CHAPTER 1

General introduction

1 Preamble

The global demand for high quality food and fibre has been a major driving force for research and development of sustainable animal husbandry and management practices (Bath, Hansen, Krecek, Van Wyk & Vatta 2001; Kaplan, Burke, Terrill, Miller, Getz, Mobini, Valencia, Williams, Williamson, Larsen & Vatta 2004). Gastrointestinal (GIT) parasitism is an important disease of livestock, leading to production losses (Bishop & Stear 2003). Several GIT nematodes of the family Trichostrongylidae parasitise sheep (Fontenot, Miller, Peña, Larsen & Gillespie 2003) and of these, *Haemonchus contortus* (Rudolphi 1803) is the predominant and economically the most important nematode parasite of sheep and goats in tropical and subtropical regions of the world (Besier & Dunsmore 1993a; Achi, Zinsstag, Yao, Dorchies & Jacquiet 2003; Fontenot *et al*. 2003; Terrill, Larsen, Samples, Husted, Miller, Kaplan & Gelaye 2004).

This parasite has been responsible for extensive production losses in sheep and McLeod (1995) estimated that in Australia, costs of treatment and loss of production due to nematode infections amounted to approximately AUS $222 million annually. In the southern United States, findings from a recent 7-year review of clinical cases at Auburn University indicated that *H. contortus* infection was the primary reason for examination of 70 % of sheep and 91 % of goats treated by hospital clinicians, and that abomasal or intestinal worm infection was the predominant disease condition on 74 % of sheep farms (Kaplan *et al*. 2004). In West Africa, *Haemonchus* is the dominant genus in small ruminants, and causes major economic losses in the Gambia, Mauritania, Nigeria, and Ivory Coast (Achi *et al*. 2003). In South Africa, with approximately 29 million sheep and 6 million goats (Vatta 2001), the effect of morbidity and resultant losses in production in small ruminants due to haemonchosis is considerable (Vatta 2001; Vatta, Letty, Van der Linde, Van Wijk, Hansen & Krecek 2001; Van Wyk & Bath 2002).

Production losses due to haemonchosis in small ruminants have also been documented in Brazil (Pessoa, Morais, Bevilaqua & Luciano 2002; Sotomaior, Caldas, Iark, Benvenutti & Rodrigues 2003a; Sotomaior, Milczewski, Iark, Caldas, Benvenutti, Sillas & Schwartz 2003b; Sotomaior, Milczewski, Morales & Schwartz 2003c; Molento, Tasca, Gallo, Ferreira, Bononi

& Stecca 2004a), and other central and south American countries (Milczewski, Sotomaior, Schwartz, Barros Filho, Morales & Schmidt-Popazoglo 2003; Sotomaior *et al.* 2003a,b,c).

Control of haemonchosis has traditionally been achieved by frequent anthelmintic treatment of all individuals (Waller 1997, 1999; Hoste, Chartier, Lefrileux, Godeau, Pors, Bergaud & Dorchies 2002; Van Wyk & Bath 2002; Fontenot *et al.* 2003; Geary & Thompson 2003; Terrill *et al.* 2004). However, due to escalating anthelmintic resistance in South Africa (Van Wyk, Stenson, Van der Merwe, Vorster & Viljoen 1999; Vatta 2001; Vatta *et al.* 2001; Van Wyk & Bath 2002), and in many other regions of the world where small ruminants are kept (Hoste *et al.* 2002; Bishop & Stear 2003; Fontenot *et al.* 2003), alternative strategies, which are not entirely based on frequent anthelmintic treatment of entire flocks, must be considered. Development of new, unrelated anthelmintics is unlikely to arrest the development of anthelmintic resistance in small ruminants in the near-to-medium future, due to the high costs involved in the screening and development of these drugs (Waller 1997). The apparent perception of the pharmaceutical manufacturers is that the size of the small ruminant industry does not justify the necessary investment (Waller 1997), although there are indications that higher profitability in small ruminant husbandry enterprises could provide the financial incentive to justify new product development (Besier 2007).

Along with the intensification of small ruminant production systems such as those in the southern United States (Kaplan *et al.* 2004), and the failure of intensively used chemotherapeutic agents to sustainably control nematode parasites because of parasite resistance (Waller 1997; Taylor, Hunt & Goodyear 2002b), anthelmintic resistance has made it essential to explore and develop novel ways of worm management to reduce selection for resistance.

The present study was initiated to evaluate the feasibility of integrating supplemental, software-based risk analysis techniques with the FAMACHA© system of selective drenching of sheep to manage haemonchosis, as a first step towards developing software for on-farm worm management by the farmer.

