was virtually identical on all farms (Fig. 4.12; Appendix 5, Table 4). Apart from a brief rise in oocyst counts in the Kimbolton farm kids in February, on all farms mean *E. arloingi* counts were generally <10,000 opg from February on. The adults did not show much seasonal variation in *E. arloingi* counts although on both farms occasional peaks in mean count occurred associated with raised counts in a few individual animals (Fig. 4.25; Appendix 6, Table 4).

The prevalence of *E. hirci* in samples from kids was initially low on two farms coinciding with the predominance of other species (such as *E. christenseni* & *E. arloingi*) at that time but, for most of the year, it was found in more than 90% of samples. In both kids and adults, the trends in oocyst counts were similar to those of *E. arloingi* (Fig. 4.13; Appendix 5, Table 5 and Fig. 4.26; Appendix 6, Table 5).

The prevalence of *E.n2* in kid samples (Fig. 4.20; Appendix 5, Table 12) was also initially low but from January on it was generally found in >90% of samples. The oocyst production attributable to this species was remarkably constant with mean counts in the region of 1000 - 10,000 opg throughout most of the year on all farms. This species was also common in adult samples but oocyst counts were generally much lower (Fig. 4.33; Appendix 6, Table 12).

Group II This group comprising *E. christenseni*, *E. jolchejevi*, *E. n1*, *E. alijeve* and *E. tunisiensis* were, overall, less numerous than species in group 1 either because they showed marked seasonal variations in relative proportions or were present in only low to moderate numbers for much of the year, particularly in samples from the kids.

Among these species the most striking seasonal change was evident with *E. christenseni* in the Old West Road kids which showed extremely high initial counts with a steep fall to low levels (Fig. 4.9; Appendix 5, Table 1). The trends were less dramatic on the other farms where the initial *E. christenseni* counts were much lower. However on all farms the sample prevalence of this species became low by the second half of the year. The oocyst counts in adults showed no seasonal trend (Fig. 4.22; Appendix 6, Table 1) though it is interesting to note the relatively high predominance of this species in the adult counts from the Old West Road farm at the time where they were also high in the kids.

In kids, the initial mean counts of *E. jolchejevi* (Fig. 4.11; Appendix 5, Table 3) appeared to fluctuate widely probably because of the influence of more dominant species, but from January on they fluctuated less, tending to rise through the autumn and then declining. The mean counts in adults (Fig. 4.24; Appendix 6, Table 3) were low and relatively consistent on the Ballantrae farm but variable on the Old West Road farm. The sample prevalence of the species was relatively high for much of the year in both kids and adults.

Oocysts of *E. n1* and *E. alijeve* were observed regularly in samples from both kids and adults. Apart from the relatively high counts of *E. n1* in Old West Road kids up until January, mean counts for this species were remarkably constant in both kids (Fig. 4.19; Appendix 5, Table 11) and adults (Fig. 4.32 & Appendix 6, Table 11). Although representing a comparatively small percentage of total oocyst counts, for much of the year *E. n1* was present in over 60 % of samples from both age-groups. In kids, *E. alijeve* was not observed until November. Thereafter, the mean counts decreased slowly with time and were very similar on all farms. For most of the year the sample prevalence was >40%. In adults, low levels of infection were apparent for most of the year, the fluctuations probably reflecting changes in the numbers of more predominant species.

The mean counts of *E. tunisiensis* in kids showed a steady downward trend from November on (Fig. 4.10; Appendix 5, Table 2). It represented only a small proportion of total counts on all farms but achieved a prevalence of >30% for most of the year. In adults, low levels of oocyst count were detected throughout the year with minor fluctuations (Fig. 4.23, Appendix 6, Table 2). As a percentage of total counts, *E. tunisiensis* never exceeded 15% and was generally much lower. The prevalence of infection was low in spring but over 50% for most of the year (Fig. 4.23; Appendix 6, Table 2).

Group III The species which were found with relatively low oocyst counts were *E. caprina*, *E. caprovina*, *E. ninakohlyakimovae*, *E. apsheronica* and *E. n3*. In association with their low numbers, the seasonal pattern and sample prevalence of these species in kids fluctuated greatly, tending to obscure any seasonal trends (Figs. 4.14, 4.15, 4.16, 4.17, 4.21). However, the mean counts *E. caprina*, *E. caprovina* and *E. ninakohlyakimovae* tended to fall as the year progressed but increased again in late winter. Numbers of *E. apsheronica* were low and a seasonal pattern was not detected. *E. n3* was not recorded before January and it became more numerous in the autumn and then declined in winter.

In adults, the low counts again made detection of any seasonal trends difficult. There was a tendency for *E. caprina* and *E. caprovina* numbers to increase through the year; with

E. ninakohlyakimovae there also appeared to be a slight downward trend but with an increase in winter (Figs. 4.27, 4.28, 4.30; Appendix 6, Tables 6, 7, 9). With *E. apsheronica* numbers increased in the Ballantrae adults from October to May, but in Old West Road adults were at relatively constant levels from November to April with a decline in April/May. On both farms numbers of all but *E. n3* in this group also declined in October (Figs. 4.27, 4.28 & 4.29).

E. n3 was by far the least common coccidian recorded in adults (Fig. 4.34; Appendix 6, Table 13) and was only recorded in one month in Ballantrae adults though considerably more common in the kids on that farm.

4.4 DISCUSSION

Interpreting the observed seasonal patterns of total oocyst counts in terms of individual species is complicated, especially with respect to kids. It should also be borne in mind that as only 100 oocysts were identified in each sample, both the estimated prevalences of species present in samples and their proportions will be considerably influenced by changes in the proportion of other species. These effects are unavoidable and will be greatest on species present in low numbers. For example, in adults, the mean counts of all species (except *E. n3*) in the least prevalent group fell to zero in October (Figs. 4.27, 4.28 & 4.29) largely because species in the other two groups, especially *E. arloingi*, *E. hirci*, *E. n2* and *E. christenseni*, accounted >80% of all the species identified. In addition a number of host-parasites factors are likely to affect the seasonal patterns and relative predominance of individual species, particularly the inherent reproductive potential of each species and the rate of acquisition of resistance to them.

Bearing in mind the difficulties of interpretation mentioned above, it is nevertheless evident from the results that the species did not all behave similarly although the patterns on the different farms were, on the whole, remarkably similar. For example, some species, such as *E. christenseni* and *E. arloingi* (Fig 3.16 & 3.19) together accounted for the majority of the oocysts present in spring-early summer when total oocyst counts were high. Subsequently, however, the level of *E. christenseni* fell steeply becoming a very minor contributor to oocyst counts by mid-late summer. In contrast to this, the proportion of *E. arloingi* remained at a much higher level and oocysts of this species continued to be produced in substantial numbers for the whole observation period although total counts had fallen substantially by autumn.

With the species designated *E. n2* (Fig. 4.20), on the other hand, oocyst counts remained almost constant throughout the year. It contributed an extremely small proportion of the oocysts excreted early in the year but its relative contribution increased steadily from January on as the proportions of other species declined. Similarly, the autumn rise in the proportions of *E. jolchejevi* and *E. hirci* (Figs. 4.11 & 4.13) in Ballantrae kids was mainly attributable to a decrease in other species as actual oocyst counts changed comparatively little over this time.

The results seem to indicate that substantial differences in host-parasite relationships and parasite population dynamics exist between species, particularly in young animals. Judging from the seasonal trends in oocyst counts, it appears that some species are capable of stimulating a higher level of host resistance (e.g. *E. christensenii*, *E. tunisiensis*, *E. ninakohlyakimovae*) than others (e.g. *E. arloingi*, *E. hirci*, *E. alijevi* and *E. n1*). The fact that similar patterns occurred in all three farms indicates that these differences are not the result of different circumstances. To discover the biological basis of these differences would require detailed study of single species infections but it may be a combination of immunogenicity and reproductive potential. It is interesting to observe that in adult animals, individual species oocyst counts were generally low and comparatively constant throughout the year and such differences between species not evident. This indicates that, given time, a similar equilibrium between host and parasite is established.

As the predominance of the various species varied considerably during the year, it is difficult to make meaningful statements about their relative overall importance. One approach to this is to consider the mean proportions (percentage of total counts) of each species found over the whole year (Table 4.1) which reflects both the level of oocyst production attributable to each species and its duration. As can be seen from the table, the overall prevalence of infection in individual animals is not a useful measure where repeated sampling is done as prevalences are 100% for most species.

Many authors (Table 1.3) have commented on the relative predominance of different species in a given locality as observed in their samples. Invariably these involve samples taken on one or a few occasions i.e. measure point prevalences. However, the differences in the seasonal patterns of individual species will produce widely differing assessments of prevalence and predominance from one time of year to another. This means that such data derived from one or a few samplings do not necessarily reflect either the true overall prevalence or overall predominance of a species or provide a satisfactory basis for comparisons between different localities. A further consequence is that it is not possible to make valid comparisons between the results from the present study and those of others.

Nevertheless it is interesting to note that in the published data (Table 1.3), *E. arloingi* is rated as the most predominant and prevalent species. In the present study the same species also represented the highest mean percentage oocyst count (Table 4.1) and showed a sample prevalence (Fig. 4.9 & Fig. 4.25; Appendix 5, Table 4 & Appendix 6, Table 4) throughout the year of >75% in all groups studied. A possible explanation for this could be that the species is of low immunogenicity and high biotic potential so that it is always present in relatively high numbers in the coccidial population.

Contrary to the observations of O'Callaghan (1989), all the species recorded in the study were found in all three breeds which is attributable to the long sampling period in the present study.

FIG. 4.9 SEASONAL VARIATIONS OF *E. CHRISTENSENI*
FROM KIDS

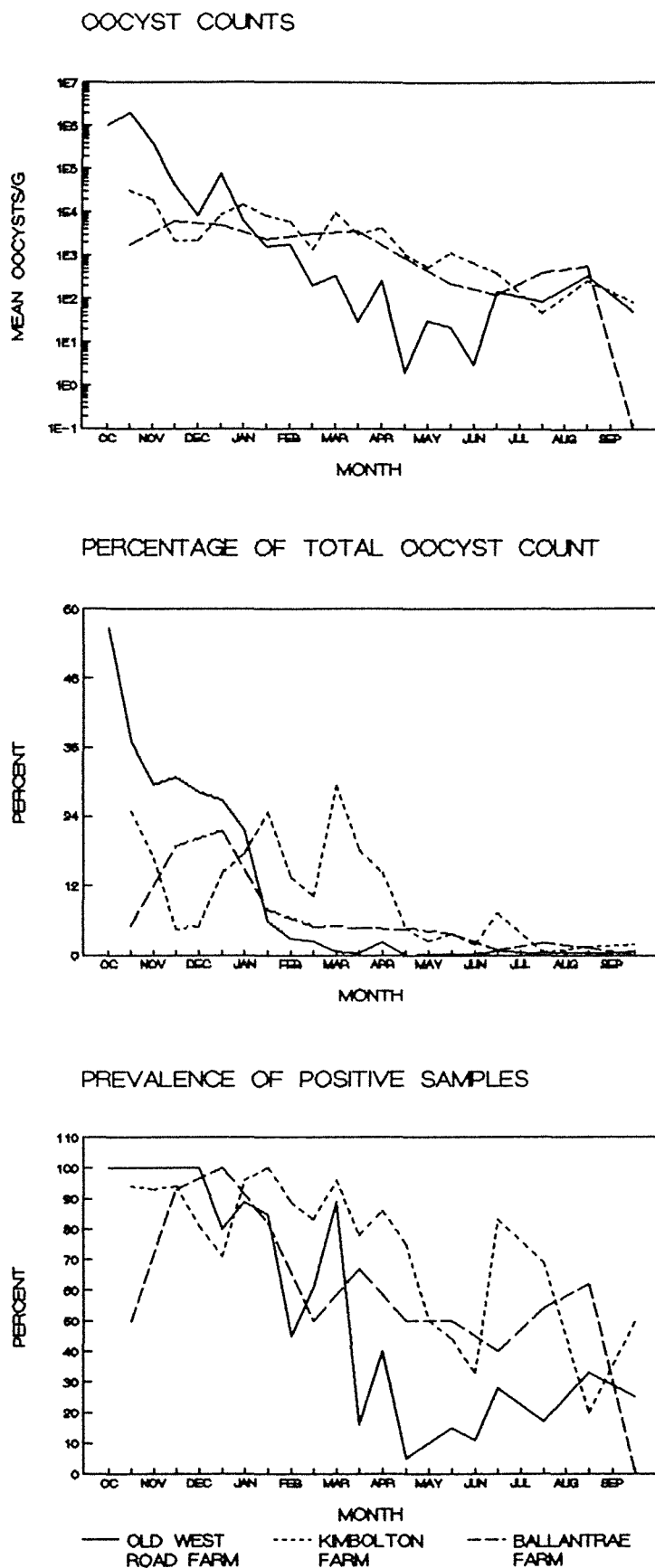


FIG. 4.10 SEASONAL VARIATIONS OF *E. TUNISIENSIS* FROM KIDS

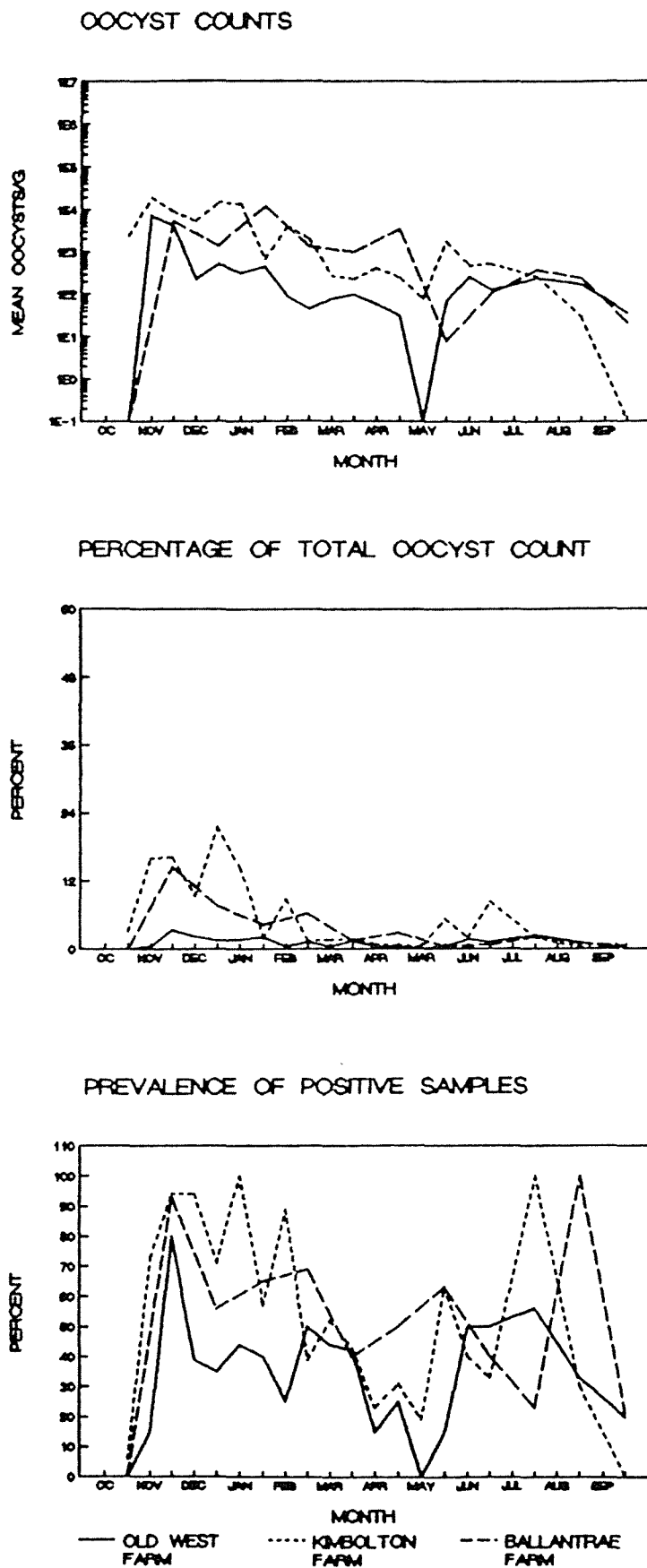


FIG. 4.11 SEASONAL VARIATIONS OF *E. JOLCHEJEVI* FROM KIDS

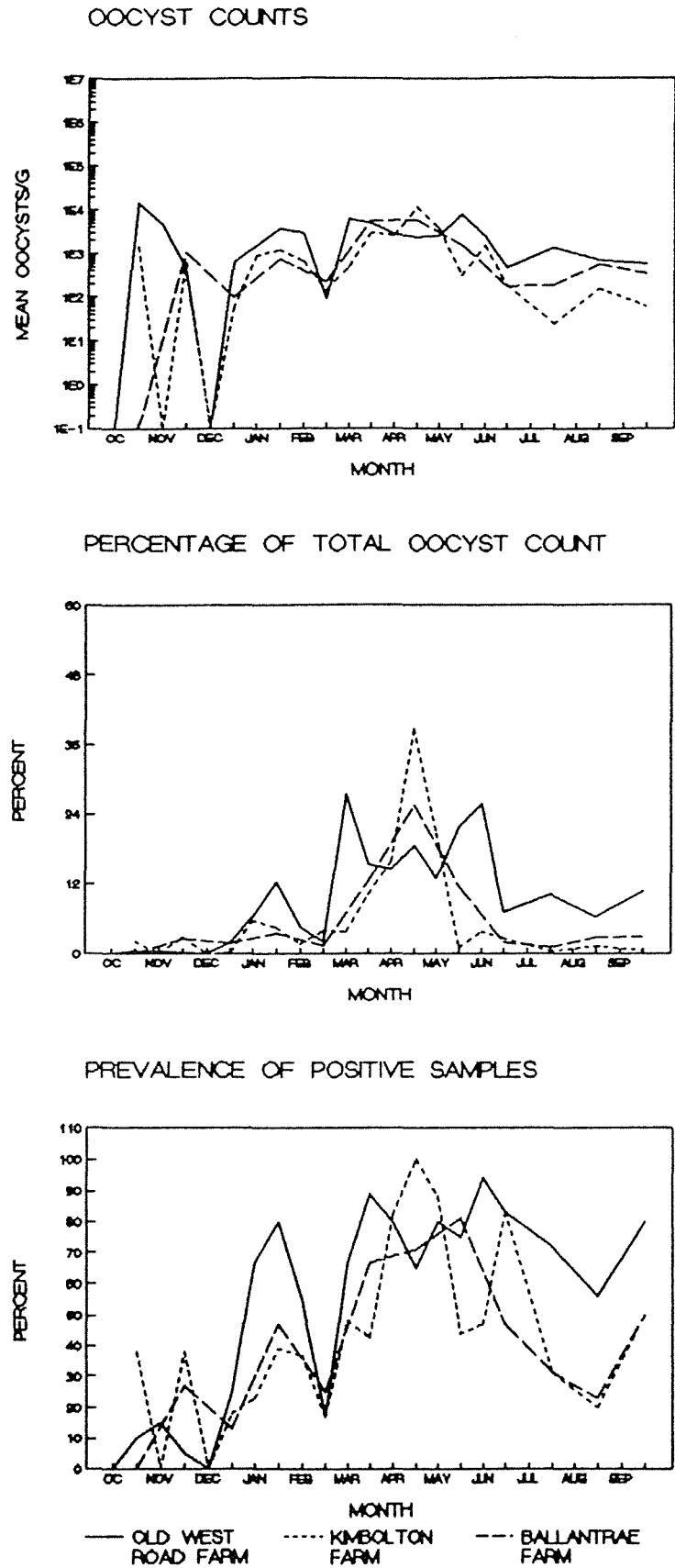
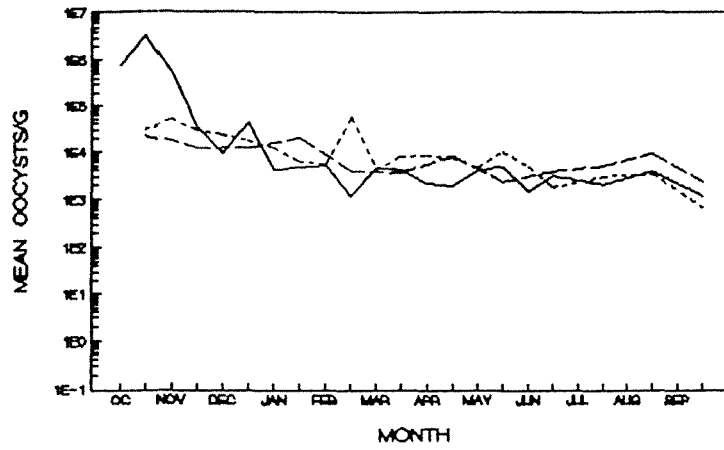
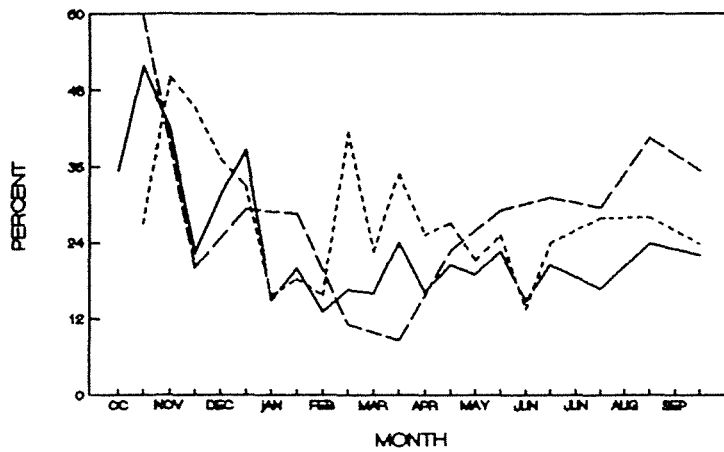