1.1 *Haemonchus contortus*: the parasite and its epidemiology

Haemonchus contortus occurs in the abomasum of sheep, cattle, goats and other ruminants in most parts of the world (Soulsby 1982; Hansen & Perry 1994). This species has been known to infect sheep since the early years of the previous century (Theiler 1912). Clunies-Ross (1932) reported that on a particular sheep rearing station in Queensland, Australia, *H. contortus* infection prevented rearing of young sheep unless carbon tetrachloride was introduced as an anthelmintic. Achi *et al*. (2003) found that *H. contortus* preferentially infected sheep and goats rather than cattle in northern Ivory Coast, and that monospecific infection with *H. contortus* occurred in 68 % of 28 sheep populations studied. *Haemonchus contortus* also accounts for 75-100 % of the total faecal nematode egg counts on the majority of sheep and goat farms in the southern United States (Kaplan *et al*. 2004).

1.1.1 Ecology and pathology of Haemonchus contortus

Haemonchus contortus is commonly known as the "stomach worm" or "wireworm" of ruminants, and "barber's pole worm" in Australia (Donald, Southcott & Dineen 1978). It is one of the most pathogenic parasites of small ruminants, because of its blood sucking habit (Veglia 1918; Soulsby 1982), and it is particularly pathogenic in young hosts (Whitfield 1994). Male worms are 10–20mm in length and more or less uniformly light brown in colour, while females are from 18–30mm long with whitish ovaries and uteri that are spirally wound around a red intestine, giving the appearance of a barber's pole (Soulsby 1982). Sexually mature female worms in the abomasum produce large numbers of eggs that are voided with the faeces, followed by egg-hatch and development into free-living larval stages on pastures if suitable climatic conditions prevail. The rate and success of free-living larval development and survival is largely determined by climatic variables such as temperature and moisture (Donald *et al.* 1978). The ecology of free-living stages of the major trichostrongylid parasites has recently been reviewed by O'Connor *et al*. (2006). Under optimal environmental conditions, larvae on pasture develop to the infective third stage larvae (L_3) in four to six days, while low temperatures below 9ºC result in little or no development (Soulsby 1982). The pre-patent period of *H. contortus* is about two weeks after ingested L_3 have moulted into fourth-stage larvae (Dunn 1969; Hansen & Perry 1994). Within six hours of entering the host, the L_3 enter the mucous membrane or glands in the wall of the abomasum, where they moult into fourth stage larvae (L_4) within about four days. This is followed by the fourth moult about nine to 11 days after infection of the host (Veglia 1915), followed by the emergence of maturing young adult worms on the mucosal surface. Under adverse climatic conditions, hypobiosis, also known as arrested larval development, occurs (Chappel 1994). This phenomenon, which has been described by Horak (1981b) in *H. contortus* in South Africa, is characterised by arrested early fourth stage larvae (L_4) within the abomasal glands. These arrested larvae begin to develop during periods of natural immunosuppression, such as during parturition, but the phenomenon also appears to be seasonal (Anderson, Dash, Donald, Southcott & Waller 1978; Chappel 1994) and may also depend on parasite related factors such as genetic composition and density dependence (Reinecke 1983). Photoperiod and temperature may be the most important factors acting upon free-living stages that subsequently enter a hypobiotic state in the animal (Horak 1980). Arrested *H. contortus* larvae resume development and moult into adults at the start of the ensuing grazing season (Chappel 1994).

Moderate to large numbers of *H. contortus* larvae entering the abomasum reduce the appetite and efficiency of protein metabolism of the ruminant host (Martin & Clunies-Ross 1934; Reinecke 1983; Hansen & Perry 1994; Kaplan *et al*. 2004). This results in severe clinical, and sometimes fatal, anaemia (Veglia 1915; Andrews 1942; Baker, Cook, Douglas & Cornelius 1959; Reinecke 1983). Animals infected with large numbers of larvae, which subsequently become adult worms, may suffer from anaemia before parasite eggs are detected in the faeces (Hansen & Perry, 1994; Coop & Kyriazakis 1999). The anaemia results both from the blood ingested by the worms and from the damage caused to the abomasal mucosa by blood sucking of adult stages, which continually shift from spot to spot, leaving large numbers of small haemorrhaging petechial lesions, leading to severe blood loss (Reinecke 1983). Blood loss during the early stages of infection may be relatively small, however (Dargie & Allonby 1975). Erythrocyte loss occurrs at a high rate during the late pre-patent period, leading to progressive anaemia which stimulates a haemopoietic response in terms of an increased erythrocyte production rate followed by exhaustion or death of the animal (Albers *et al*. 1990). Economically, the greatest effects of parasitic diseases are manifested in production losses, especially in the developing world. However, in countries such as New Zealand and eastern Australia, sporadic but significant sheep losses occur due to acute haemonchosis (Kahn *et al*. 2007)

1.2 Control of haemonchosis

1.2.1 Chemical control

Control of haemonchosis in small ruminants over the last three decades has largely been effected by the use of broad-spectrum anthelmintic drugs, with thiabendazole being one of the first broad-spectrum anthelmintics to be introduced (Athanasiadou, Kyriazakis, Jackson & Coop 2000). Table 1.1 lists the major groups of anthelmintics that have been commercially available for the control of nematode parasites. The use of experimental compounds such as rotenone, when used to control endoparasites in livestock, are at present limited by human health safety concerns (Kotze, Dobson & Chandler 2006).

TABLE 1.1 Anthelmintic drug groups and their modes of action.

Anthelmintic group	Mode of action	Activity spectrum	Reference
Benzimidazoles	Bind tubulin dimers, reduce absorption of nutrients in parasite in GIT of host	Broad spectrum	Taylor *et al.* (2002b) Anziani *et al.* (2004) Panchadcharam (2004)
Tetrahydropyrimidines/ imidazothiazoles	Paralysis in exposed parasites in GIT of host	Broad spectrum	Panchadcharam (2004)
Macrocyclic lactones	Impair development, feeding ability, and motility of free living stages	Broad spectrum	Panchadcharam (2004) Sheriff *et al.* (2005)
Substituted salicylanilides	Bind to blood proteins in treated host	Narrow spectrum, effective against *H. contortus*	Love *et al.* (2003) Panchadcharam (2004)
Organophosphorous drugs	Inhibit acetyl cholinesterase enzymes	Narrow spectrum	Gibson (1975) Martin *et al.* (2002)

However, anthelmintic resistance, defined as “the ability of the parasite to survive dosages of drugs that would normally kill parasites of the same species and stage of development” (Panchadcharam 2004), has escalated in South Africa (Van Wyk 1999; Van Wyk, Van Wijk, Stenson & Barnard 2001b; Van Wyk 2001, 2002), and elsewhere in the world (Dobson, Besier, Barnes, Love, Bell & Le Jambre 2001; Hoste *et al.* 2002; Pook, Power, Sangster, Hodgson & Hodgson 2002; Van Wyk & Bath 2002; Bishop & Stear 2003; Fontenot *et al.* 2003). In Western Australia, the farm-level prevalence of resistance of *Teladorsagia* (= *Ostertagia*) *circumcincta* to macrocyclic lactones had reached 38 % by 2000 (Suter, Besier, Perkins, Robertson & Chapman 2004). Anthelmintic resistance is selected for and genetically inherited when survivors of drug treatment pass the resistance genes to their offspring (Panchadcharam 2004), and is the result of the fact that helminth parasites are known to possess several different mechanisms that are able to detoxify harmful xenobiotics (Kotze *et al.* 2006). Resistant individuals continue to initiate new infections until these ultimately succumb to host immunity, while susceptible individuals are eradicated by anthelmintic treatment (Hastings 2001).

Although control failures were temporarily alleviated by higher drug doses and more frequent treatment as resistance developed, the beneficial effect of this strategy was short-lived. The exclusive use of anthelmintics to control nematodes has selected worm populations that simultaneously exhibit increasing levels of resistance to several classes of anthelmintics (Van Wyk *et al*. 1997; Chartier, Pors, Hubert, Rocheteau, Benoit & Bernard 1998; Fontenot *et al*. 2003), and in some cases to all major anthelmintic activity groups (Van Wyk *et al*. 1997a, b).

Continued use of anthelmintics has had the effect of increasing the frequency of resistant alleles in parasite populations due to the selective effect of the drugs, and anthelmintic resistance has become sufficiently widespread and serious as to threaten the viability of sustainable small ruminant production in many countries (Waller 1999). However, the failure of anthelmintics to control GIT nematodes may also be due to reasons other than resistance, such as poor maintenance of drenching equipment, and underdosing due to errors in assessing body mass (Taylor, Hunt & Goodyear 2002a).