FIG. 4.12 SEASONAL VARIATIONS OF *E. ARLOINGI* FROM KIDS

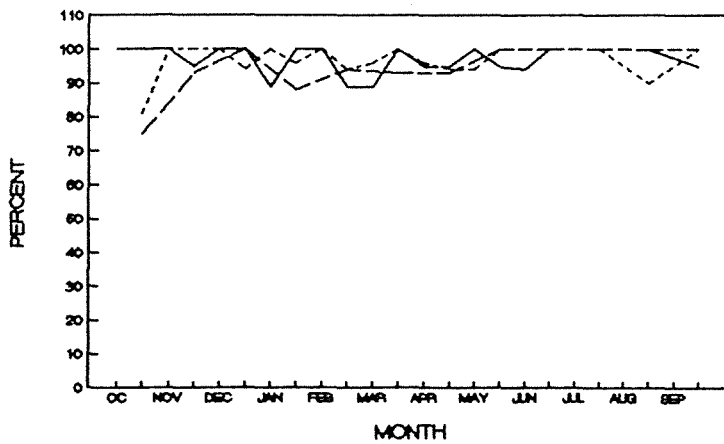
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT



PREVALENCE OF POSITIVE SAMPLES



— OLD WEST ROAD FARM ···· KIMBOLTON FARM - - - BALLANTRAE FARM

FIG. 4.13 SEASONAL VARIATIONS OF *E. HIRCI* FROM KIDS

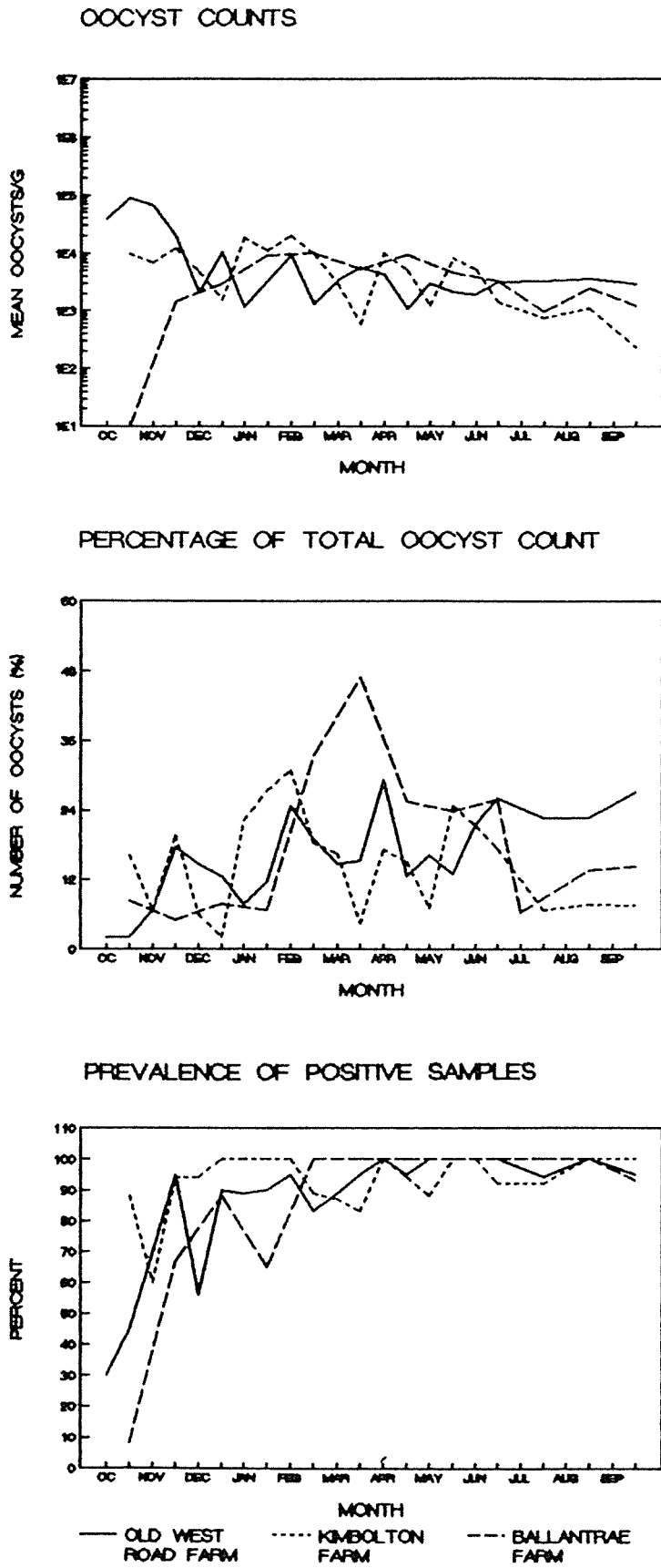
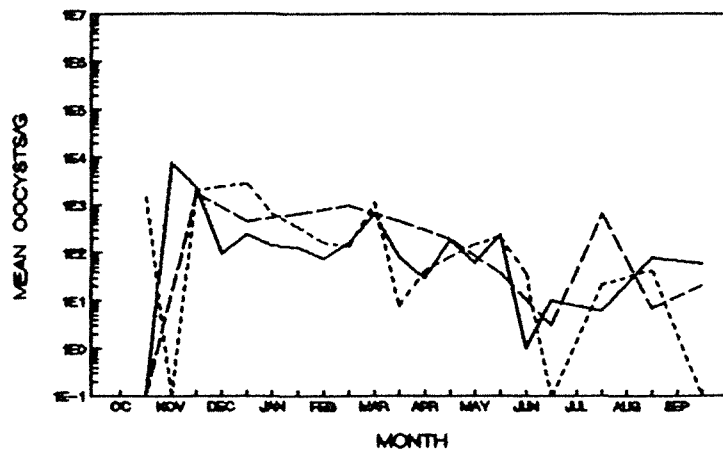
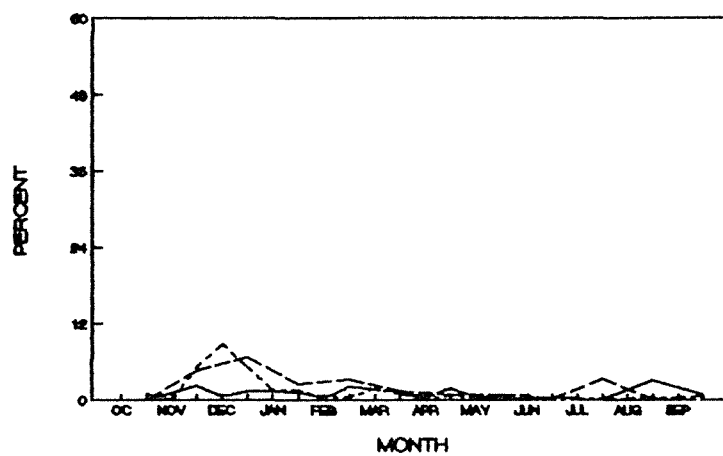


FIG. 4.14 SEASONAL VARIATIONS OF *E. CAPRINA* FROM KIDS

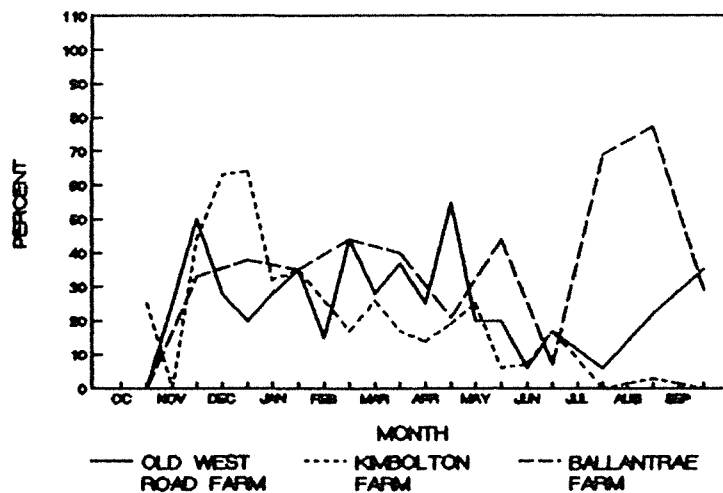
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT



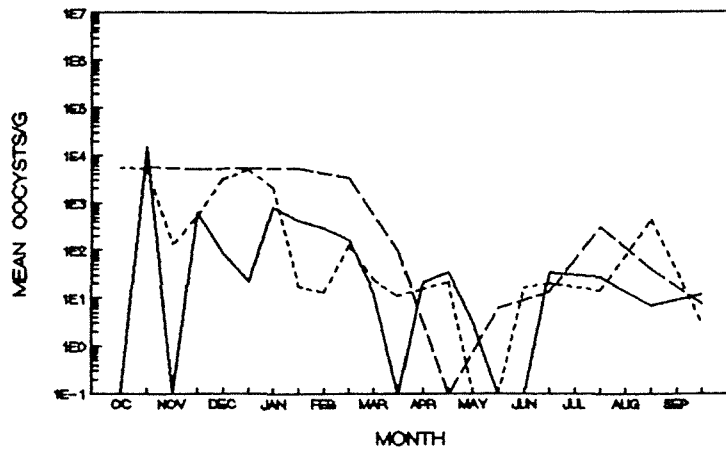
PERCENTAGE OF POSITIVE SAMPLES



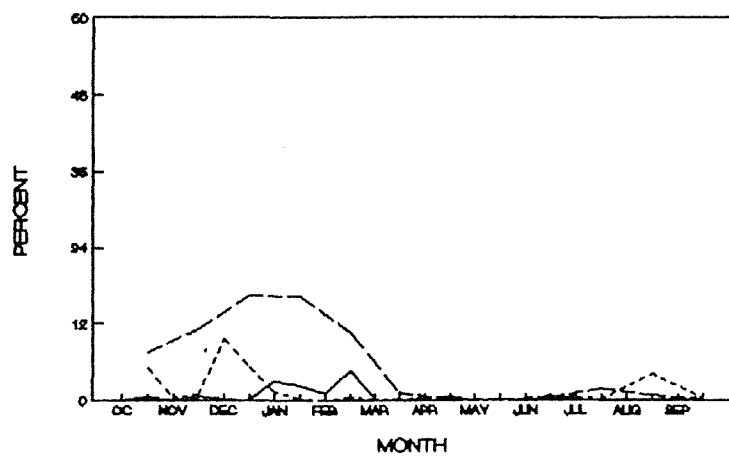
— OLD WEST ROAD FARM ···· KIMBOLTON FARM - - - BALLANTRAE FARM

FIG. 4.15 SEASONAL VARIATIONS OF *E. CAPROVINA* FROM KIDS

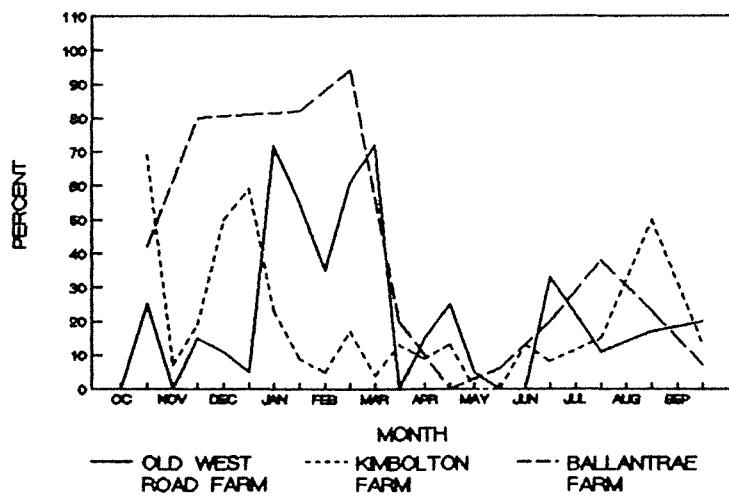
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT



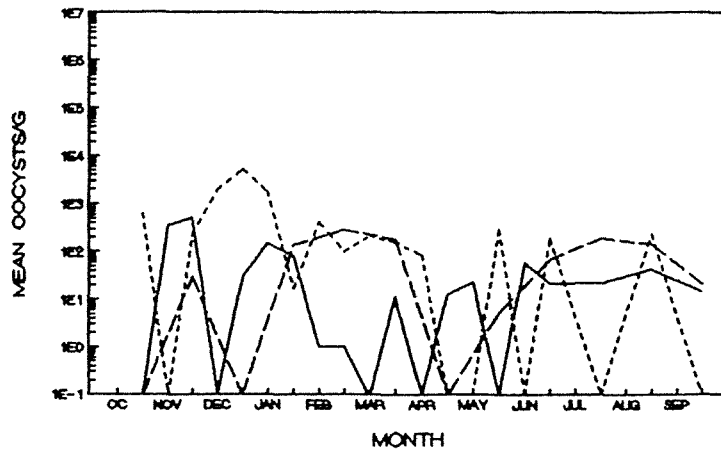
PREVALENCE OF POSITIVE SAMPLES



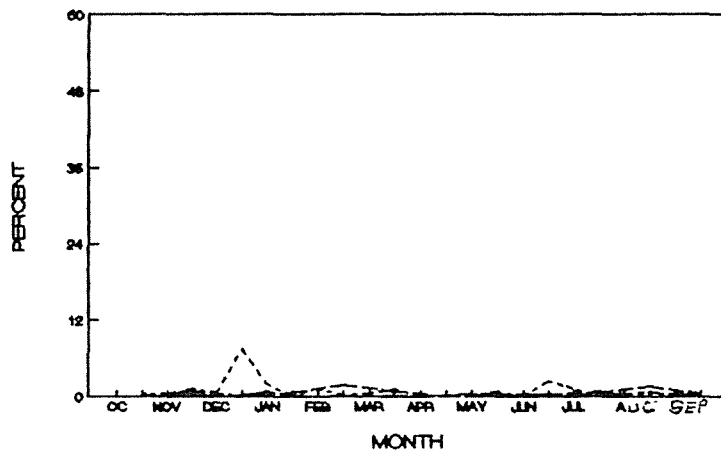
— OLD WEST ROAD FARM ····· KIMBOLTON FARM - - - BALLANTRAE FARM

FIG. 4.16 SEASONAL VARIATIONS OF *E. APSHERONICA* FROM KIDS

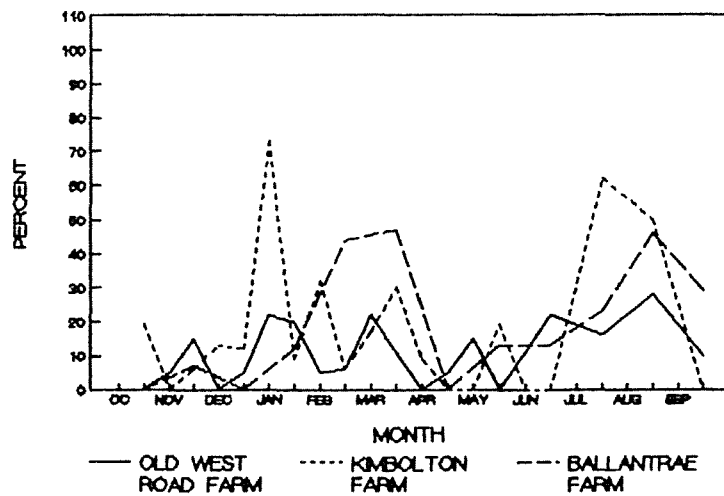
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT



PREVALENCE OF POSITIVE SAMPLES



— OLD WEST ROAD FARM - - - KIMBOLTON FARM - · - · BALLANTRAE FARM

FIG. 4.17 SEASONAL VARIATIONS OF *E. NINAKOHLIYAKIMOVAE* FROM KIDS

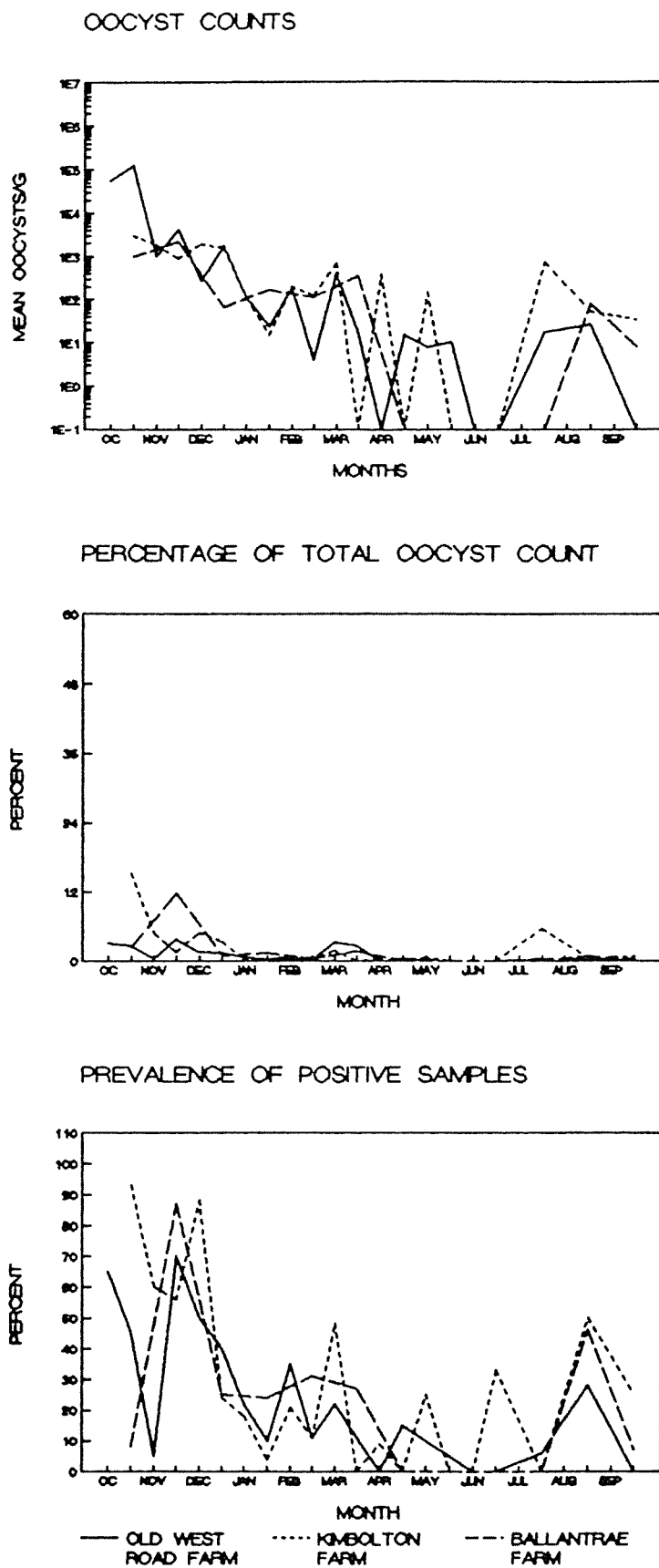


FIG. 4.18 SEASONAL VARIATIONS OF *E. ALIJEVI* FROM KIDS

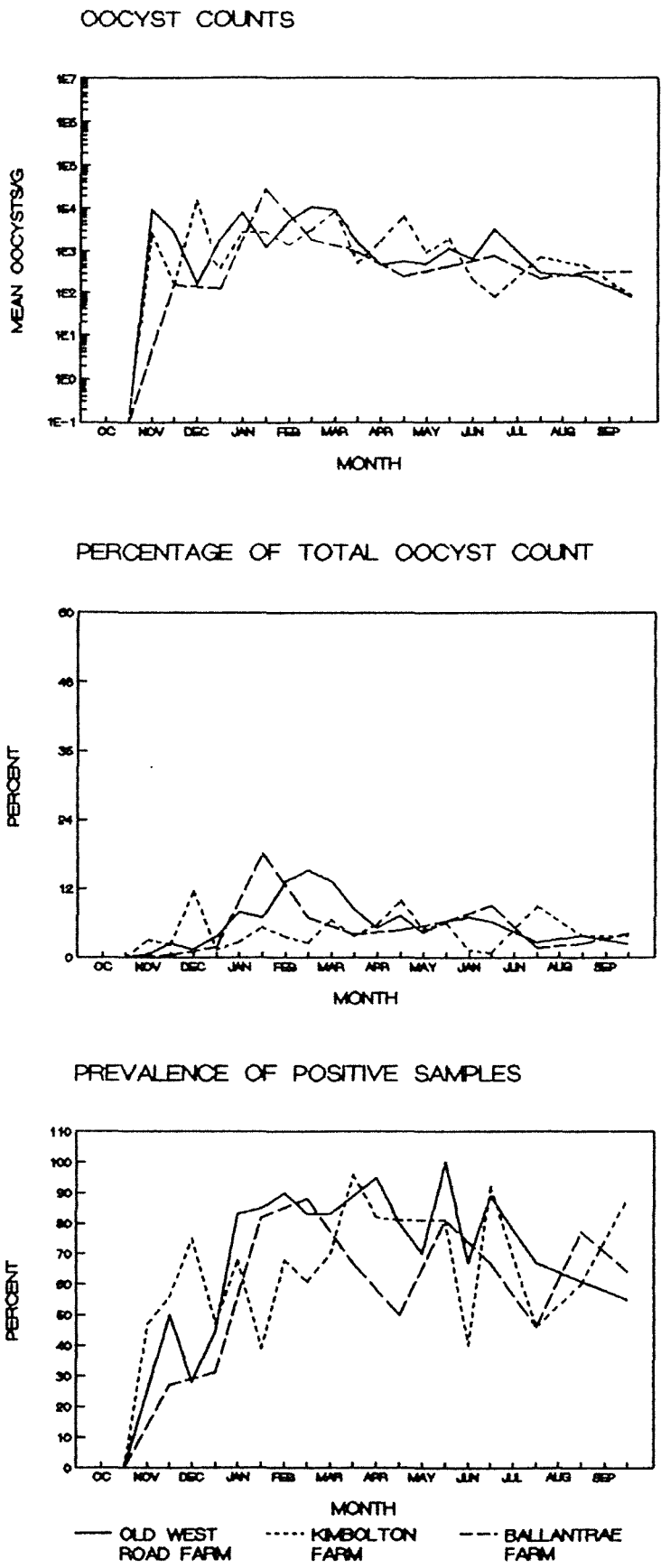


FIG. 4.19 SEASONAL VARIATIONS OF *E. N1* FROM KIDS

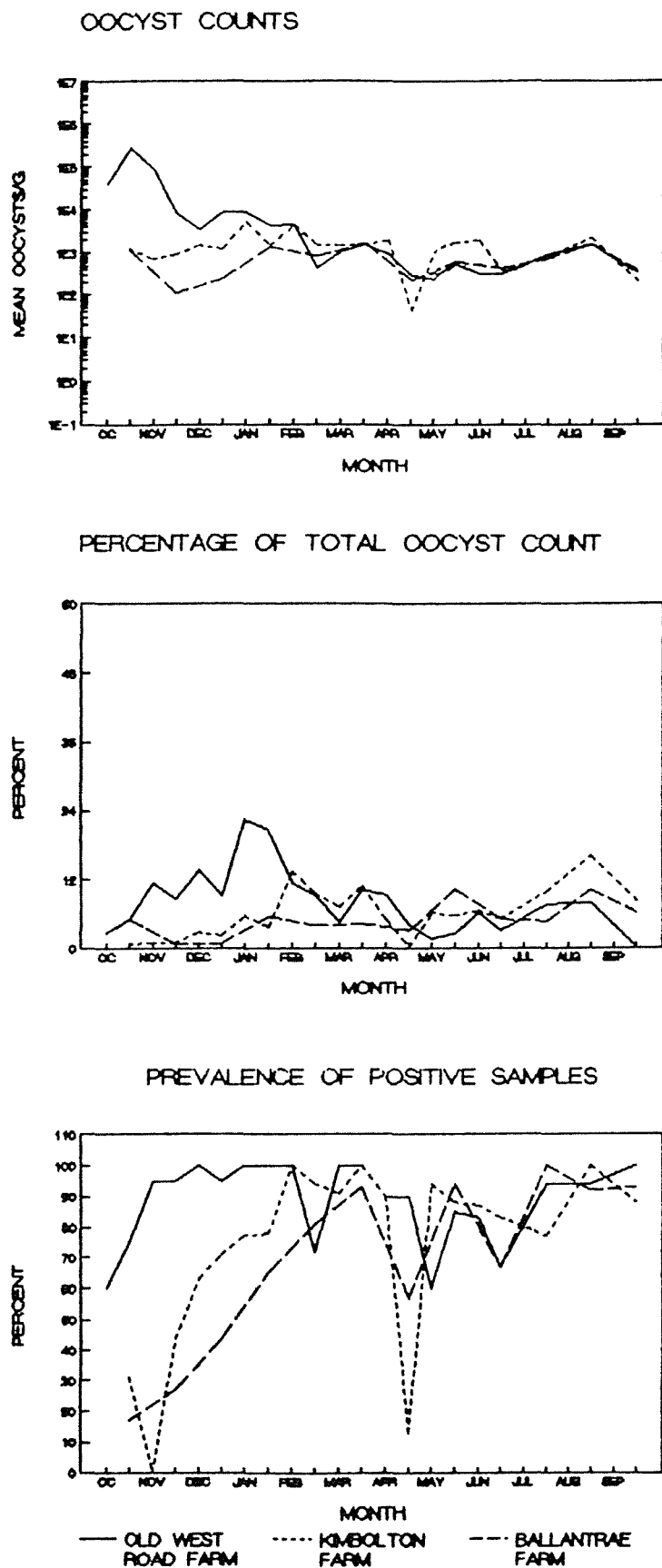


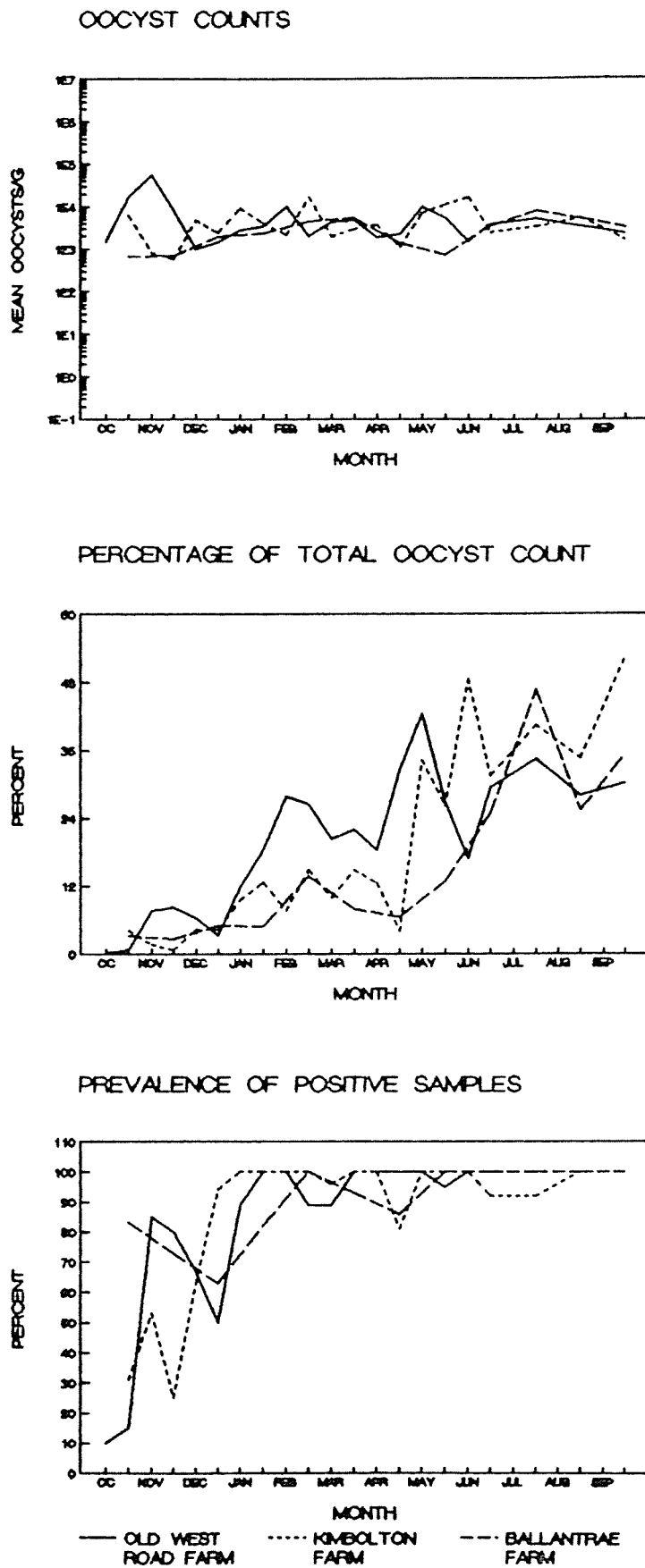
FIG. 4.20 SEASONAL VARIATIONS OF *E. N2* FROM KIDS

FIG. 4.21 SEASONAL VARIATIONS OF *E. N3* FROM KIDS

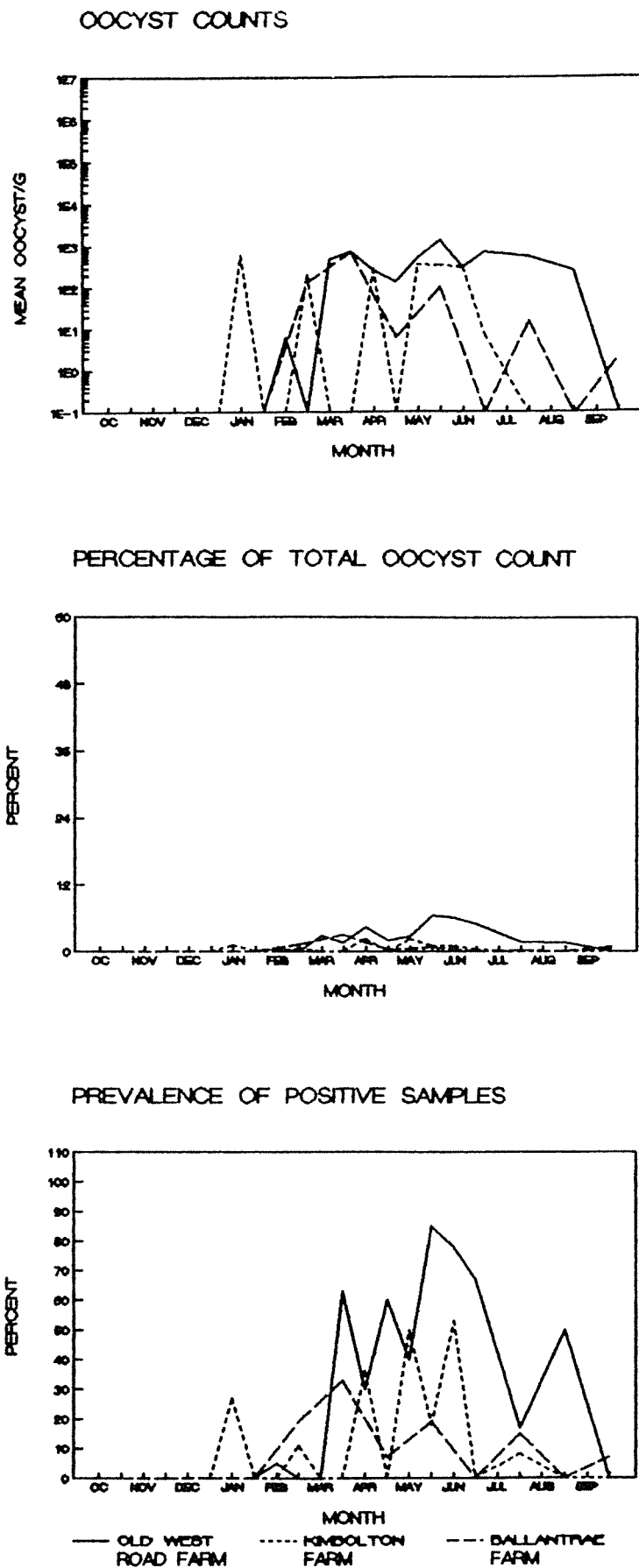
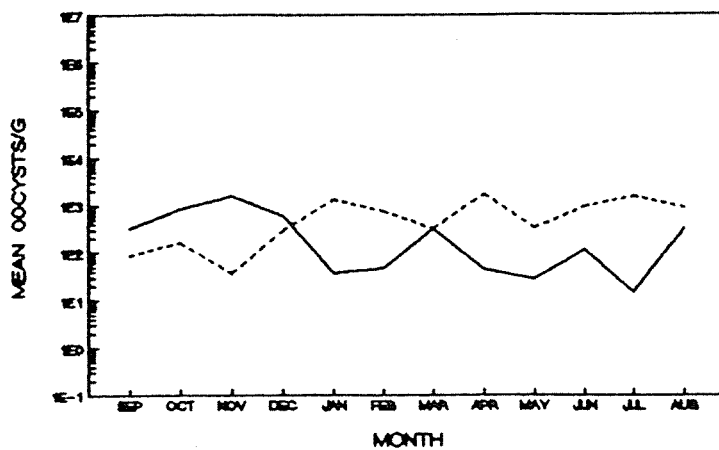
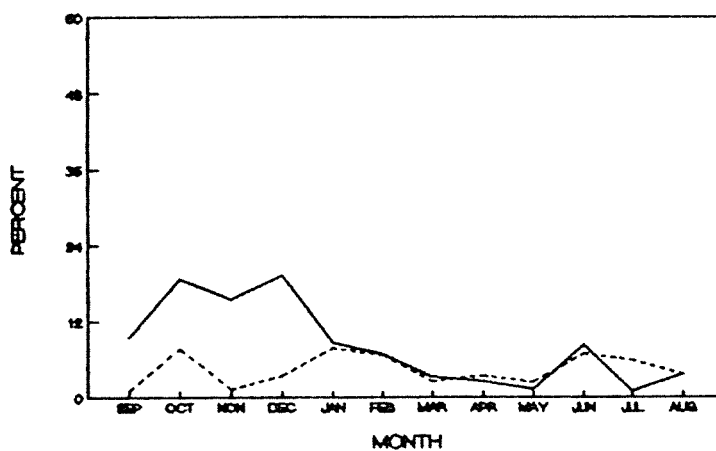


FIG. 4.22 SEASONAL VARIATIONS OF *E. CHRISTENSENI* FROM ADULTS

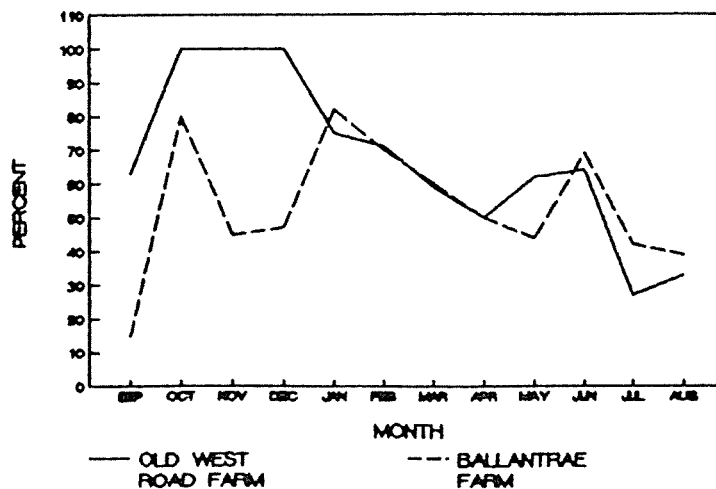
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT



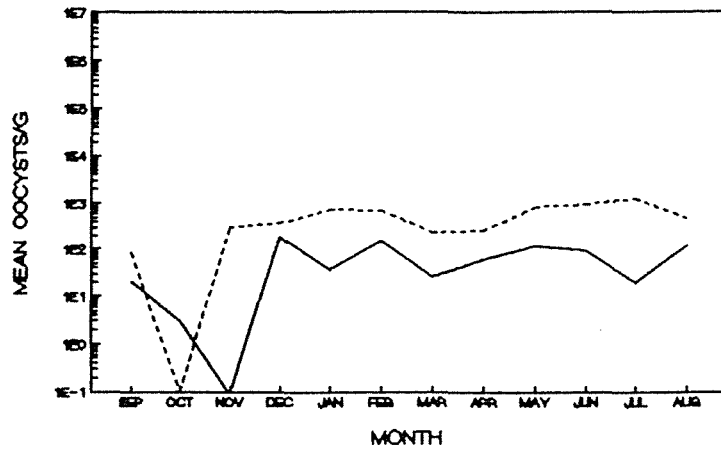
PREVALENCE OF POSITIVE SAMPLES



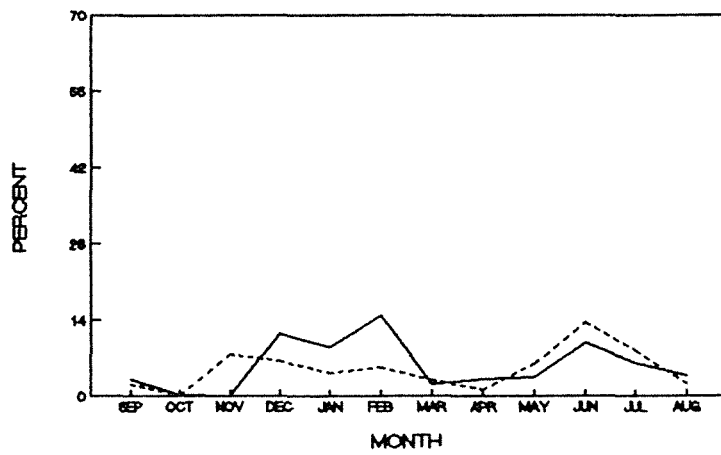
— OLD WEST ROAD FARM - - - BALLANTRAE FARM

FIG. 4.23 SEASONAL VARIATIONS OF *E. TUNISIENSIS*
FROM ADULTS

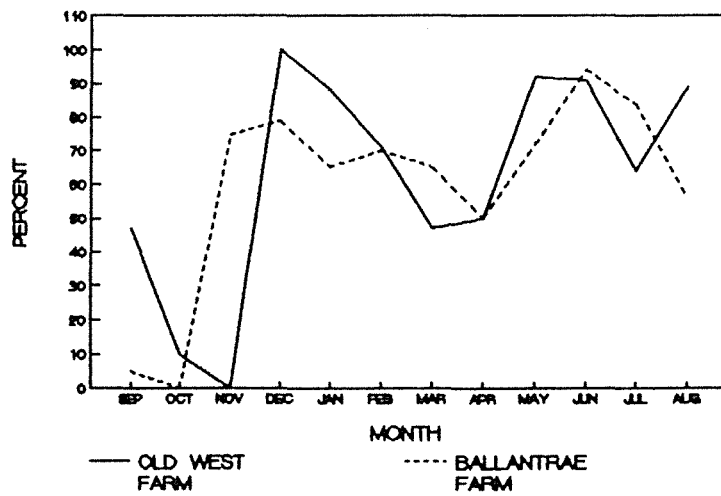
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT



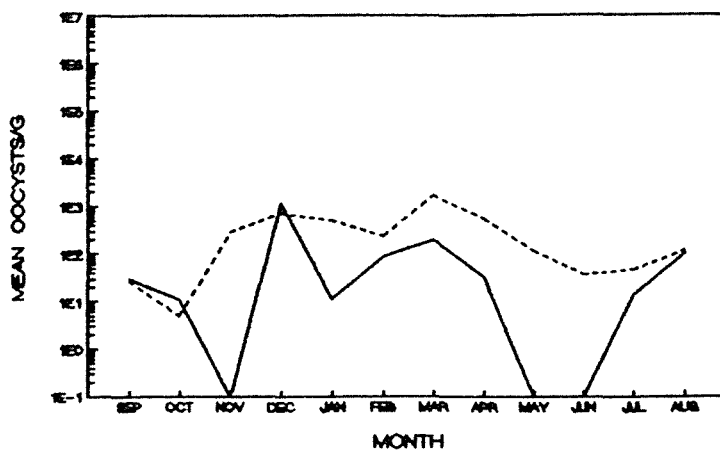
PREVALENCE OF POSITIVE SAMPLES



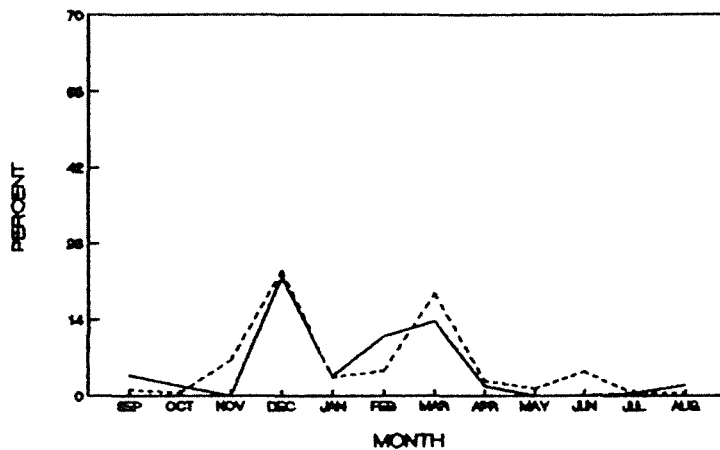
— OLD WEST FARM - - - BALLANTRAE FARM

FIG. 4.24 SEASONAL VARIATIONS OF *E. JOLCHEJEVI*
FROM ADULTS

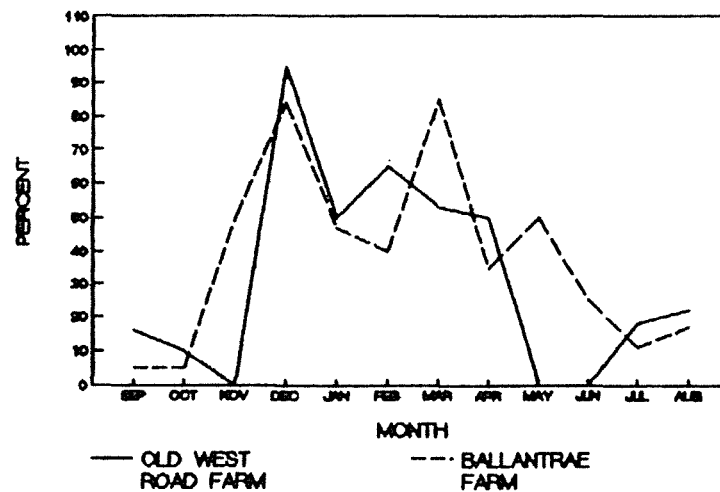
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT



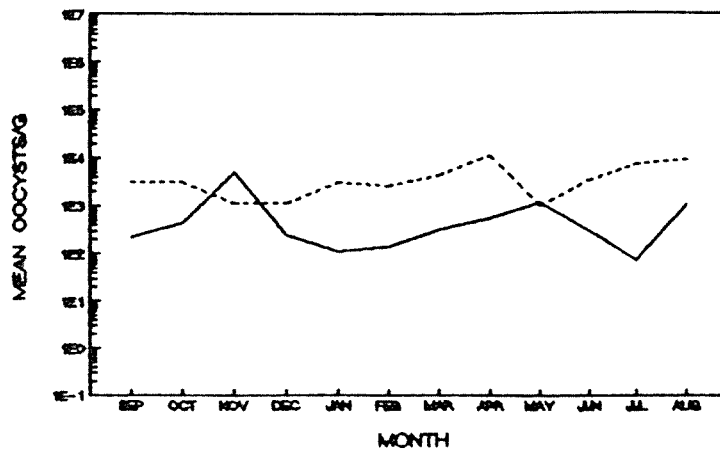
PREVALENCE OF POSITIVE SAMPLES



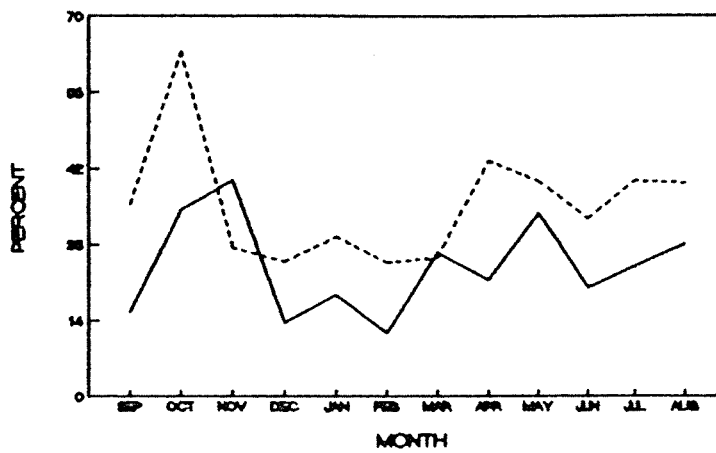
— OLD WEST ROAD FARM --- BALLANTRAE FARM

FIG. 4.25 SEASONAL VARIATIONS OF *E. ARLOINGI* FROM ADULTS

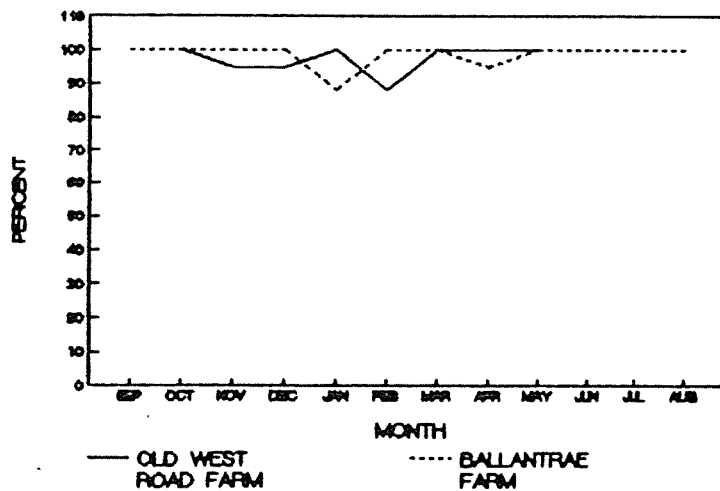
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT



PREVALENCE OF POSITIVE SAMPLES



— OLD WEST ROAD FARM - - - BALLANTRAE FARM

FIG. 4.26 SEASONAL VARIATIONS OF *E. HIRCI* FROM ADULTS

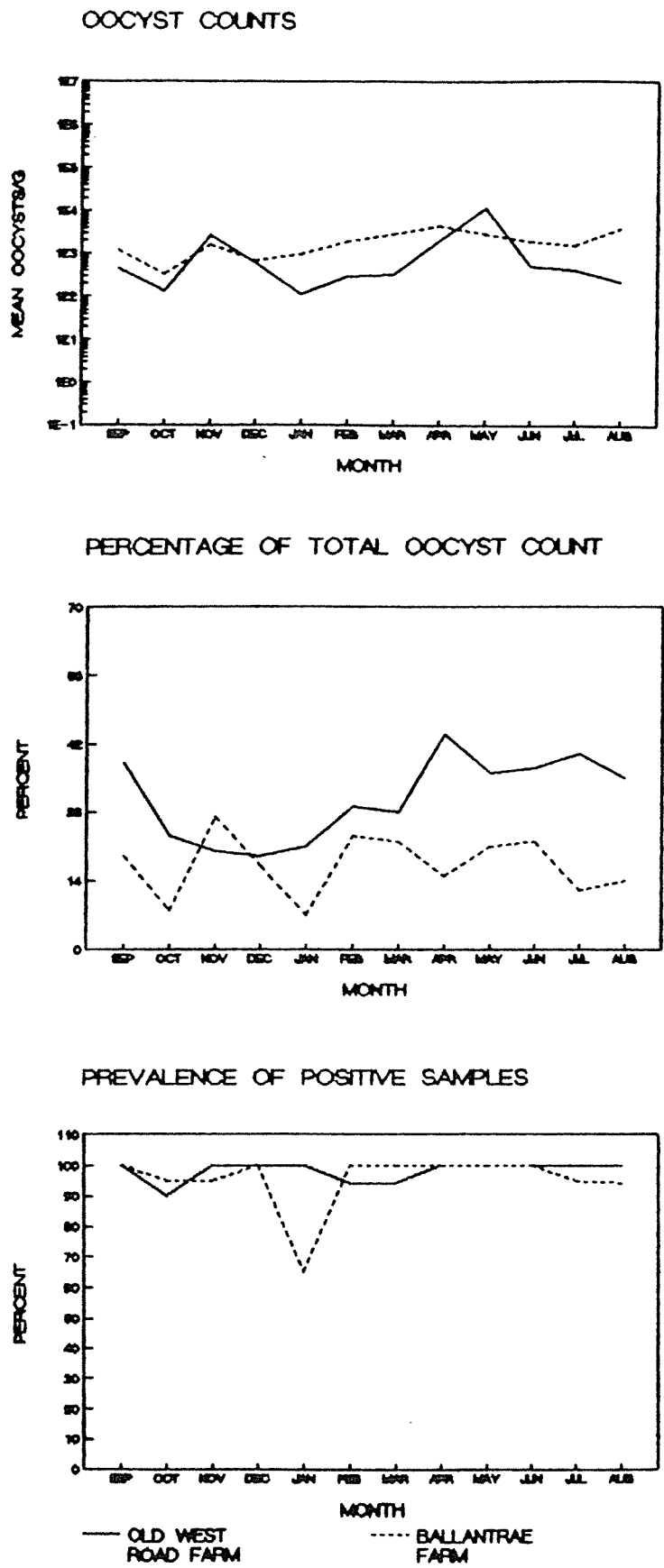


FIG. 4.27 SEASONAL VARIATIONS OF *E. CAPRINA* FROM ADULTS

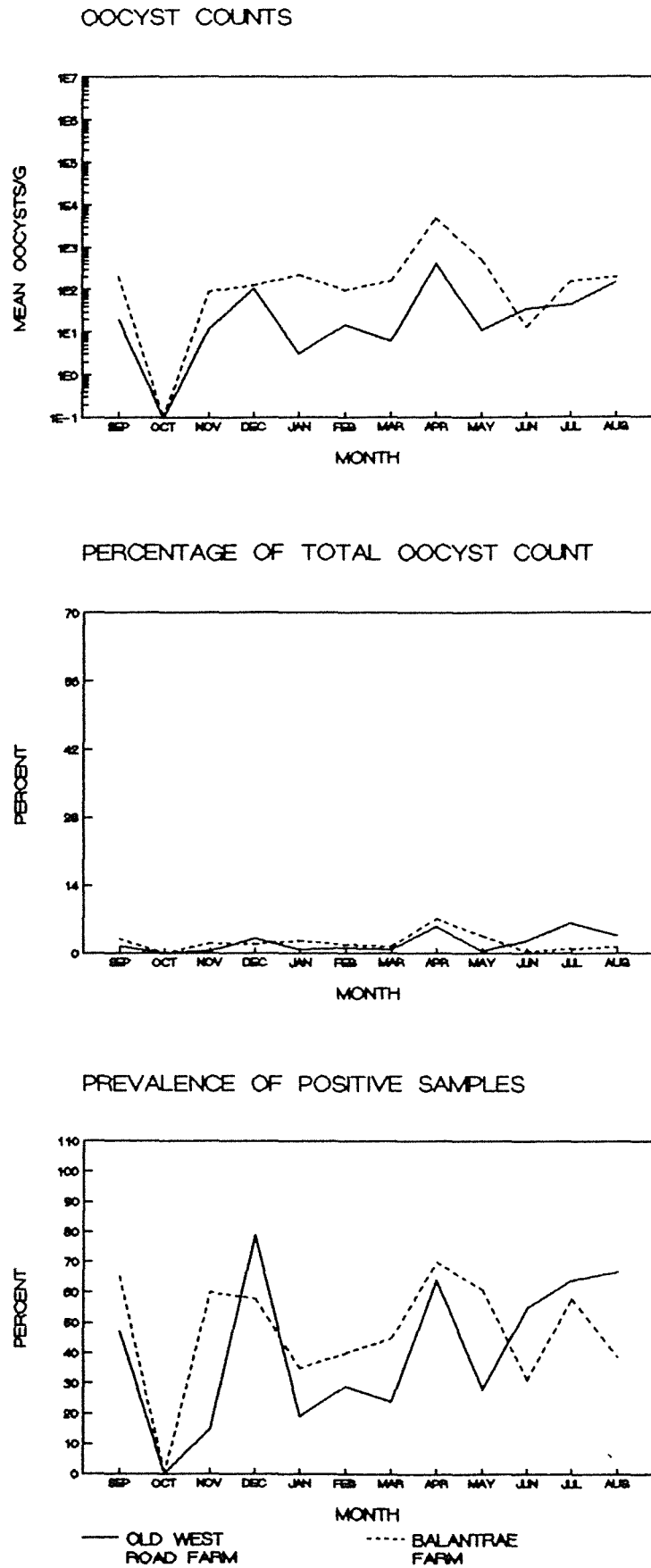
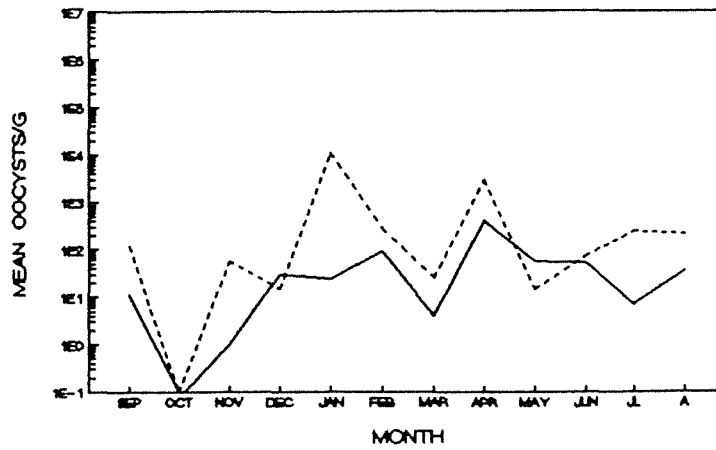
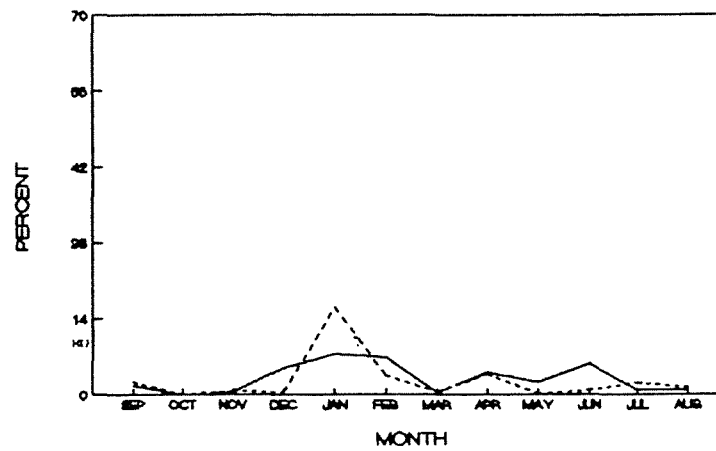


FIG. 4.28 SEASONAL VARIATIONS OF *E. CAPROVINA* FROM ADULTS

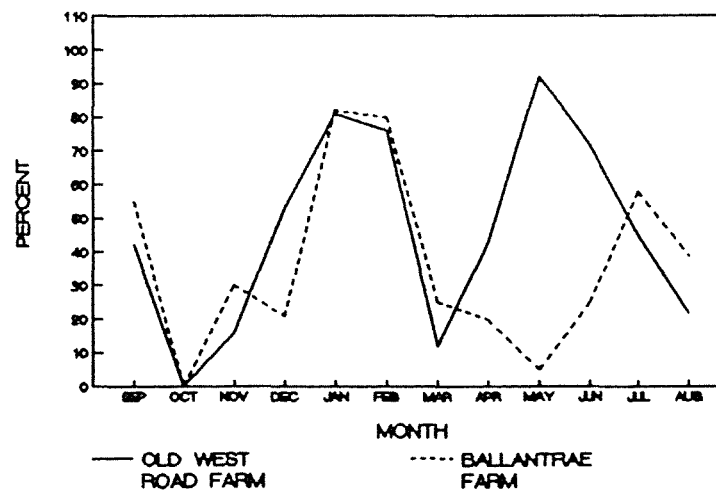
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT

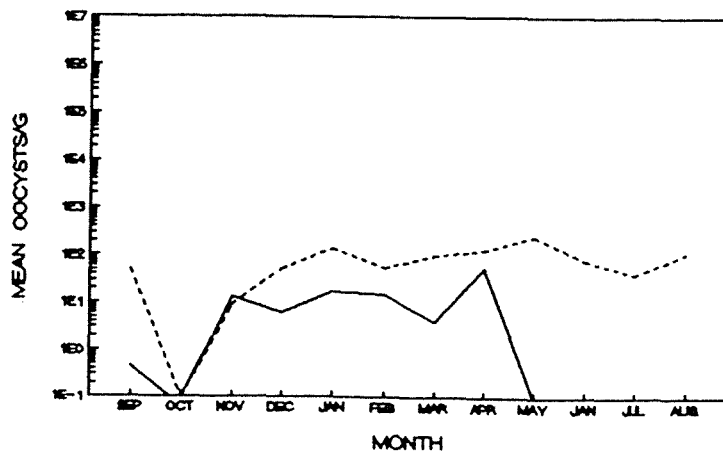


PREVALENCE OF POSITIVE SAMPLES

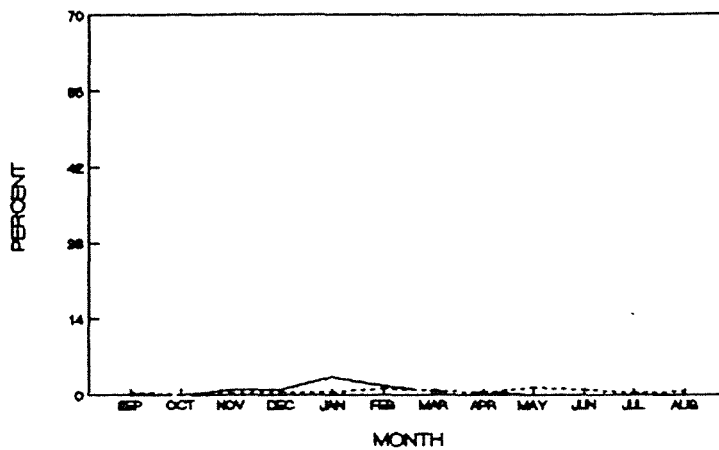


— OLD WEST ROAD FARM - - - BALLANTRAE FARM

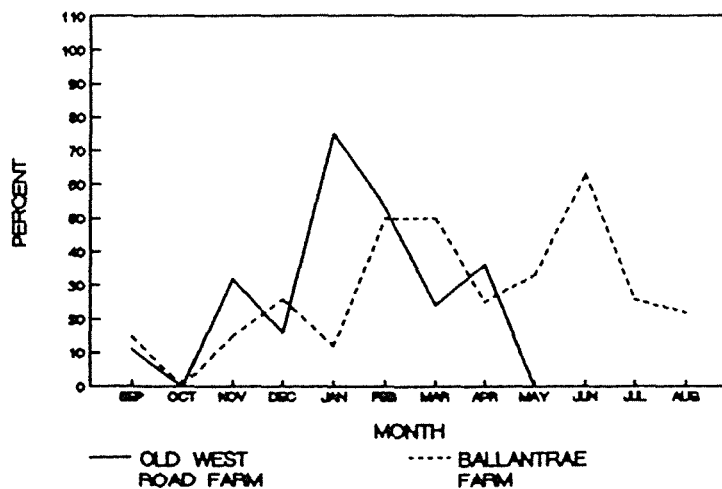
FIG. 4.29 SEASONAL VARIATIONS OF *E. APSHERONICA* FROM ADULTS
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT



PREVALENCE OF POSITIVE SAMPLES



— OLD WEST ROAD FARM
- - - BALLANTRAE FARM

FIG. 4.30 SEASONAL VARIATIONS OF *E. NINAKOHLIYAKIMOVAE* FROM ADULTS

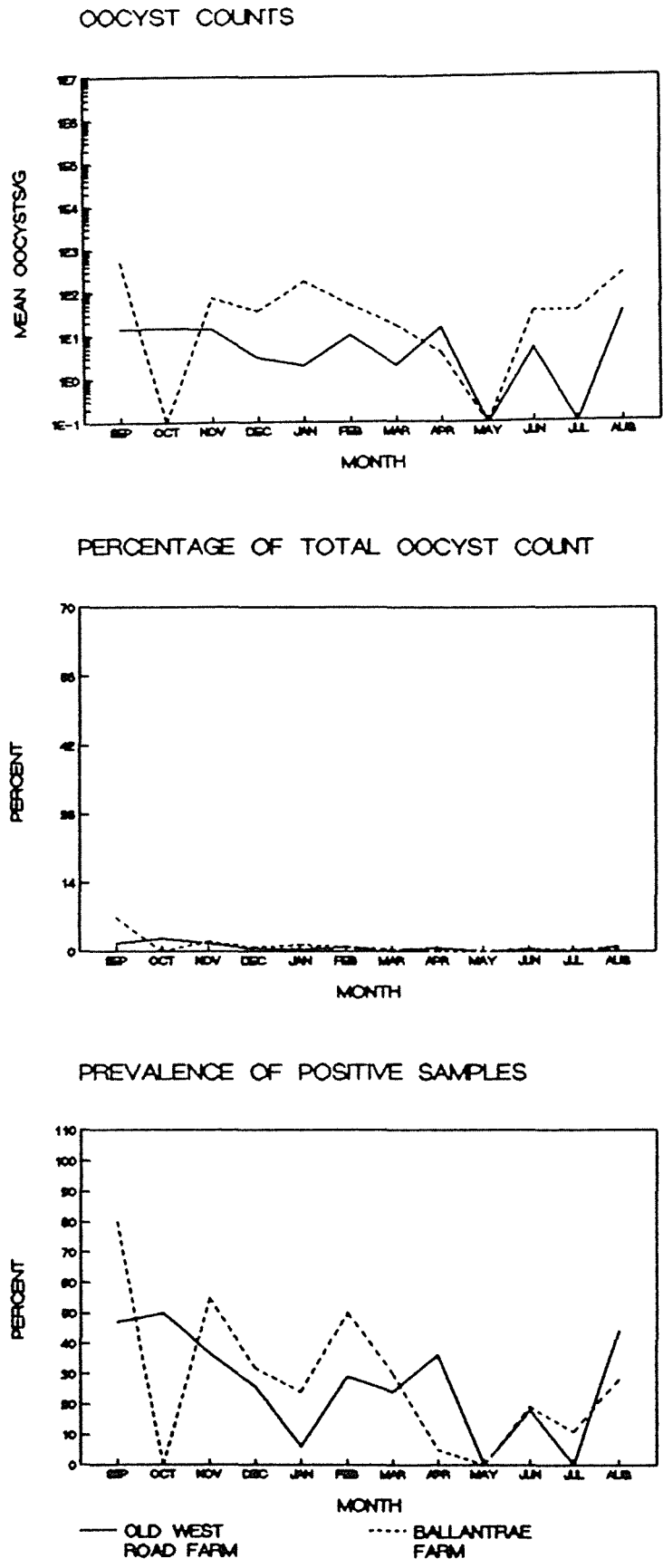


FIG. 4.31 SEASONAL VARIATIONS OF *E. ALIJEVI* FROM ADULTS

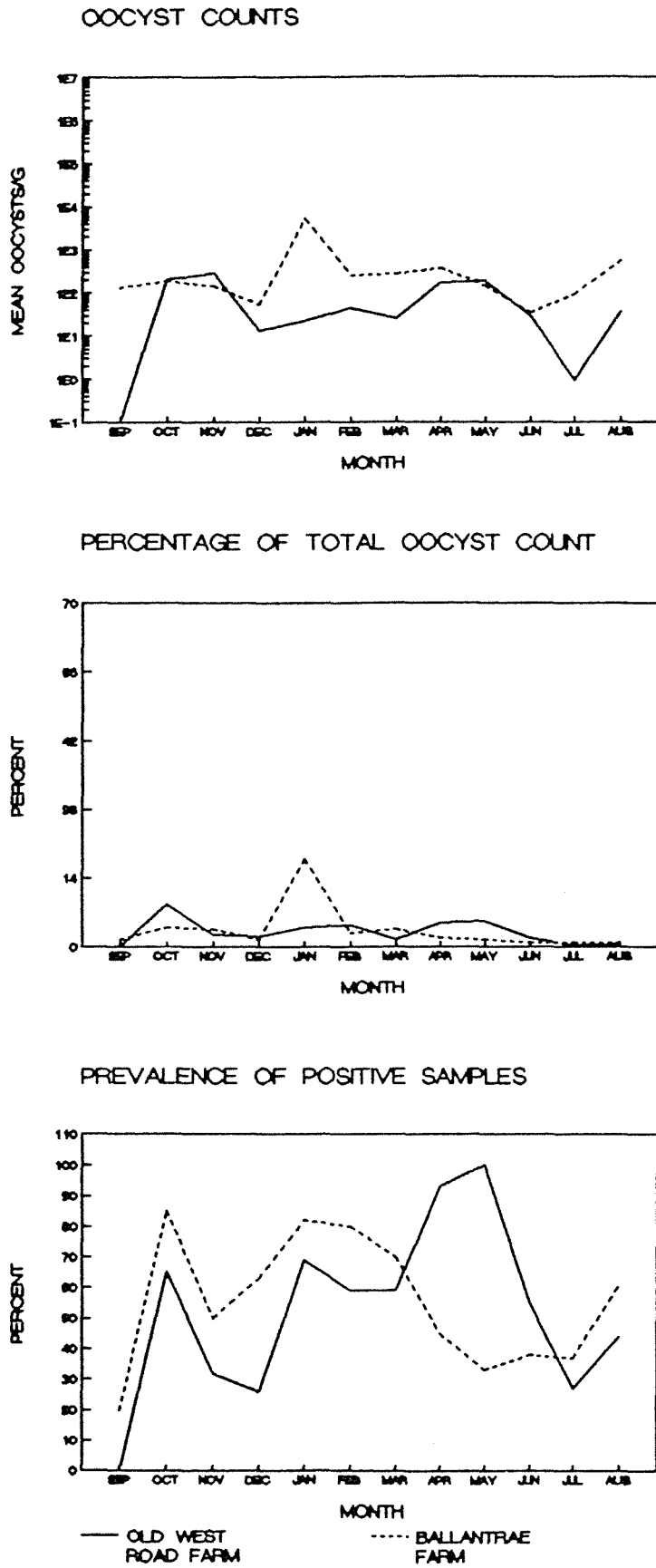
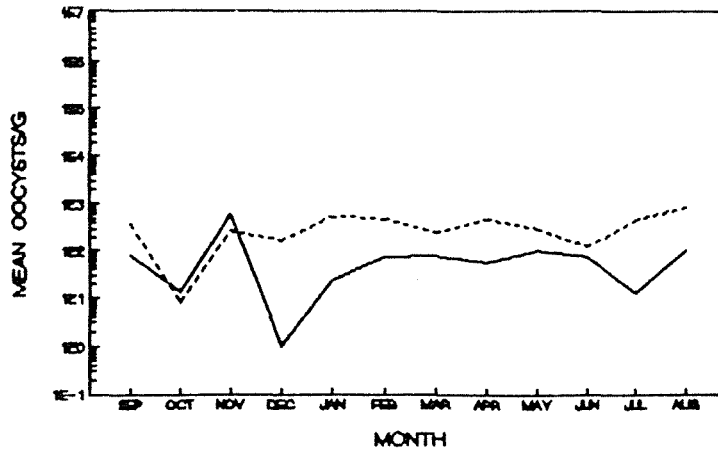
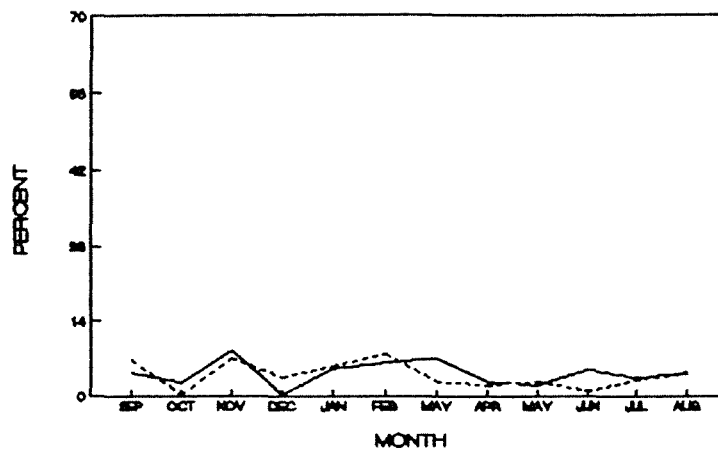


FIG. 4.32 SEASONAL VARIATIONS OF *E. N1* FROM ADULTS

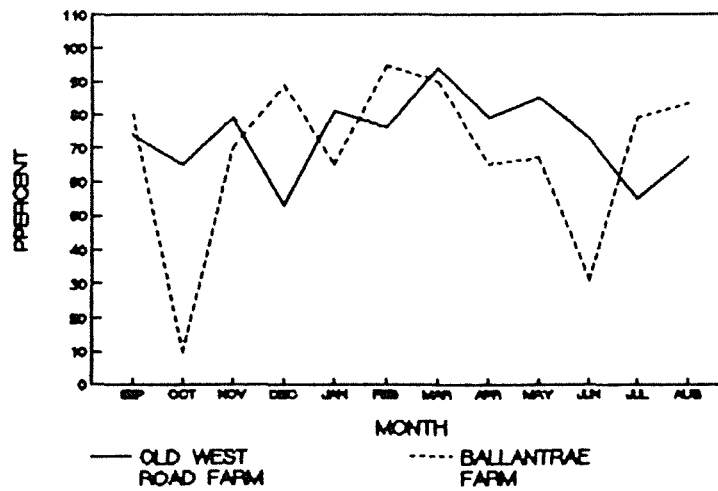
OOCYST COUNTS



PERCENTAGE OF TOTAL OOCYST COUNT



PREVALENCE OF POSITIVE SAMPLES



— OLD WEST ROAD FARM - - - BALLANTRAE FARM

FIG. 4.33 SEASONAL VARIATIONS OF *E. N2* FROM ADULTS

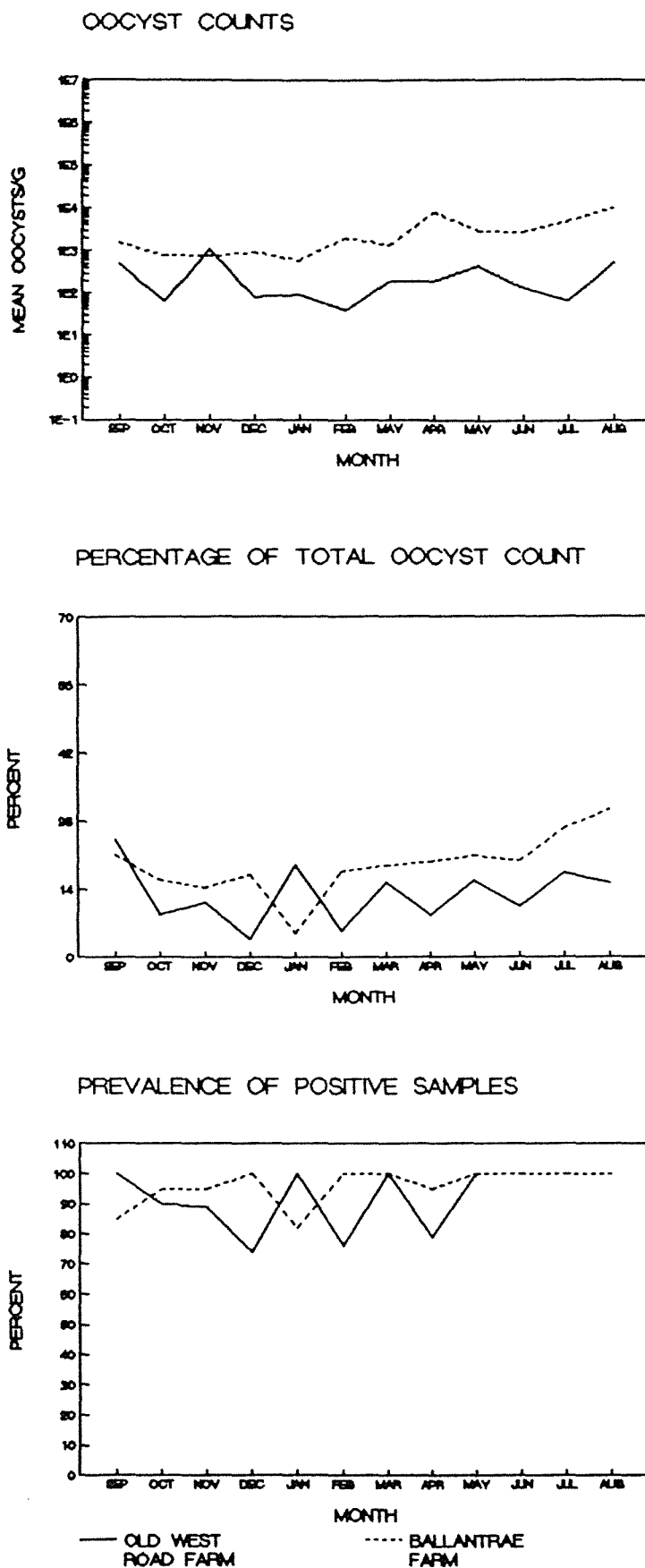
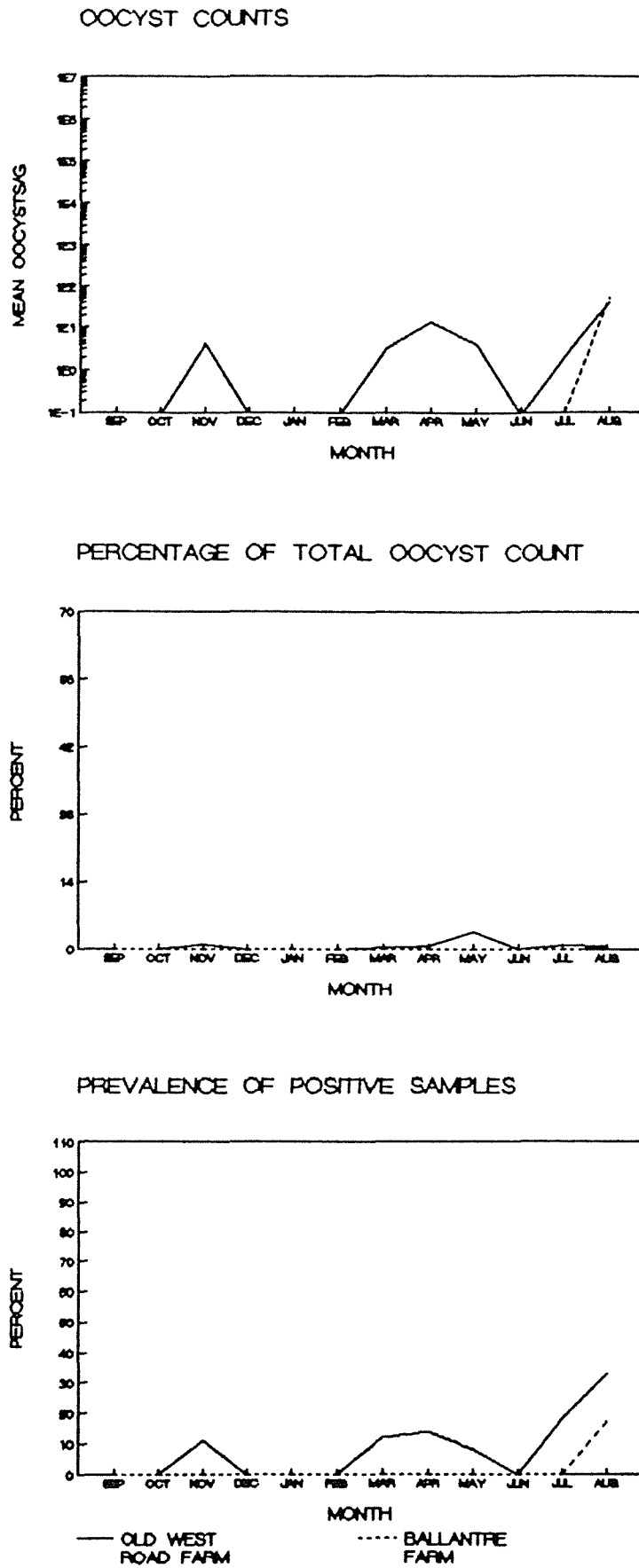


FIG. 4.34 SEASONAL VARIATIONS OF *E. N3* FROM ADULTS



CHAPTER FIVE

**RESULTS OF EXPERIMENT
FOR THE DETERMINATION OF SPORULATION TIME AND THE
SPORULATION STAGES OF *E. CHRISTENSENI* OOCYSTS AT VARIOUS
TEMPERATURES**

The procedures used in this experiment are described in section 2.4. During the sporulation process a series of eight recognisable stages was observed (Figs 5.1, 5.2 & Table 5.1):-

- Stage 1 No sign of alteration in the sporont which was spherical with fine granules.
- Stage 2 The sporont contracted away from the wall and became irregular in shape; it contained coarse granules and some globules.
- Stage 3 Cytokinesis started and the sporont was segmented into four lobes.
- Stage 4 Four spherical sporoblasts were formed.
- Stage 5 Sporoblasts became ovoid with a thickening at one end (presumably forming the stieda body at a later stage). A demarcation became evident along the middle of each sporoblast.

Table 5.1 Time (days) to achieve the different stages of sporulation of *E. christensenii* oocysts at various temperatures

Temperature(°C)	Stages *							
	1	2	3	4	5	6	7	8
4	-	14	16	18	20	22	26	32
10	1	4	5	7	8	10	11	14
15	1	3	4	5	6	7	8	11
20	1	-	-	2	3	4	5	10
27	-	-	-	1	2	3	4	7
37	-	-	-	0.5	1	1.5	2	-

* 90% of oocysts at each stage.

- Stage 6 Sporocysts were formed. Within each sporocyst, two sporozoites developed each containing 2 or more globules. Granules were scattered throughout the sporocysts.
- Stage 7 Granules in sporocysts became centrally located but were still coarse (these later formed the sporocyst residuum). Globules in sporozoites were well defined.
- Stage 8 Granules in each sporocyst appeared finer and sporozoites were more distinct. Sporozoite globules became clear and refractile. In some sporocysts the granules encircled the refractile globule and the sporocyst residuum appeared as a rosette (Fig. 5.3a). No further morphological changes were seen even in oocysts kept at 27°C for 14 days.

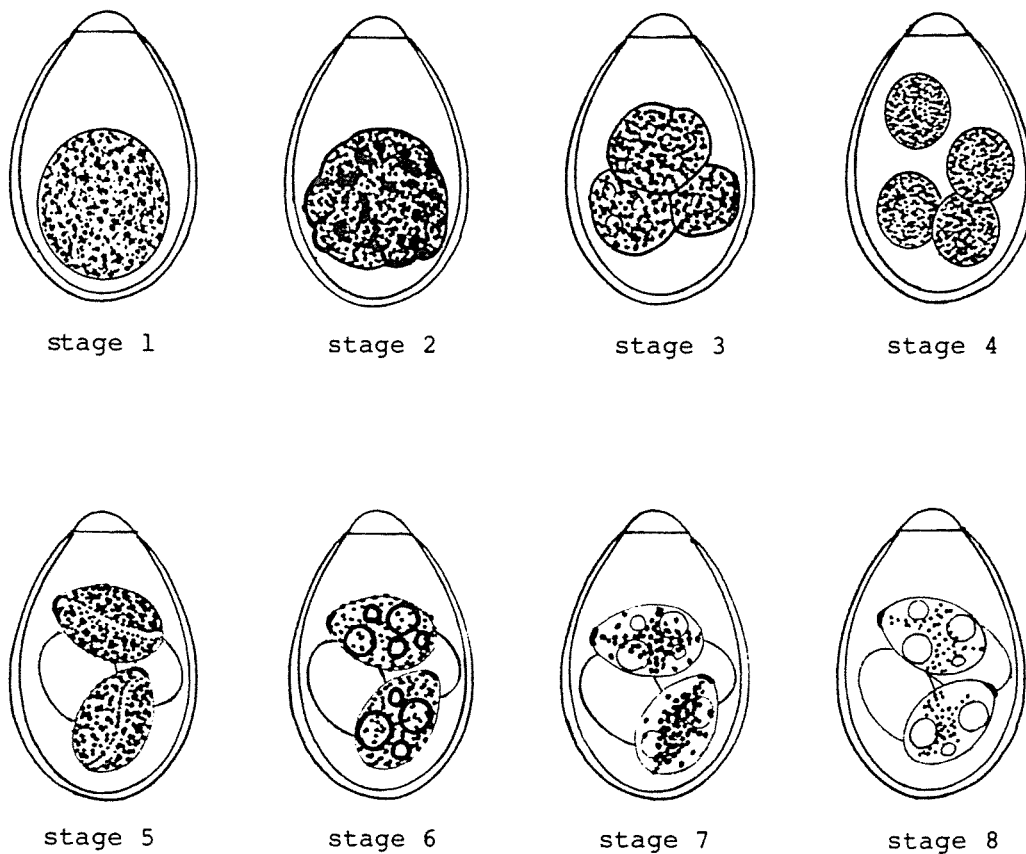


Fig. 5.1 Schematic diagram of sporulation stages of *E. christensenii* oocysts

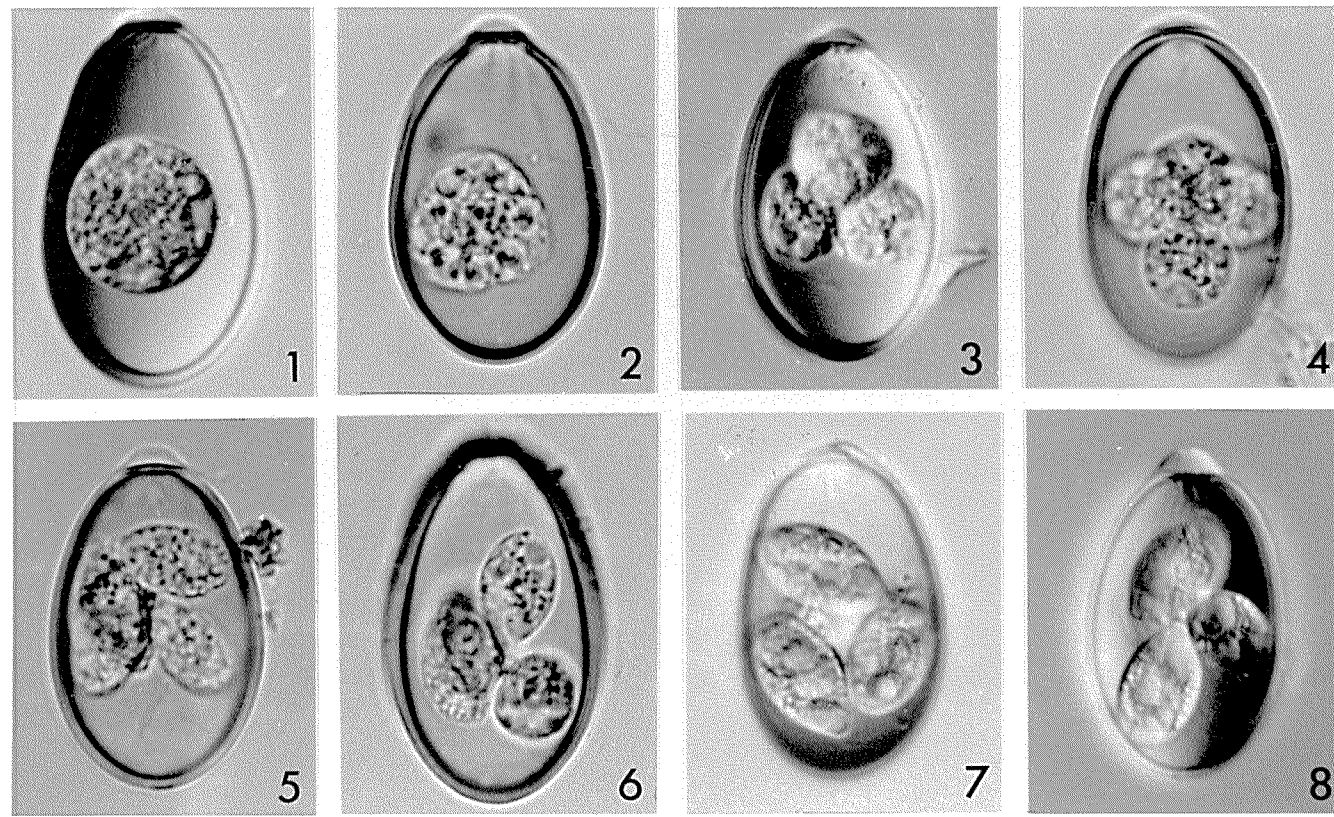


Fig. 5.2 Photomicrographs of sporulation stages of *E. christensenii* oocysts. Stages 1 - 8 correspond with those in Fig. 5.1 X 1122

These stages are, of course, arbitrary divisions of a continuous process. Although most of the stages have clearly defined characteristics, it was not always easy to separate stages 7 & 8 as assessment of how fine the granules were was inevitably subjective. The development of each stage was considered complete when 90% of the oocysts had attained it.