In spite of the development of anthelmintic resistance, chemotherapeutic drugs remain the most important treatment option for worm control and management (Van Wyk 2001), and will probably remain so for the foreseeable future (Taylor *et al*. 2002a). Alternative treatment strategies that include vaccines, biological control and selective breeding for parasite resistant animals are not likely to be generally available in the short term, and even if they do, these methods will of necessity need to be integrated with chemotherapy.

1.2.2 Biological control

The development of anthelmintic resistance in parasite populations worldwide has brought about concerted interest in the development of biological agents that have the potential to control GIT nematodes of livestock. One of the main thrusts of research towards biological control has been directed at nematode-destroying microfungi such as *Duddingtonia flagrans* (Panchadcharam 2004). It is a nematode-trapping fungus that produces thick-walled chlamydospores that destroy larval nematodes in faecal matter by trapping them in a sticky hyphal network (Fontenot *et al*. 2003), as has been demonstrated *in vitro* (Faedo 2001; Peña, Miller, Fontenot, Gillespie & Larsen 2002). Spores of this fungus are able to survive passage through the ruminant gastrointestinal tract, and germinate in faecal material deposited on pastures (Faedo 2001; Panchadcharam 2004). A limiting factor affecting the use of this microorganism is that doses as high as10^8 chlamydospores per gram of faeces had to be used before a response could be elicited (Larsen, Faedo & Waller 1994). Studies have shown, however, that *D. flagrans* chlamydospores fed to lambs successfully reduced levels of *Teladorsagia* and *Trichostrongylus* larvae on pastures grazed by the lambs (Githigia, Thamsborg, Larsen, Kyvsgaard & Nansen 1997). A further advantage of using *D. flagrans* is that its chlamydospores may have a high environmental persistence (Faedo, Larsen & Thamsborg 2000). The latter authors found that experimental deposition of faeces containing *D. flagrans* chlamydospores onto pasture prevented transmission of nematode L_3, including *Nematodirus* spp., from the faecal pellets to the pasture by hyphal trapping of emerging nematodes. However, despite the fact that promising results have emerged from this research, the commercial realization of nematphagous fungi as effective biological control agents has yet to be achieved (Besier 2006).

Other biological control methods that have been reported, with varying degrees of success, include feeding animals with plants that have anthelmintic properties, as well as the use of various plant extracts. For the purpose of the current study, experimental plant extracts such as condensed tannins (Hammond, Fielding & Bishop 1997), are considered to be biological control methods, as they are usually solvent-extracted directly from living plant material (Athanasiadou *et al*. 2000). These techniques have included the use of plant extracts such as essential oil of *Ocimum gratissimum* and eugenol against *H. contortus* (egg-hatch test) (Pessoa *et al*. 2002), anthelmintic activity of essential oil of *O. gratissimum* against the free-living nematode *Caenorhabditis elegans* (Asha, Prashanth, Murali, Padmaja & Amit 2001), and feeding of condensed tannins to sheep infected with *Trichostrongylus colubriformis* (Athanasiadou *et al*. 2000; Paolini, Bergeaud, Grisez, Prevot, Dorchies & Hoste 2003). Although these methods may hold promise for the future, plant preparations have not yet been developed for general on-farm use against nematode parasite infections in ruminants (Panchadcharam 2004).

The use of vaccines may also be a potentially useful control measure applied to nematode infections in small ruminants (Smith 1999). However, in a series of repeated trials, Emery (1996) found that protection rates were variable, ranging from 40–70 % when lambs were vaccinated with an extract prepared from the gut of *H. contortus.* Vaccination has not yet proved to be successful in practice (Walkden-Brown & Eady 2003). The large-scale production of anti-nematode vaccines, due to complexities in their production, is likely to delay the advent of these products into the distant future (Besier 2006). However, the possibility of control by vaccination is currently being investigated and several protective antigens have been identified (Smith & Zarlenga 2006). The mode of action of vaccines is very different to that of conventional anthelmintics, and it is currently assumed that experimental vaccines would be effective against both anthelmintic resistant and susceptible isolates of *Haemonchus contortus* (Smith 2007). This has been confirmed for at least one such antigen (Newton, Morrish, Martin, Montagues & Rolph 1995).

1.2.3 Resistance management

The move towards organic farming systems in recent years (Athanasiadou *et al*. 2000), coupled to the aforementioned problems caused by the development of anthelmintic resistant populations of nematodes, has brought about an increased demand for alternatives to the exclusive use of blanket treatment of flocks with anthelmintics to control nematode infections. The following definitions for host resistance, resilience and tolerance are used in this work (Albers, Gray, Piper, Barker, Le Jambre & Barger 1987):

Resistance: the initiation and maintenance of responses provoked in the host to suppress the establishment of parasites and/or eliminate parasite load.

Resilience: the ability of the host to maintain a relatively undepressed production level under parasite challenge.

Tolerance: the ability of the host to survive in the face of parasite challenge.

Acquired immunity

Acquired immunity to nematode infections is important, as it has been demonstrated that pen-reared lambs infected with trickle infections of *T. colubriformis* responded by exhibiting reduced establishment of newly ingested larvae, an increase in the arrested development of third-stage larvae, reduced egg production by adult female worms, and rejection of established worms (Dobson, Waller & Donald 1990a,b,c). The duration of acquired immunity to *H. contortus* may be short-lived when compared to *T. colubriformis*, but the strength of acquired immunity in well-nourished sheep that have had sufficient exposure to allow immunity to develop could be managed to minimise the effect of parasitic worms (Barnes & Dobson 1993). Furthermore, Dobson & Barnes (1995) found that establishment of *H. contortus* in worm-free young lambs was reduced if there had been prior, and prolonged, infection with *Teladorsagia circumcincta* because of immunological cross-protection or abomasal deterioration. However, the latter authors also concluded that the presence of

T. circumcincta in the challenge could partially be responsible for reduced establishment of *H. contortus* due to inter-specific competition, and that the effect of cross-protection between the species is minor compared to the direct effects of inter-specific competition.

Nutritional supplementation

Reduced appetite has a pronounced effect on the nitrogen metabolism of infected animals, since there is a decrease in the total amount of available substrate for metabolic processes (Knox & Steel 1996). A lowered supply of dietary protein may also delay the development of capacity to expel worms in lambs exposed to continuous infection with *H. contortus* (Abbot, Parkins & Holmes 1985). However, the reduced nutrient availability to the host through reductions both in feed intake and the efficiency of absorbed nutrients is to some extent dependent on the parasite species and its location in the digestive tract of the host (Coop & Kyriazakis 1999). There is evidence that gastrointestinal nematodoses are challenge or infection density diseases, and there may be threshold levels of challenge or infection for the parasite species commonly associated with economic losses (Jackson & Miller 2006). The effects of reduced voluntary food intake and the reduction in digestion and utilization of nutrients can be overcome by supplementation with additional protein (Van Houtert, Barger, Steel, Windon & Emery 1995; Knox & Steel 1996) or minerals (Suttle, Knox, Jackson, Coop & Angus 1992; McClure, McClure & Emery 1999).

Grazing management

Grazing management, incorporating the principle of rotational grazing as an evasive method to move animals before they encounter high pasture infectivity, has for many years been used as a means of limiting host-parasite contact. However, the principle is difficult to apply in many extensive production systems due to insufficient land for conservation (Jackson & Miller 2006). Under these conditions, non-susceptible stock such as cattle are often used to reduce larval challenge to grazing sheep. Grazing weaned lambs on swards previously grazed by cattle only during the pre-weaning period has been found to reduce internal parasites in the lambs, and mixed grazing with cattle and sheep throughout the grazing can improve lamb liveweight gain (Marley, Fraser, Davies, Rees, Vale & Forbes 2006). These authors found that the highest growth rates were observed in lambs where mixed cattle/sheep grazing occurred. In areas such as the tropics, development of nematode eggs to infective larvae occurs over a short time period, often less than one week, and the survival time for the infective larvae is also relatively short at about four weeks (Banks, Singh, Barger, Pratap & Le Jambre 1990). These shortened development times have been used to manage parasite suprapopulations by rotational grazing in countries such Fiji, where animals were grazed for 3–4 days, were then moved, and were not returned to the same pasture for 31–32 days (Barger, Siale, Banks & Le Jambre 1994). Thus, grazing management as a means of restricting host-parasite contact is well established, and has been developed to the point where it is an acceptable parasite control measure (Jackson & Miller 2006).