The majority of the oocysts developed from one stage to another at about the same time. Table 5.1 shows that at 20 and 27°C only 24 and 48 hours respectively were required for segmentation to occur (stage 4), but for those at 15°C, 10°C and 4°C, 5, 7 and 18 days, respectively were required. Generally, development to stage 7 occurred at the rate of one stage per day after stage 4 was attained. In all samples, in 3-3.5% of the oocysts the sporont had disintegrated by the time the first samples were examined (Fig. 5.3b).

Oocysts in cultures kept at 37°C developed at an accelerated rate. Stage 4 was seen after 12 hours and stages 4 to 7 developed normally within 48 hours. However, at 60 hours, only 2.5% of oocysts were completely sporulated. The rest were abnormal, most sporocysts containing coarse, scattered granules and large globules (Fig. 5.3c). In some oocysts, only one very large globule occupied the entire sporocyst. Seven percent of the oocysts appeared as in Fig. 5.3b.

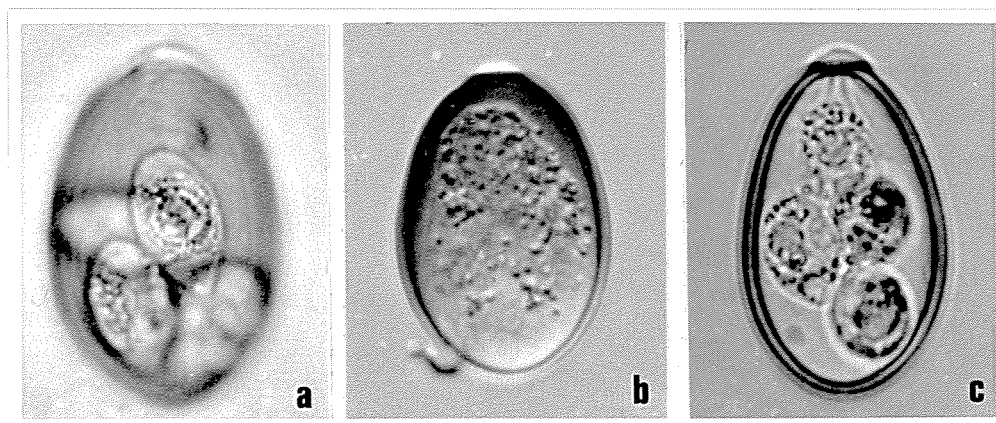


Fig. 5.3 *E. christenseni* oocysts: (a) Fully sporulated showing rosette-like arrangement of sporocyst residuum. (b) Oocyst with disintegrated sporont. (c) Oocyst with abnormal appearance of sporocyst contents caused by incubation at 37°C. X1122

The relationship between temperature and time taken for completion of sporulation is shown in Table 5.1 and Fig. 5.4. Values of both temperatures and days taken for 90% of oocysts to achieve complete sporulation were converted into \log_{10} values for statistical calculations as with this transformation the data most closely approximated to a straight line. The temperature:development time relationship yielded a correlation coefficient of $r = -0.99$ and a regression of $\log y = 4.48 + (-0.76) \log x$ (Fig. 5.4) where $y = \text{days}$ and $x = \text{temperature (}^{\circ}\text{C)}$. As can be seen from the table, at 27°C sporulation was complete in 7 days, required 10-14 days at $10\text{-}20^{\circ}\text{C}$ and 32 days at 4°C .

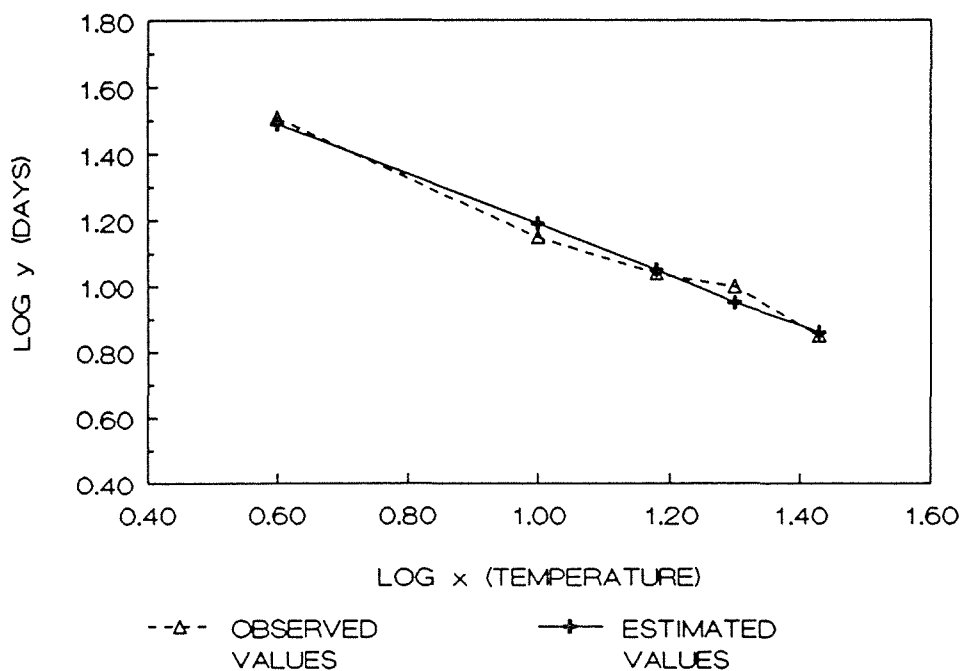


Fig. 5.4 *E. christensenii* oocysts: Regression of log development rate on log temperature

Discussion

The published information on the sporulation time of *E. christensenii* is very limited. Chevalier (1966) gave a figure of 3-6 days at "room temperature" without specifying what

the range of temperatures that represented or what criterion he used to determine the endpoint of sporulation or the proportion of oocysts sporulated. Levine (1985) quoted the same figures without indicating the origin of them or sporulation conditions; he may well have been quoting Chevalier. Lima (1981) also gave the same figure as Chevalier for oocysts kept at 30°C but provided no description of the criteria used for the endpoint of sporulation.

There is a similar lack of detailed information on the sporulation of other species of coccidia infecting goats (and sheep). Since this is a subject of some epidemiological importance, there is clearly a need for careful work to be done, at least on species that are of major importance.

CONCLUSION

The present study revealed not only that most of the previously described *Eimeria* species infecting goats have established in New Zealand but also the presence of additional species. Although the morphological characteristics of these additional species and statistical analysis of their dimensions comparison show that they are significantly different from previously described species, further work on their biology and clinical significance would provide further confirmation of their status as species infecting goats.

The taxonomic confusion relating to the identification of the species involved is a major problem confronting anyone working on the coccidia of goats. In part this is due to a lack of detail in the morphological descriptions of *Eimeria* species from goats, a failure to provide adequate differentiation between species and data derived from small numbers of oocysts in many papers. Much of this confusion will never be resolved though one would hope that it will not be added to by further publications of this kind. Another problem to that very few workers have studied the field intensively or viewed the published data critically in relation to their own work. As a result the possibility that there may be species additional to those already described has been little explored.

The research described in this thesis confirms the influence of husbandry systems on the epidemiology and intensity of coccidial infections. Although, coccidia are recognised as potential causes of serious clinical problems in goats, especially kids, they remain a relatively neglected subject for research.

Very few species have been isolated in pure culture and little is known of the details of their life cycles or their relative pathological significance. Even in terms of sporulation times, which are of some epidemiological importance, the amount of published data is minimal and much that has been published is of little value since the conditions under which the oocysts were kept are inadequately described.

Clearly, there is a lot more work to be done on the coccidia of goats. Priority needs to be given to work on single species directed towards elucidating development cycles and pathogenicity and other aspects of the host-parasite relationship. Further work is also needed on immunological aspects of infection to see if this could be exploited to prevent outbreaks of disease.

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APPENDIX 1

Descriptive statistics of oocysts and sporocysts of
Eimeria species found in the present study

A - Named species with micropylar cap

E. christenseni

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO*	39.02	1.901	100	39.04	34.72	43.60
WO*	25.40	1.437	100	25.24	22.16	29.52
LS*	15.26	8.452E-01	100	15.28	12.96	18.48
WS*	9.385	5.504E-01	100	9.400	8.400	11.28
LWRO*	1.539	8.036E-02	100	1.539	1.377	1.765
LWRS*	1.628	8.380E-02	100	1.626	1.424	1.815

E. tunisiensis

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	34.07	1.550	100	34.16	30.40	38.40
WO	23.61	8.501E-01	100	23.68	20.72	26.08
LS	15.99	6.672E-01	100	15.92	14.16	17.20
WS	8.239	4.246E-01	100	8.240	7.120	10.00
LWRO	1.444	6.241E-02	100	1.445	1.286	1.584
LWRS	1.944	9.902E-02	100	1.942	1.624	2.337

E. jolchejevi s.s.

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	31.33	1.259	100	31.36	28.00	34.42
WO	22.61	7.969E-01	100	22.64	20.40	24.48
LS	15.23	8.452E-01	100	15.36	11.92	16.72
WS	8.155	4.329E-01	100	8.160	7.120	9.520
LWRO	1.385	6.247E-02	100	1.376	1.119	1.527
LWRS	1.874	1.480E-01	100	1.876	1.505	2.337

E. jolchejevi 'large form'

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	36.52	2.298	100	36.56	32.00	41.36
WO	26.28	1.114	100	26.44	23.60	28.96
LS	16.37	0.962	100	16.36	14.56	18.48
WS	9.044	4.843E-01	100	9.120	7.440	10.00
LWRO	1.390	7.560E-02	100	1.385	1.239	1.569
LWRS	1.813	1.169E-01	100	1.823	1.570	2.049

E. arloingi

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	29.06	1.317	100	29.04	26.24	31.92
WO	19.33	1.189	100	19.32	16.16	23.04
LS	14.27	9.246E-01	100	14.24	12.56	16.80
WS	6.998	3.945E-01	100	6.960	6.000	8.160
LWRO	1.508	9.300E-02	100	1.512	1.212	1.713
LWRS	2.043	1.350E-01	100	2.033	1.747	2.386

E. hirci 'small form'

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	20.05	1.232	141	20.08	17.36	22.48
WO	17.11	1.062	141	17.12	14.96	19.84
LS	9.822	7.106E-01	141	9.840	8.000	12.56
WS	6.589	4.579E-01	141	6.640	5.360	7.760
LWRO	1.174	5.973E-02	141	1.171	1.019	1.343
LWRS	1.495	1.163E-01	141	1.500	1.087	1.760

E. hirci 'large form'

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	25.72	1.170	159	25.68	22.56	31.36
WO	18.55	0.983	159	18.56	16.40	21.52
LS	11.38	7.617E-01	159	11.44	9.680	13.12
WS	7.563	4.203E-01	159	7.600	6.320	8.480
LWRO	1.389	9.683E-02	159	1.400	1.137	1.742
LWRS	1.507	9.791E-01	159	1.495	1.313	1.951

B - Named species without micropylar cap

E. caprina

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	32.79	1.196	100	32.64	28.72	35.52
WO	23.11	7.564E-01	100	23.00	20.88	25.20
LS	15.25	5.565E-01	100	15.28	14.00	16.72
WS	8.302	4.049E-01	100	8.240	7.280	9.440
LWRO	1.420	6.612E-02	100	1.414	1.251	1.574
LWRS	1.841	1.055E-01	100	1.838	1.570	2.163

E. caprovina

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	28.01	1.826	100	28.24	21.76	32.00
WO	22.00	1.540	100	22.40	17.84	24.56
LS	14.15	7.764E-01	100	14.40	12.00	15.44
WS	7.849	5.239E-01	100	7.920	6.000	8.800
LWRO	1.276	7.209E-02	100	1.262	1.030	1.445
LWRS	1.807	1.007E-01	100	1.817	1.529	2.200

E. apsheronica

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	29.68	2.253	100	29.40	24.72	35.52
WO	22.22	1.257	100	22.24	19.20	25.36
LS	14.87	1.040	100	14.88	12.32	16.80
WS	8.111	5.204E-01	100	8.160	6.720	9.280
LWRO	1.336	7.063E-02	100	1.338	1.154	1.615
LWRS	1.836	1.161E-01	100	1.829	1.556	2.098

E. ninakohlyakimovae

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	23.87	1.346	100	23.80	20.64	26.88
WO	18.64	9.282E-01	100	18.80	16.40	20.54
LS	12.56	7.107E-01	100	12.64	10.72	14.08
WS	6.742	4.515E-01	100	6.800	5.440	7.840
LWRO	1.281	6.160E-02	100	1.268	1.149	1.469
LWRS	1.868	1.142E-01	100	1.858	1.625	2.265

E. alijevi

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	17.02	1.089	100	17.24	14.88	19.52
WO	14.05	6.311E-01	100	14.04	12.80	15.44
LS	9.327	5.891E-01	100	9.320	8.320	11.12
WS	5.050	2.784E-01	100	5.120	4.320	5.680
LWRO	1.211	5.290E-02	100	1.209	1.116	1.365
LWRS	1.851	1.358E-01	100	1.859	1.507	2.117

Previously undescribed species with micropylar cap

E. n1

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	22.32	1.555	100	22.40	18.48	25.76
WO	17.39	0.964	100	17.32	15.12	19.52
LS	12.15	8.378E-01	100	12.08	10.00	13.60
WS	6.154	4.724E-01	100	6.160	4.800	7.168
LWRO	1.284	6.486E-02	100	1.283	1.013	1.477
LWRS	1.980	1.330E-01	100	1.976	1.663	2.550

E. n2

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	29.27	1.625	100	29.56	25.28	34.08
WO	19.79	1.108	100	19.76	17.60	24.64
LS	12.76	6.399E-01	100	12.80	11.28	14.00
WS	7.593	4.118E-01	100	7.600	6.720	8.960
LWRO	1.482	8.738E-02	100	1.467	1.317	1.732
LWRS	1.682	1.102E-01	100	1.691	1.358	1.930

Previously undescribed without micropylar cap

E. n3

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
LO	22.97	0.990	100	22.92	20.24	25.76
WO	17.48	5.248E-01	100	17.44	16.24	18.96
LS	12.64	5.394E-01	100	12.68	11.28	13.84
WS	6.343	3.329E-01	100	6.320	5.360	7.120
LWRO	1.315	6.489E-02	100	1.308	1.140	1.493
LWRS	1.998	1.210E-01	100	2.000	1.724	2.507

* LO = oocyst length; WO = oocyst width; LS = sporocyst length
 WS = sporocyst width; LWRO = length width ratio of oocyst;
 LWRS = length width ratio of oocyst

APPENDIX 2

Results of two sample t-tests to determine the significance of the differences of oocyst and sporocyst measurements

Table 1 - Between *E. arloingi* and *E. tunisiensis*

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. arloingi</i>	29.06	100	1.317	-24.60	198	0.000
<i>E. tunisiensis</i>	34.07	100	1.550			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. arloingi</i>	19.33	100	1.189	-29.29	179.30	0.000*
<i>E. tunisiensis</i>	23.61	100	0.850			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. arloingi</i>	1.508	100	0.093	5.72	173.1	0.000*
<i>E. tunisiensis</i>	1.444	100	0.062			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. arloingi</i>	14.27	100	0.924	-15.04	180.1	0.000*
<i>E. tunisiensis</i>	15.99	100	0.667			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. arloingi</i>	6.998	100	0.395	-21.41	198	0.000
<i>E. tunisiensis</i>	8.239	100	0.425			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. arloingi</i>	2.043	100	0.135	5.92	181.6	0.000*
<i>E. tunisiensis</i>	1.944	100	0.097			

* Adjusted for unequal variances.

Table 2 - Between *E. jolchejevi* s.s. and *E. jolchejevi* 'large form'

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> s.s.	31.33	100	1.259	-19.87	153.5	0.000*
<i>E. jolchejevi</i> 'large form'	36.52	100	2.298			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> s.s.	22.61	100	0.795	-26.76	179.3	0.000*
<i>E. jolchejevi</i> 'large form'	26.28	100	1.114			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> s.s.	1.385	100	0.062	-0.52	191.2	0.611
<i>E. jolchejevi</i> 'large form'	1.390	100	0.076			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> s.s.	15.23	100	0.845	- 8.84	198	0.000
<i>E. jolchejevi</i> 'large form'	16.37	100	0.962			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> s.s.	8.155	100	0.433	-13.69	198	0.000
<i>E. jolchejevi</i> 'large form'	9.044	100	0.484			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> s.s.	1.874	100	0.148	3.21	188	0.001*
<i>E. jolchejevi</i> 'large form'	1.813	100	0.117			

* Adjusted for unequal variances.

Table 3 - Between *E. hirci* 'small form' and *E. hirci* 'large form'

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. hirci</i> 'small form'	20.05	141	1.232	-32.59	286.4	0.000*
<i>E. hirci</i> 'large form'	25.72	159	1.710			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. hirci</i> 'small form'	17.11	141	1.062	-12.15	298	0.000
<i>E. hirci</i> 'large form'	18.55	159	0.983			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. hirci</i> 'small form'	1.174	141	0.059	-23.43	267.2	0.000*
<i>E. hirci</i> 'large form'	1.389	159	0.097			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. hirci</i> 'small form'	9.822	141	0.710	-19.20	298	0.000
<i>E. hirci</i> 'large form'	11.38	159	0.761			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. hirci</i> 'small form'	6.589	141	0.457	-19.20	298.3	0.000
<i>E. hirci</i> 'large form'	7.563	159	0.420			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. hirci</i> 'small form'	1.495	141	0.116	-0.93	298	0.355*
<i>E. hirci</i> 'large form'	1.507	159	0.098			

* Adjusted for unequal variances.

Table 4 - Between *E. n1* and *E. hirci* 'small form'

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	22.32	100	1.555	-12.11	181.4	0.000*
<i>E. hirci</i> 'small form'	20.05	141	1.232			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	17.39	100	0.964	-2.05	224.9	0.039
<i>E. hirci</i> 'small form'	17.11	141	1.062			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	1.284	100	0.065	-13.58	239	0.000
<i>E. hirci</i> 'small form'	1.174	141	0.060			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	12.15	100	0.084	-23.62	190.7	0.000*
<i>E. hirci</i> 'small form'	9.822	141	0.711			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	6.154	100	0.472	7.17	239	0.000
<i>E. hirci</i> 'small form'	6.589	141	0.456			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	1.980	100	0.113	-30.03	239	0.000
<i>E. hirci</i> 'small form'	1.495	141	0.116			

* Adjusted for unequal variances.