Parasite community replacement

Attempts by Bird, Shulaw, Pope & Bremer (2001) to control anthelmintic resistant sheep endoparasites through parasite community replacement using a technique developed by Van Wyk & Van Schalkwyk (1990) have achieved success. In their study, endemic combined populations of anthelmintic resistant *Haemonchus* spp., *Teladorsagia* spp., and *Trichostrongylus* spp. populations on two experimental pastures on the same farm were reduced to nondetectable levels with a combination of strategically timed anthelmintic treatments (ivermectin) and pasture management. Faecal egg count reduction tests had previously indicated resistance to levamisole (0 % reduction), albendazole (89 % reduction), and susceptibility to ivermectin (>99 % reduction). The predominant genus in the preparasitic larval populations was *Haemonchus*, which ranged between 96 and 98 % of the total parasite community. On one of the “clean” pastures, each sheep in a new group (n = 102) was infected with 5 000–10 000 anthelmintic susceptible third-stage larvae, consisting of *Haemonchus* spp. (82 %), *Trichostrongylus* spp. (8 %), and *Teladorsagia* spp. (10 %). The sheep on the second pasture were not dosed with susceptible larvae, and maintained the endemic resistant parasite populations. The experimentally infected sheep seeded pastures with susceptible populations, and a reversion to susceptibility was obtained that matched that observed on the donor farm, where *Haemonchus* spp. and *Teladorsagia* spp. were susceptible to all three drugs, and only *Trichostrongylus* spp. was resistant only to levamisole. In a similar trial undertaken under farming conditions, Van Wyk *et al.* (2001b) obtained increased efficiency of albendazole, ivermectin, levamisole and rafoxanide when sheep were artificially infected with an unselected population of *H. contortus*.

Selective breeding to withstand parasite challenge

The ability of animals to withstand challenge from nematode parasites may be enhanced by selective breeding for resistance to parasite infection (Eady, Woolaston, Lewer, Roadsma, Swan & Ponzoni 1998) and dietary supplementation (Kahn, Knox, Gray, Lea & Walkden-Brown 2003). Although dietary supplementation in susceptible breeds may improve the resilience of sheep to GIT parasite infection, published data suggest that those breeds that have been selected for resilience are likely to show only a marginal improvement in resisting nematode challenge. Eady *et al.* (1998) suggested that the diminished response to an increased supply of metabolically available protein could be because the partitioning of amino acids between the GIT and other tissues may be altered during the course of selective breeding. Mugambi *et al*. (2005) demonstrated that when Dorper and Red Maasai were mated to produce backcross lambs under *H. contortus* challenge, quantitative trait loci that control resistance to endoparasites could be identified, and the variation in resistance and resilience to endoparasites was then used to select the most extreme resistant and susceptible lambs for genotyping using microsatellite genetic markers.

Due to the fact that chemotherapy is likely to remain the main control option for the treatment of nematode infections in small ruminants for the foreseeable future, there is a need to develop supplemental management techniques to be applied to anthelmintic treatments within the broader context of integrated pest (parasite) management, which are able to extend the life of those drugs that are still effective on individual farms. A summary of non-anthelmintic parasite control measures is given in Table 1.2.

TABLE 1.2 Summary of non-anthelmintic nematode control measures applied to small ruminants

Control measure	Method	Results	Reference
Grazing management	Sequential paddock rotational grazing	Decreased faecal worm egg counts, decreased frequency of anthelmintic treatments	Barger (1996) Panchadcharam (2004)
Nutritional supplementation	Dietary supplementation with rumen undegradable protein	Resilience of susceptible sheep improved; less marked improvement in more resilient breeds	Coop & Kyriazakis (1999)
	Low-cost feed supplements (urea/molasses feed blocks)	Enhanced use of available diet; increased ability to withstand infection	Panchadcharam (2004)
Breeding for resistance to parasites coupled with nutritional supplementation	Periparturient ewes selected for resistance to *H. contortus* and supplemented with cottonseed meal feed	Increased resistance to infection due to protein supplementation	Kahn *et al.* (2003)
Selection of breed for resistance and/or resilience to parasites	Selective establishment of breeds innately resistant/resilient to nematode infection	Decreased faecal worm egg counts/susceptibility to worms, but genetic response is a long-term process	Zajac *et al.* (1988)