Table 5 - Between *E. n1* and *E. hirci* 'large form'

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	22.32	100	1.555	-16.17	257.9	0.000
<i>E. hirci</i> 'large form'	25.72	159	1.710			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	17.39	100	0.964	-9.32	257	0.000
<i>E. hirci</i> 'large form'	18.55	159	0.983			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	1.284	100	0.065	-10.47	256.5	0.000*
<i>E. hirci</i> 'large form'	1.389	159	0.097			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	12.15	100	0.084	7.64	257	0.000
<i>E. hirci</i> 'large form'	11.38	159	0.761			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	6.154	100	0.472	-25.02	257	0.000
<i>E. hirci</i> 'large form'	7.563	159	0.420			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	1.980	100	0.013	30.73	165.9	0.000*
<i>E. hirci</i> 'large form'	1.507	159	0.098			

* Adjusted for unequal variances.

Table 6 - Between *E. n1* and *E. arloingi*

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	22.32	100	1.555	-33.11	192.8	0.000*
<i>E. arloingi</i>	29.06	100	1.317			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	17.39	100	0.964	-12.67	189.9	0.000*
<i>E. arloingi</i>	19.33	100	1.189			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	1.284	100	0.065	-19.75	176.9	0.000*
<i>E. arloingi</i>	1.508	100	0.093			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	12.15	100	0.838	-17.00	198	0.000
<i>E. arloingi</i>	14.27	100	0.925			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	6.154	100	0.472	-13.72	191.9	0.000*
<i>E. arloingi</i>	6.998	100	0.395			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n1</i>	1.980	100	0.133	-3.30	198	0.001
<i>E. arloingi</i>	2.043	100	0.135			

* Adjusted for unequal variances.

Table 7 - Between *E. n2* and *E. arloingi*

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	29.27	100	1.625	1.00	189.8	0.321*
<i>E. arloingi</i>	29.06	100	1.317			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	19.79	100	1.108	2.84	198	0.005
<i>E. arloingi</i>	19.33	100	1.189			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	1.482	100	0.090	-2.06	198	0.034
<i>E. arloingi</i>	1.508	100	0.093			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	12.76	100	0.640	-13.47	176.1	0.000*
<i>E. arloingi</i>	14.27	100	0.925			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	7.593	100	0.412	10.43	198	0.000
<i>E. arloingi</i>	6.998	100	0.395			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	1.682	100	0.110	-20.68	190.3	0.000*
<i>E. arloingi</i>	2.043	100	0.135			

* Adjusted for unequal variances.

Table 8 - Between *E. n2* and *E. hirci* 'large form'

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	29.27	100	1.625	16.56	257	0.000
<i>E. hirci</i> 'large form'	25.72	159	1.710			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	19.79	100	1.108	9.41	257	0.000
<i>E. hirci</i> 'large form'	18.55	159	0.983			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	1.471	100	0.087	7.77	257	0.000
<i>E. hirci</i> 'large form'	1.389	159	0.097			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	12.76	100	0.064	15.66	236.4	0.000*
<i>E. hirci</i> 'large form'	11.380	159	0.761			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	7.593	100	0.412	0.57	257	0.5752
<i>E. hirci</i> 'large form'	7.563	159	0.420			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. n2</i>	1.682	100	0.110	13.38	257	0.000
<i>E. hirci</i> 'large form'	1.507	159	0.098			

* Adjusted for unequal variances.

Table 9 - Between *E. tunisiensis* and *E. christenseni*

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. tunisiensis</i>	34.07	100	1.550	-20.21	190.3	0.000*
<i>E. christenseni</i>	39.02	100	1.901			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. tunisiensis</i>	23.61	100	0.850	-10.72	160.8	0.000*
<i>E. christenseni</i>	25.40	100	1.437			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. tunisiensis</i>	1.444	100	0.062	-9.37	186.6	0.000*
<i>E. christenseni</i>	1.539	100	0.083			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. tunisiensis</i>	15.99	100	0.667	6.78	187.9	0.000*
<i>E. christenseni</i>	15.26	100	0.845			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. tunisiensis</i>	8.24	100	0.425	-16.48	186	0.000*
<i>E. christenseni</i>	9.39	100	0.550			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. tunisiensis</i>	1.944	100	0.100	24.33	192.7	0.000*
<i>E. christenseni</i>	1.539	100	0.084			

* Adjusted for unequal variances.

Table 10 - Between *E. jolchejevi* 'large form' and *E. tunisiensis*

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> 'large form'	36.52	100	2.298	8.87	173.6	0.000*
<i>E. tunisiensis</i>	34.07	100	1.550			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> 'large form'	26.28	100	1.114	19.06	185.1	0.000*
<i>E. tunisiensis</i>	23.61	100	0.850			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> 'large form'	1.390	100	0.756	-5.44	191.1	0.000*
<i>E. tunisiensis</i>	1.444	100	0.062			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> 'large form'	16.37	100	0.962	3.24	176.4	0.000*
<i>E. tunisiensis</i>	15.99	100	0.667			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> 'large form'	9.044	100	0.484	12.49	198	0.000
<i>E. tunisiensis</i>	8.239	100	0.425			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi</i> 'large form'	1.813	100	1.169	5.92	181.6	0.000*
<i>E. tunisiensis</i>	1.944	100	0.092			

* Adjusted for unequal variances.

Table 11 - Between *E. jolchejevi s.s.* and *E. tunisiensis*

For oocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi s.s.</i>	31.33	100	1.259	-13.71	190	0.000*
<i>E. tunisiensis</i>	34.07	100	1.550			

For oocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi s.s.</i>	22.61	100	0.800	-8.54	198	0.000*
<i>E. tunisiensis</i>	23.61	100	0.850			

For oocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi s.s.</i>	1.385	100	0.062	-6.62	198	0.000
<i>E. tunisiensis</i>	1.444	100	0.062			

For sporocyst length

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi s.s.</i>	15.23	100	0.845	-6.99	187.9	0.000*
<i>E. tunisiensis</i>	15.99	100	0.667			

For sporocyst width

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi s.s.</i>	8.155	100	0.433	-1.39	198	0.161
<i>E. tunisiensis</i>	8.239	100	0.425			

For sporocyst shape index

VARIABLE	MEAN	N	S.D.	T	DF	P
<i>E. jolchejevi s.s.</i>	1.874	100	0.148	-3.94	172.9	0.000*
<i>E. tunisiensis</i>	1.944	100	0.099			

* Adjusted for unequal variances.

APPENDIX 3

Summary statistics of oocyst counts for each sampling

A - Kids: Old West Road Farm

MONTH	MEAN	S.D.	N	MINIMUM	MAXIMUM
OCT1	1.891E+06	1.426E+06	20	3.960E+05	6.129E+06
OCT2	6.009E+06	5.262E+06	20	1.822E+06	1.740E+07
NOV1	1.124E+06	1.070E+06	20	4.500E+04	3.648E+06
NOV2	1.255E+05	9.784E+04	20	2.100E+04	3.930E+05
DEC1	2.408E+04	2.485E+04	18	1.500E+03	9.300E+04
DEC2	1.464E+05	2.199E+05	20	5.400E+03	9.360E+05
JAN1	4.142E+04	6.124E+04	18	3.200E+03	1.922E+05
JAN2	2.348E+04	1.977E+04	20	3.600E+03	7.040E+04
FEB1	4.014E+04	5.446E+04	20	2.000E+03	2.292E+05
FEB2	1.449E+04	3.280E+04	17	0.000	1.376E+05
MAR1	2.964E+04	5.331E+04	19	1.000E+03	2.400E+05
MAR2	1.694E+04	2.243E+04	19	1.000E+03	8.400E+04
APR1	1.320E+04	1.413E+04	20	1.400E+03	5.240E+04
APR2	9.470E+03	6.610E+03	20	2.800E+03	2.580E+04
MAY1	2.320E+04	1.976E+04	20	4.800E+03	8.480E+04
MAY2	1.416E+04	3.086E+04	20	2.600E+03	1.440E+05
JUN1	8.961E+03	1.091E+04	18	600.0	4.640E+04
JUN2	1.511E+04	1.751E+04	18	800.0	7.200E+04
JUL	1.226E+04	1.247E+04	18	2.700E+03	4.800E+04
AUG	1.436E+04	2.695E+04	18	2.000E+03	1.152E+05
SEP	7.570E+03	7.488E+03	20	1.200E+03	3.160E+04

B - Kids: Kimbolton Farm

MONTH	MEAN	S.D.	N	MINIMUM	MAXIMUM
OCT2	1.112E+05	1.310E+05	16	0.000	5.270E+05
NOV1	9.176E+04	9.847E+04	15	1.000E+03	3.320E+05
NOV2	5.731E+04	4.926E+04	16	6.000E+03	1.860E+05
DEC1	6.350E+04	1.033E+05	16	9.000E+03	4.440E+05
DEC2	2.632E+05	2.822E+05	16	7.200E+03	9.324E+05
JAN1	6.977E+04	8.302E+04	22	600.0	2.760E+05
JAN2	3.604E+04	3.074E+04	23	2.800E+03	1.080E+05
FEB1	4.617E+04	5.938E+04	19	5.600E+03	2.428E+05
FEB2	1.231E+05	1.934E+05	19	8.000E+03	8.868E+05
MAR1	2.951E+04	3.718E+04	23	2.000E+03	1.660E+05
MAR2	1.330E+04	8.510E+03	23	2.800E+03	3.640E+04
APR1	2.896E+04	5.310E+04	22	4.000E+03	2.048E+05
APR2	3.241E+04	1.953E+04	16	1.000E+04	9.120E+04
MAY1	1.481E+04	8.403E+03	16	3.800E+03	3.200E+04
MAY2	3.716E+04	2.461E+04	16	1.040E+04	1.060E+05
JUN1	3.132E+04	3.697E+04	15	4.000E+03	1.548E+05
JUN2	7.485E+03	4.031E+03	13	2.200E+03	1.440E+04
JUL	9.617E+03	6.455E+03	12	1.200E+03	2.120E+04
AUG	1.390E+04	8.356E+03	10	3.800E+03	3.200E+04
SEP	3.025E+03	1.964E+03	8	600.0	6.200E+03

C - Kids: Ballantrae Farm

MONTH	MEAN	S.D.	N	MINIMUM	MAXIMUM
OCT	2.927E+04	5.624E+04	13	0.000	2.060E+05
NOV	3.500E+04	3.947E+04	15	0.000	1.500E+05
DEC	3.332E+04	5.766E+04	16	1.400E+03	2.092E+05
JAN	7.872E+04	1.828E+05	17	4.200E+03	7.644E+05
FEB	3.156E+04	2.972E+04	16	1.200E+03	8.280E+04
MAR	7.885E+04	1.217E+05	15	2.200E+03	4.548E+05
APR	4.514E+04	1.125E+05	14	1.600E+03	4.320E+05
MAY	1.061E+04	1.324E+04	16	600.0	4.200E+04
JUN	1.259E+04	1.356E+04	15	400.0	4.800E+04
JUL	1.652E+04	9.662E+03	13	3.000E+03	3.220E+04
AUG	2.054E+04	1.913E+04	13	4.400E+03	7.680E+04
SEP	7.957E+03	5.942E+03	14	2.000E+03	2.200E+04

D - Adults: Old West Road Farm

MONTH	MEAN	S.D.	N	MINIMUM	MAXIMUM
AUG	5.289E+03	4.866E+03	9	700.0	1.520E+04
SEP	1.639E+03	2.132E+03	19	100.0	8.450E+03
OCT	1.692E+03	4.538E+03	20	50.00	2.075E+04
NOV	1.134E+04	2.488E+04	19	150.0	8.150E+04
DEC	2.934E+03	9.200E+03	19	150.0	4.080E+04
JAN	484.4	463.9	16	100.0	2.000E+03
FEB	929.4	759.7	17	0.000	2.700E+03
MAR	1.182E+03	1.139E+03	17	200.0	4.900E+03
APR	3.914E+03	4.895E+03	14	600.0	1.920E+04
MAY	3.185E+03	4.467E+03	13	600.0	1.780E+04
JUN	1.282E+03	627.4	11	200.0	2.000E+03
JUL	654.5	973.0	11	100.0	3.500E+03
AUG	4.600E+03	5.250E+03	9	400.0	1.800E+04

E - Adults: Ballantrae Farm

MONTH	MEAN	S.D.	N	MINIMUM	MAXIMUM
AUG	4.503E+03	4.214E+03	19	600.0	1.370E+04
SEP	7.520E+03	8.671E+03	20	1.700E+03	3.960E+04
OCT	4.610E+03	6.763E+03	20	300.0	2.950E+04
NOV	4.688E+03	5.252E+03	20	250.0	2.445E+04
DEC	4.553E+03	5.070E+03	19	300.0	2.240E+04
JAN	2.406E+04	4.565E+04	17	800.0	1.956E+05
FEB	8.967E+03	1.369E+04	20	1.700E+03	6.280E+04
MAR	1.092E+04	2.078E+04	20	51.00	9.540E+04
APR	3.468E+04	5.423E+04	20	2.400E+03	1.920E+05
MAY	1.323E+04	1.422E+04	18	400.0	6.040E+04
JUN	1.012E+04	1.248E+04	16	800.0	4.820E+04
JUL	1.812E+04	1.890E+04	19	2.000E+03	7.770E+04
AUG	1.974E+04	1.482E+04	18	1.700E+03	6.200E+04

APPENDIX 4

Results of two sample t-tests to determine the significance
of differences of oocyst counts

A - Between male and female kids from Ballantrae farm

For October

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	7700	5	7636	-1.41	7.3	0.200*
Female	42750	8	69640			

For November

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	66000	5	53680	1.92	4.4	0.120*
Female	19200	10	17620			

For December

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	5779	7	80720	1.39	6.6	0.209*
Female	14299	9	20370			

For January

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	157000	7	276700	1.28	6.0	0.246*
Female	23560	10	13280			

For February

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	30930	6	34250	-0.06	14	0.954
Female	31940	10	28640			

For March

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	139000	6	170900	1.39	5.7	0.217*
Female	14290	9	20370			

For April

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	24400	4	28510	-0.65	10.8	0.527*
Female	53440	10	133200			

For May

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	13300	6	15800	0.62	14	0.548*
Female	19200	10	17620			

For June

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	15900	6	18200	0.76	13	0.461
Female	10380	9	20370			

For July

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	18730	3	11690	0.44	11	0.671
Female	15860	10	9591			

For August

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	14850	4	11040	-0.70	11	0.499
Female	23070	9	21900			

B - Between male and female kids from Kimbolton farm**For October**

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	90140	11	78080	-0.69	4.5	0.526*
Female	157500	5	212700			

For November

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	106400	10	112500	0.81	13	0.435
Female	19200	10	17620			

For December

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	84500	10	128300	1.38	9.1	0.202*
Female	28500	6	7530			

For January

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	73150	15	90250	0.27	20.0	0.788
Female	62540	7	70910			

For February

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	35690	13	42880	-1.14	17	0.270
Female	68870	6	85900			

For March

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	27650	15	44030	-0.39	20.9	0.700*
Female	14290	9	20370			

For April

VARIABLE	MEAN	N	S.D.	T	DF	P
Male	23490	14	45540	-0.63	20.0	0.535
Female	38550	8	67910			

* Adjusted for unequal variances.

Appendix 5

Data on seasonal variations in mean oocyst counts, mean percentage of total counts and sample prevalence for individual *Eimeria* species in kids

Key: OWR - Old West Road Farm KBT - Kimbolton Farm BALT - Ballantrae Farm

Table 1. *E. christenseni*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	1047000			56.70			100		
	2044000	29200	1830	36.95	23.25	5.17	100	94	50
Nov	396500	29007		29.35	16.73		100	93	
	42100	2211	6164	30.80	4.63	18.73	100	94	93
Dec	8310	2221		28.11	5.03		100	81	
	77170	8690	4957	26.70	14.21	21.50	80	71	100
Jan 1988	6248	13300		21.44	17.61		89	96	
	1586	8169	2341	5.85	24.48	7.77	85	100	82
Feb	1796	5825		2.95	13.37		45	89	
	179	1378	3168	2.22	10.11	5.00	61	83	50
Mar	341	9637		0.74	29.22		89	96	
	29	2975	3606	0.47	18.48	4.80	16	78	67
Apr	261	4200		2.30	14.14		40	86	
	2	1063	868	0.05	4.63	4.57	5	75	50
May	32	538		0.10	2.50		10	50	
	23	1174	230	0.15	3.81	3.75	15	44	50
June	3	666		0.17	1.93		11	33	
	151	49	124	0.83	0.23	1.00	28	83	40
July	90	409	422	0.28	0.50	2.23	17	69	54
Aug	357	277	578	0.28	1.50	1.39	33	20	62
Sept	52	87	0	0.75	2.00	0.00	25	50	0

Table 2. *E. tunisiensis*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	0			0.00			0		
	0	2448	0	0.00	3.20	0.00	0	6	0
Nov	7191	18670		0.45	16.00		15	73	
	4023	8867	5200	3.40	16.21	14.47	80	94	93
Dec	227	5216		2.17	9.34		39	94	
	509	14340	1402	1.55	21.57	7.63	35	71	56
Jan 1988	316	13510		1.72	14.41		44	100	
	432	658	1930	2.10	1.83	4.35	40	57	65
Feb	86	3923		0.35	8.84		25	89	
	38	1994	1288	1.18	1.33	6.25	50	39	69
Mar	77	260		0.42	1.65		44	52	
	95	218	954	1.37	1.62	1.47	42	43	40
Apr	56	396		0.40	0.90		15	23	
	31	237	3223	0.35	0.56	2.71	25	31	50
May	0	80		0.00	0.38		0	19	
	71	1693	8	0.35	5.31	0.38	15	63	63
June	265	470		1.72	2.00		50	40	
	128	517	106	1.28	8.39	0.67	50	33	40
July									
	222	266	376	2.42	1.92	2.15	56	100	23
Aug									
	172	30	242	1.06	0.30	1.00	33	30	100
Sept									
	35	0	21	0.00	0.00	0.50	20	0	21

Table 3. *E. jolchejevi*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	0			0.00			0		
	14730	1451	0	0.35	2.13	0.00	10	38	0
Nov	4705	0		0.35	0.00		5	0	
	486	641	1088	0.20	2.81	2.53	5	38	27
Dec	0	0		0.00	0.00		0	0	
	640	56	100	1.95	0.14	1.75	25	18	13
Jan 1988	1543	903		6.39	5.75		67	23	
	3657	1231	772	12.30	4.52	3.47	80	39	47
Feb	3062	674		4.55	1.63		55	37	
	84	146	228	1.83	3.89	1.44	17	17	25
Mar	6055	496		27.47	3.78		67	48	
	4730	2925	5465	15.32	10.14	12.40	89	43	67
Apr	2750	2513		14.51	15.91		80	82	
	2359	1170	5728	18.55	38.56	25.50	65	100	71
May	2493	3647		12.90	20.56		80	88	
	7645	315	1599	21.70	1.00	11.50	75	44	81
June	2557	1512		25.83	3.80		94	47	
	467	193	176	7.06	2.69	2.00	83	83	47
July									
	1243	25	190	10.22	0.42	1.08	72	31	31
Aug									
	695	156	568	6.33	1.25	2.77	56	20	23
Sept									
	571	60	342	10.70	0.60	2.86	80	50	50

Table 4. *E. arloingi*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	789500			35.40			100		
	3398000	29650	21390	51.75	27.07	59.58	100	81	75
Nov	466200	45170		42.00	49.94		100	100	
	32340	26740	10820	22.30	45.31	20.07	95	100	93
Dec	1037	21500		31.39	37.19		100	100	
	42710	16340	11450	38.55	32.93	29.25	100	94	100
Jan 1988	3933	11820		14.94	15.66		89	100	
	4526	6177	19970	19.85	18.22	28.59	100	96	88
Feb	5032	5527		13.05	15.79		100	100	
	1078	62030	4116	14.78	41.33	11.19	89	94	94
Mar	4761	4372		16.00	22.61		89	96	
	4278	8042	3703	23.95	34.81	8.60	100	100	93
Apr	2090	8217		16.20	25.27		95	96	
	1917	7435	8077	20.50	27.00	22.71	95	94	93
May	4095	4636		18.90	21.19		100	94	
	4888	10030	2318	22.60	25.06	29.12	95	100	100
June	1422	4835		14.72	13.53		94	100	
	3076	1804	3942	20.50	23.92	31.07	100	100	100
July									
	1899	3018	4951	16.78	27.83	29.46	100	100	100
Aug									
	3831	3364	9224	23.78	28.00	40.46	100	90	100
Sept									
	1162	660	2320	22.05	23.75	35.43	95	100	100