1.2.4 The FAMACHA© system of selective treatment

The FAMACHA© system, developed in South Africa (Bath, Malan & Van Wyk 1996; Bath *et al*. 2001; Malan, Van Wyk & Wessels 2001; Van Wyk & Bath 2002) from initial clinical trials by Malan & Van Wyk (1992), allows clinical diagnosis of anaemia in individual sheep. The system is based on comparing the colour of the conjunctivae of individual animals with a full-colour chart which has five colour categories from red, or non-anaemic (Category 1), to pale, or severely anaemic (Category 5) (Bath *et al.* 1996). It can be applied at farm level to detect individual animals needing treatment for haemonchosis. Animals are “scored” into numbered, and thus ordinated, categories from 1 through to 5, with category 1 being the least anaemic, and category 5 being the most anaemic, and the ordinated scores are in turn based on haematocrit values. The three intermediate categories, 2, 3 and 4 each represent 5 percentage points of the haematocrit. Category 1 represents haematocrit values above and including 28 %, with a theoretical upper limit of the maximum haematocrit for a given animal, while category 5 represents all haematocrit values below and including 12 % (Bath *et al*. 2001). The implication is that only those individuals that are unable to cope with their worm burdens at the time of evaluation are selectively treated with anthelmintics, since only individuals identified as being anaemic by FAMACHA$^©$ are treated. The FAMACHA$^©$ system has been shown to enable the producer to reduce the number of anthelmintic treatments that are administered (Van Wyk 2001; Kaplan *et al*. 2004), and thus increase the proportion of the worm population that escapes drug selection by being in refugia (Van Wyk 2001). The effect is that of reducing selection for parasite resistance, as a part of the worm population escapes the selective effect of the drug and thus voids “unselected” eggs onto pasture (Van Wyk 2001). In the process, unselected individuals are given an opportunity to pass on susceptible alleles to their offspring, which, with the continued application of the FAMACHA$^©$ system, should enhance the chance of susceptible worms to remain in the population. The FAMACHA$^©$ system represents a major departure from conventional strategic drenching with anthelmintics in small ruminant husbandry, and is likely to be one of the defining steps towards sustainable management of haemonchosis in small ruminants.

1.3 Scope of the study

Several issues of importance in the application of the FAMACHA$^©$ system of targeted selective treatment were investigated in this study. Although much of the work was concerned with the application of supplemental epidemiological techniques to the FAMACHA$^©$ system, further validation of the system in South Africa was also undertaken, as well as the application of a stochastic model to the data gathered during five years of FAMACHA$^©$ trials. The main aim of this work was to evaluate the applicability of these supplemental techniques, specifically Receiver Operating Characteristic curve analysis, stochastic estimation of worm burdens, and temporal availability of rainfall, which could be used in a computerised predictive system to treat flocks on a selective basis. It is envisaged that such a “black box” predictive system would eventually be specific enough to enable producers to make decisions based on inputs into the “black box” model, which would then allow the producer to decide when the flock should be evaluated, which class of animal is most at risk, which FAMACHA$^©$ categories of animal should be drenched, how many animals should be sampled to evaluate the anaemia status of the flock, etc.

The initial results obtained from the stochastic model indicated that it adequately reflected the field epidemiological situation with regard to the risk of disease. As a result of this research, it was also decided to explore alternative ways to represent the interaction between rainfall, one of the main risk factors in the development of haemonchosis, and haematocrit, indicated by the FAMACHA$^©$ score, as the main clinical indicator of the disease status, and to use these results as a further refinement to the application of the FAMACHA© system. Although the FAMACHA© system has largely been validated and widely disseminated in South Africa (Bath *et al*. 2001; Van Wyk & Bath 2002) and elsewhere, such as in the southern United States (Kaplan *et al*. 2004), a large body of data has accumulated during the development and subsequent further validation of the system in South Africa. These data have been continually added to as data from on-going farm-based trials have become available.

Extensive use was made of software in this work, such as STATA (Stata Statistical Software: Release 8.0. College Station, TX: StataCorp LP), and @Risk (Palisade Corporation). Data from two farms in the summer rainfall region of South Africa was selected from the data set, not only as the basis for further validation of the FAMACHA© system, but also to produce a predictive model to estimate probable worm burdens, and thus also the risk of disease, in groups of sampled sheep.

The thesis consists of seven chapters, and the research findings are presented in Chapters 2–6. In Chapter 1 an overview of the epidemiology and control options of haemonchosis are presented. Chapter 2 comprises of a review of factors likely to be useful in the development of an automated decision-support system. Conventionally, when evaluating flocks with FAMACHA©, each sheep selected for evaluation is classified into an appropriate FAMACHA© category and depending on the risk of clinical disease with regard to rainfall effects, time in season, class of animal, etc., a decision would be made to drench “high-risk” categories, and to exclude the rest of the animals from drenching. Previously, this approach has usually meant that at the beginning of the “worm season”, only sheep in FAMACHA© categories 4 and 5 are drenched (Van Wyk, Bath, Groeneveld, Stenson & Malan 2001a). However, Van Wyk & Bath (2002) suggested that the categories of animals to be drenched should be varied in relation to the worm challenge to be expected at different time of the year, for instance to routinely drench FAMACHA© categories 3–5, with FAMACHA© category 2 added if deemed necessary according to reigning climatic conditions at the peak of the *Haemonchus* season. Then, if FAMACHA© results indicate that *Haemonchus* challenge is overwhelming animals, all animals in a given flock can be drenched at that time. Although this approach has proven effective, present labour requirements for ensuring low levels of risk when numerous animals are left undrenched at times of serious worm challenge are relatively high. Hence there remains the potential for development of the mentioned software-based system that can be applied at the farm level to enable drenching decisions to be made quantitatively, by including applicable statistical methods in the decision making process.