Table 5. *E. hirici*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	38660			2.10			30		
	90450	9931	4	2.05	16.20	0.08	45	88	8
Nov	67040	6834		6.65	6.73		70	60	
	20110	12210	1466	17.55	19.44	5.07	95	94	67
Dec	2101	4903		14.56	5.98		56	94	
	10480	1605	3046	12.50	2.14	7.81	90	100	88
Jan 1988	1180	18560		7.60	22.09		89	100	
	3313	11230	9163	11.50	27.30	6.65	90	100	65
Feb	9353	19800		24.65	30.74		95	100	
	1293	9592	9954	18.87	18.28	33.37	83	89	100
Mar	3370	2897		14.63	16.39		89	87	
	5584	582	5246	15.16	4.43	46.90	95	83	100
Apr	4273	9944		29.10	17.14		100	100	
	1073	5018	9120	12.50	15.06	25.43	95	94	100
May	2985	1281		16.20	7.13		100	88	
	2090	8044	4535	13.00	24.75	23.87	100	100	100
June	1935	5139		21.50	21.27		100	100	
	3241	1444	3367	26.06	17.23	25.73	100	92	100
July									
	2488	755	985	22.61	6.67	6.31	94	92	100
Aug									
	3636	1110	2507	22.61	7.70	13.54	100	100	100
Sept									
	2916	229	1232	27.00	7.50	14.21	95	100	93

Table 6 *E. caprina*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	0			0.00			0		
	0	1473	0	0.00	1.00	0.00	0	25	0
Nov	7691	0		1.15	0.00		25	0	
	2394	2031	1888	2.25	5.19	4.60	50	44	33
Dec	96	2521		0.60	8.80		28	63	
	247	2886	480	1.40	5.14	6.69	20	64	38
Jan 1988	143	681		1.33	1.55		28	32	
	134	358	664	1.20	1.48	2.53	35	35	35
Feb	77	166		0.25	0.42		15	26	
	146	136	987	2.06	0.39	3.25	44	17	44
Mar	680	1105		1.73	1.78		28	26	
	81	8	459	1.16	0.10	1.40	37	17	40
Apr	30	41		0.45	0.72		25	14	
	188	84	198	1.85	0.31	0.79	55	19	21
May	61	149		0.45	0.38		20	25	
	241	219	38	0.50	0.38	0.88	20	6	44
June	1	35		0.06	0.67		6	7	
	10	0	3	0.22	0.00	0.07	17	17	7
July									
	6	22	630	0.11	0.17	3.15	6	0	69
Aug									
	77	41	7	3.06	0.30	0.15	22	3	7
Sept									
	55	0	20	0.80	0.00	0.50	35	0	29

Table 7. *E. caprovina*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	0			0.00			0		
	14890	5290	5627	0.40	0.00	7.50	25	69	42
Nov	0	140		0.00	0.33		0	7	
	651	533	5154	0.50	0.88	11.27	15	19	80
Dec	85	3219		0.17	9.65		11	50	
	22	5034	5521	0.05	5.20	16.44	5	59	81
Jan 1988	827	2114		2.89	1.20		72	23	
	431	18	5345	2.20	0.17	5.81	55	9	82
Feb	297	13		0.95	0.05		35	5	
	154	124	3477	4.56	0.22	16.24	61	17	94
Mar	13	24		0.16	0.22		72	4	
	0	11	100	0.06	0.14	10.56	0	13	20
Apr	21	15		0.25	0.27		15	9	
	34	21	0	0.35	0.13	1.07	25	13	0
May	3	0		0.05	0.00		5	0	
	0	0	6	0.00	0.00	0.00	0	0	6
June	0	16		0.00	0.13		0	13	
	34	20	13	0.44	0.54	0.13	33	8	20
July									
	7	14	316	0.11	0.83	0.67	11	15	38
Aug									
	7	432	40	0.17	4.10	0.69	17	50	23
Sept									
	12	3	8	0.20	0.25	0.07	20	13	7

Table 8. *E. apsheronica*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	0			0.00			0		
	140	619	0	0.00	0.47	0.00	0	19	0
Nov	360	0		0.10	0.00		5	0	
	510	233	30	0.50	1.25	1.00	15	6	7
Dec	0	1972		0.00	0.56		0	13	
	29	5246	0	0.10	7.43	0.00	5	12	0
Jan 1988	151	1802		0.72	1.95		22	73	
	81	17	136	0.20	0.17	0.53	20	9	12
Feb	1	417		0.75	1.21		5	32	
	1	99	291	0.13	0.11	1.81	6	6	44
Mar	0	213		0.00	0.43		22	17	
	11	156	179	0.11	1.05	0.80	11	30	47
Apr	0	82		0.00	0.10		0	9	
	12	0	0	0.10	0.00	0.00	5	0	0
May	22	0		0.25	0.00		15	0	
	0	282	5	0.00	0.75	0.31	0	19	13
June	55	0		0.28	0.00		11	0	
	21	187	68	0.22	2.39	0.20	22	0	13
July									
	22	0	184	0.22	0.00	0.69	16	62	23
Aug									
	42	233	141	0.61	0.50	1.46	28	50	46
Sept									
	14	0	21	0.15	0.00	0.43	10	0	29

Table 9. *E. ninakohlyakimovae*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	54500			0.30			65		
	122700	29810	992	2.65	15.13	2.33	45	93	8
Nov	1020	1914		0.40	4.73		5	60	
	4204	897	2232	3.75	1.56	11.63	70	56	87
Dec	276	1983		1.50	4.89		50	88	
	1781	1566	68	1.35	3.36	0.94	40	24	25
Jan 1988	117	117		0.72	0.25		22	18	
	24	15	169	0.25	0.04	1.35	10	4	24
Feb	184	201		0.65	0.37		35	21	
	4	119	109	0.19	0.11	0.38	11	11	31
Mar	397	689		3.16	1.78		22	48	
	16	0	347	2.63	0.00	1.67	11	0	27
Apr	0	379		0.00	0.27		0	9	
	15	0	0	0.30	0.00	0.00	15	0	0
May	8	145		0.10	0.56		10	25	
	10	0	0	0.05	0.00	0.00	5	0	0
June	0	0		0.50	0.00		0	0	
	0	0	0	0.00	0.00	0.00	0	33	0
July									
	17	725	0	0.22	5.53	0.00	6	0	0
Aug									
	26	52	78	0.39	0.50	0.77	28	50	46
Sept									
	0	33	8	0.00	0.75	0.29	0	25	7

Table 10. *E. alijeivi*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	0			0.00			0		
	0	0	0	0.00	0.00	0.00	0	0	0
Nov	9124	2600		0.55	3.00		25	47	
	2703	147	162	2.45	2.25	0.47	50	56	27
Dec	169	1496		1.44	11.52		28	75	
	907	363	120	3.56	1.36	1.75	45	47	31
Jan 1988	7035	2488		8.00	2.70		83	68	
	1169	2262	24380	6.95	5.34	18.06	85	39	82
Feb	4601	1323		13.25	3.58		90	68	
	9605	2849	1744	15.25	2.44	7.00	83	61	88
Mar	8538	7411		13.21	6.50		83	70	
	1595	527	930	8.31	3.81	4.07	89	96	67
Apr	473	1619		5.10	5.72		95	82	
	555	6259	249	7.28	9.94	4.79	80	81	50
May	490	918		4.25	4.81		70	81	
	1119	1864	440	6.20	5.94	6.31	100	81	81
June	651	214		6.89	1.20		67	40	
	3133	78	752	6.28	0.77	9.07	89	92	67
July									
	295	695	223	2.58	8.93	1.69	67	46	46
Aug									
	252	78	313	3.81	3.70	2.31	61	60	77
Sept									
	80	440	312	2.30	3.75	4.14	55	88	64

Table 11. *E. ni*

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	42060			2.65			60		
	332600	1165	1080	5.15	0.80	5.17	75	31	17
Nov	99170	667		11.35	0.93		95	0	
	9037	861	102	8.65	0.94	0.73	95	44	27
Dec	3655	1521		13.67	2.75		100	63	
	9875	1284	233	9.25	2.21	0.82	95	71	44
Jan 1988	9781	5787		22.39	5.64		100	77	
	4866	1505	1423	20.65	3.74	5.53	100	78	65
Feb	5118	5139		11.45	3.37		100	100	
	446	1620	873	8.17	9.56	4.06	72	94	81
Mar	1157	1672		2.32	7.26		100	91	
	1706	1723	1878	10.11	10.90	4.33	100	100	93
Apr	1073	2220		9.30	5.05		190	90	
	310	46	236	4.10	0.13	3.00	90	13	57
May	255	1181		1.65	6.13		60	94	
	547	1729	611	2.45	5.75	10.31	85	88	94
June	309	1906		6.22	6.53		83	87	
	309	392	439	3.00	5.15	5.13	67	83	67
July									
	914	798	727	7.67	9.83	4.69	94	77	100
Aug									
	1553	2260	1586	7.89	6.20	10.15	94	100	92
Sept									
	355	225	422	0.15	8.25	6.29	100	88	93

Table 12. E. n2

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	1522			0.15			10		
	16680	6135	666	0.70	4.13	3.25	15	31	83
Nov	56170	833		7.65	1.60		85	53	
	8265	581	690	8.15	0.63	2.60	80	25	73
Dec	1037	5009		6.39	4.38		67	63	
	1497	2481	2057	3.30	4.29	5.06	50	94	63
Jan 1988	2815	9651		11.89	9.49		89	100	
	3554	4180	2428	18.35	12.65	4.94	100	100	82
Feb	10500	2260		27.80	7.63		100	100	
	1825	7620	4652	23.56	14.89	13.81	89	100	100
Mar	4433	1982		20.37	9.87		89	96	
	4730	2974	5353	22.05	14.09	7.93	100	100	93
Apr	1904	3714		18.35	12.55		100	100	
	2238	1106	1390	32.65	4.00	6.57	100	81	86
May	9868	7047		42.50	34.31		100	100	
	5485	1490	724	26.85	26.25	12.75	95	100	100
June	1494	6270		16.83	48.67		100	100	
	3897	2435	3601	29.33	31.46	24.93	100	92	100
July									
	4918	3345	8132	34.39	40.33	46.62	100	92	100
Aug									
	3442	5555	5255	27.94	34.60	25.31	100	100	100
Sept									
	2313	1643	3250	30.10	52.50	35.21	100	100	100

Table 13. E. n3

MONTH	Oocyst Counts			Percentage of total oocyst count			Prevalence of positive samples		
	FARM								
	OWR	KBT	BALT	OWR	KBT	BALT	OWR	KBT	BALT
Oct 1987	0			0.00			0		
	0	0	0	0.00	0.00	0.00	0	0	0
Nov	0	0		0.00	0.00		0	0	
	0	0	0	0.00	0.00	0.00	0	0	0
Dec	0	0		0.00	0.00		0	0	
	0	0	0	0.00	0.00	0.00	0	0	0
Jan 1988	0	584		0.00	1.13		0	27	
	0	0	0	0.00	0.00	0.00	0	0	0
Feb	6	0		0.50	0.00		5	0	
	0	196	129	0.00	0.60	1.19	0	11	19
Mar	463	0		2.79	0.00		0	0	
	712	0	662	1.58	0.00	2.93	63	0	33
Apr	254	268		4.25	2.17		30	36	
	130	0	6	2.00	0.00	0.21	60	0	7
May	508	337		2.65	2.18		40	50	
	1287	322	99	6.40	1.00	0.75	85	18	19
June	291	280		5.94	0.93		78	53	
	683	7	0	4.83	0.23	0.00	67	0	0
July									
	110	0	15	1.72	0.00	0.15	17	8	15
Aug									
	245	0	0	1.50	0.00	0.00	50	0	0
Sept									
	0	0	2	0.00	0.00	0.72	0	0	7

APPENDIX 6

Data on seasonal variations in mean oocyst counts, mean percentage of total counts and sample prevalence for individual *Eimeria* species in adults

Key: OWR - Old West Road Farm; BALT - Ballantrae Farm;

E. christenseni

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	318	87	9.47	0.95	63	15
Oct	826	161	18.60	7.65	100	80
Nov	1571	37	15.47	1.30	100	45
Dec	594	289	19.21	3.37	100	47
Jan 1988	37	1268	8.56	7.77	75	82
Feb	45	736	6.88	6.65	71	70
Mar	309	314	3.18	2.55	59	60
Apr	44	1650	2.50	3.35	50	50
May	27	323	1.30	2.39	62	44
June	112	908	8.18	6.88	64	69
July	14	1515	1.00	5.90	27	42
Aug	306	859	3.67	3.61	33	39

E. tunisiensis

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Septembe	20	81	3.16	2.20	47	5
October	3	0	0.30	0.00	10	0
November	0	298	0.00	7.75	0	75
December	182	369	11.63	6.58	100	79
January	37	739	8.94	4.35	88	65
February	152	698	14.81	5.30	71	70
March	26	239	2.18	3.00	47	65
April	61	265	3.14	1.20	50	50
May	117	833	3.54	5.94	92	72
June	95	952	9.82	13.62	91	94
July	19	1217	6.00	8.32	64	84
August	114	475	3.78	2.33	89	56

E. jolchejevi

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	30	27	3.74	1.20	16	5
Oct	11	5	1.70	0.50	10	5
Nov	0	284	0.00	6.50	0	50
Dec	1118	694	21.80	22.89	95	84
Jan 1988	11	499	3.50	3.35	50	47
Feb	85	231	10.94	4.50	65	40
Mar	193	1624	13.71	18.70	53	85
Apr	32	550	1.71	2.80	50	35
May	0	110	0.00	1.28	0	50
June	0	36	0.00	4.38	0	25
July	13	46	0.55	0.42	18	11
Aug	103	114	2.00	0.56	22	17

E. arloingi

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	218	3181	15.63	35.45	100	100
Oct	436	3166	34.35	63.55	100	100
Nov	5073	1113	39.89	27.45	95	100
Dec	238	1133	13.63	24.84	95	100
Jan 1988	107	3030	18.69	29.35	100	88
Feb	133	2551	11.62	24.65	88	100
Mar	300	4312	26.47	25.50	100	100
Apr	518	10960	21.43	43.40	100	95
May	1092	952	33.62	39.67	100	100
June	281	3305	19.91	32.69	100	100
July	71	7210	23.91	39.58	100	100
Aug	982	8600	27.89	39.11	100	100

E. hirci

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	437	1187	38.00	19.00	100	100
Oct	134	329	23.00	8.00	90	95
Nov	2676	1578	20.00	27.00	100	95
Dec	588	667	19.00	17.00	100	100
Jan 1988	112	929	21.00	7.00	100	65
Feb	290	1876	29.00	23.00	94	100
Mar	309	2780	28.00	22.00	94	100
Apr	1960	4454	44.00	15.00	100	100
May	11373	2809	36.00	21.00	100	100
June	508	1897	37.00	22.00	100	100
July	396	1498	40.00	12.00	100	95
Aug	214	3660	35.00	14.00	100	94

E. caprina

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	19	204	1.53	2.95	47	65
Oct	0	0	0.00	0.00	0	0
Nov	12	91	0.47	2.10	15	60
Dec	105	129	3.00	1.90	79	58
Jan 1988	3	218	0.75	2.53	19	35
Feb	14	95	1.13	1.75	29	40
Mar	6	156	0.82	1.45	24	45
Apr	394	4799	5.57	7.20	64	70
May	11	497	0.54	3.50	28	61
June	35	13	2.55	0.31	55	31
July	43	156	6.27	1.00	64	58
Aug	144	191	3.67	1.28	67	39

E. caprovina

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	11	118	1.58	2.2	42	55
Oct	0	0	0.00	0.00	0	0
Nov	1	55	0.42	0.70	16	30
Dec	30	15	4.84	0.47	53	21
Jan 1988	24	10620	7.44	16.06	81	82
Feb	89	269	6.88	3.45	76	80
Mar	4	25	0.29	0.50	12	25
Apr	396	2833	4.00	3.65	43	20
May	57	14	2.23	0.11	92	5
June	52	74	5.66	0.75	72	25
July	7	243	0.82	2.05	45	58
Aug	35	208	0.78	1.22	22	39

E. apsheronica

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	1	50	0.21	0.45	11	15
Oct	0	0	0.00	0.00	0	0
Nov	13	9	0.89	0.30	32	15
Dec	6	52	0.90	0.53	16	26
Jan 1988	17	137	3.25	0.53	8	12
Feb	15	54	1.69	1.15	53	5
Mar	4	100	0.59	0.75	24	50
Apr	53	124	0.50	0.30	36	25
May	0	256	0.00	1.28	0	33
June	0	80	0.00	1.00	0	63
July	0	40	0.00	0.37	0	26
Aug	0	111	0.00	0.67	0	22

E. ninakohlyakimovae

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	14	492	1.63	6.85	47	80
Oct	15	0	2.55	0.00	50	0
Nov	14	77	1.68	1.90	37	55
Dec	3	37	0.42	0.84	26	32
Jan 1988	2	176	0.50	1.35	6	24
Feb	10	52	0.75	1.05	29	50
Mar	2	17	0.29	2.55	24	30
Apr	15	4	0.64	0.05	36	50
May	0	0	0.00	0.00	0	0
June	5	35	0.36	0.50	18	19
July	0	35	0.00	0.26	0	11
Aug	34	254	0.89	0.67	44	28

E. alijeви

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	0	131	0.00	1.45	0	20
Oct	201	184	8.65	3.95	65	85
Nov	281	141	2.47	3.50	32	55
Dec	13	54	2.16	1.42	26	63
Jan 1988	22	5313	3.94	7.82	69	82
Feb	44	254	4.44	2.80	59	80
Mar	25	272	1.53	3.50	59	70
Apr	169	374	4.93	1.85	93	45
May	185	141	5.15	1.44	100	33
June	28	33	1.73	0.75	55	38
July	1	89	0.36	0.75	27	37
Aug	35	531	0.67	0.84	44	61

E. n1

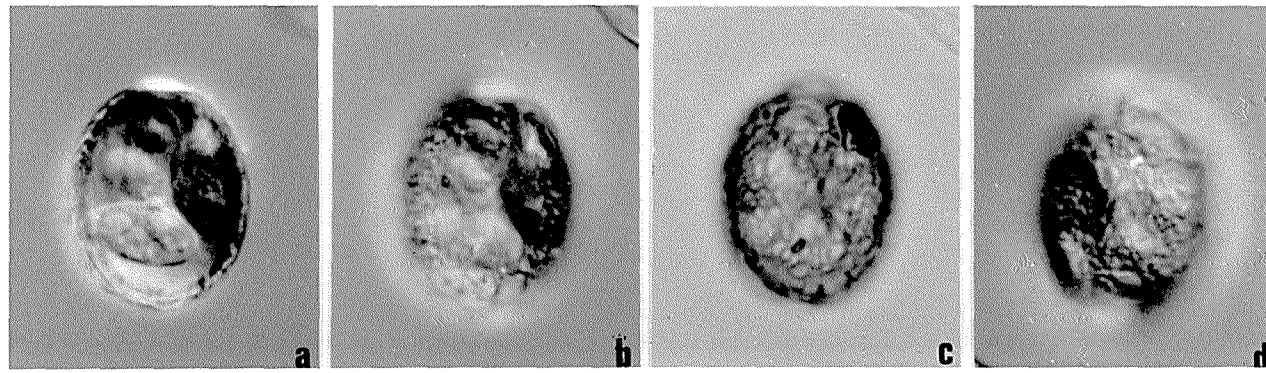
Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	79	381	4.16	6.65	74	80
Oct	13	8	2.40	0.20	65	10
Nov	617	268	8.37	6.95	79	70
Dec	1	168	0.11	3.42	53	89
Jan 1988	23	545	5.00	5.53	81	65
Feb	73	470	6.25	7.80	76	95
Mar	75	248	7.06	2.55	94	90
Apr	56	467	2.57	1.90	79	65
May	97	299	1.92	2.61	85	67
June	72	122	4.90	0.88	73	31
July	12	431	3.18	2.84	55	79
Aug	100	836	4.22	4.05	67	83

E. n2

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	507	1591	24.21	21.10	100	85
Oct	63	769	8.75	15.95	90	95
Nov	1111	762	11.21	14.35	89	95
Dec	82	925	3.79	17.00	74	100
Jan 1988	92	6158	18.94	4.88	100	82
Feb	38	1999	5.38	17.70	76	100
Mar	203	1398	15.47	18.00	100	100
Apr	203	8190	8.64	19.65	79	95
May	462	2970	15.77	20.78	100	100
June	136	2850	10.36	19.87	100	100
July	67	4981	17.45	26.47	100	100
Aug	545	10450	15.33	30.40	100	100

E. n3

Month	Oocyst counts		Percentage of total oocyst count		Prevalence of positive samples	
	Farm					
	OWR	BALT	OWR	BALT	OWR	BALT
Sept 1987	0	0	0.00	0.00	0	0
Oct	0	0	0.00	0.00	0	0
Nov	4	0	0.89	0.00	11	0
Dec	0	0	0.00	0.00	0	0
Jan 1988	0	0	0.00	0.00	0	0
Feb	0	0	0.00	0.00	0	0
Mar	3	0	0.29	0.00	12	0
Apr	13	0	0.57	0.00	14	0
May	4	0	3.54	0.00	8	0
June	0	0	0.00	0.00	0	0
July	2	0	0.80	0.00	18	0
Aug	38	49	0.67	0.17	33	17



Photomicrographs of the single *E. punctata* found subsequent to the completion of the study. Figures a - d are of the oocyst photographed in different planes to show the internal structure (a) and the texture of the surface of the oocyst (b - d). X1122