In Chapter 3 the basic descriptive work undertaken for the two farms included in this work is described, and how the information was organised according to the epidemiological variables of time, place, sheep populations and management factors. The operating characteristics of the FAMACHA© diagnostic system were evaluated in terms of sensitivity, specificity, predictive values and prevalence, to further validate the system on the two selected farms.

The data from the two farms involved indicated that the accuracy of anaemia estimation was higher for Farm 2 than for Farm 1, and that for identical haematocrit cut-off values and proportions of the sampled flock considered to be diseased, the conditional probability that a sampled animal has a positive test result (i.e. test sensitivity) was always higher for Farm 2. This meant that on the latter farm, any animal defined as anaemic by the pre-determined cut-off value for the haematocrit values which form the basis of the FAMACHA© diagnostic system, had a higher probability than on Farm 1 of being detected as suffering from haemonchosis and treated. Sheep on Farm 2 had lower overall levels of anaemia, despite being treated at a higher FAMACHA© category number (i.e. a lower haematocrit value) than sheep on Farm 1, but these sheep were more accurately "scored" into FAMACHA© categories. Sheep on Farm 2 were also evaluated at shorter intervals during periods of peak worm challenge. However, despite a considerable degree of misclassification on Farm 1, the FAMACHA© system proved to be a valuable tool for rapidly identifying anaemic sheep at farm level. The degree of misclassification on Farm 1 was very consistent, as confirmed by the method of Best Linear Unbiased Prediction analysis. These results indicated a heritability of evaluation by the FAMACHA© system to be on a par with both haematocrit determination and faecal worm egg counts.

It is also clear that application of the FAMACHA© method should be continually evaluated in terms of calibration and training of the operators, to avoid misclassification bias. These findings further underscored the recommendation of Van Wyk & Bath (2002) that animals should be examined at least weekly during periods of the highest worm challenge, commonly in January and February in South Africa in summer rainfall areas.

The aim of Chapter 4 was to investigate the feasibility of using Receiver Operating Characteristic curve analysis to select FAMACHA© categories as treatment thresholds according to a given haemtocrit cut-off and desired sensitivity of FAMACHA© classification. The use of Receiver Operating Characteristic curve analysis has in recent times been used extensively in serological testing (Greiner & Gardner 2000), but to the best of our knowledge this work is the first application of this type of analysis to the FAMACHA© system of targeted selective treatment. The calculation of the area under the Receiver Operating Characteristic curve for the FAMACHA© system for nominally selected haematocrit cut-off values of ≤22 % and ≤19 % on both of the farms indicated that the diagnostic accuracy of the system was moderate to high, implying that the system as implemented on the two farms examined here is effective in discriminating between diseased and non-diseased individuals. The area under the curve ranged from a minimum indexed value of 0.79 on Farm 1 to a maximum value of 0.90 on Farm 2, and since the area under the curve represents the probability that a randomly selected individual with the disease will have a lower haematocrit value than a randomly selected individual without the disease for a given haematocrit cut-off, the FAMACHA© test is clinically relevant and useful. The findings from Receiver Operating Characteristic curve analyses for selection of threshold FAMACHA© categories for anthelmintic treatment in Chapter 4 were in agreement with those in Chapter 3, in terms of selecting sheep in FAMACHA© categories that should be treated or not, and should add further impetus to the ease and accuracy of FAMACHA© implementation. They also supported the finding of different levels of accuracy of FAMACHA© classification on the two farms.

In Chapter 5, a model is presented, which could be used to estimate the risk of disease in real-time. In this work, a previously published linear regression model was populated with field data from FAMACHA© trials undertaken with naturally infected sheep. The model was used stochastically, to estimate the risk of haemonchosis by simulation of the mean worm burden of sheep in a sample. Monte Carlo simulation, which is a numerical integration method in which a random element is used to obtain some parameter of a random variable by sampling from a known posterior distribution (Toft, Innocent, Gettinby & Reid 2007), was used to simulate the model. Findings from the model indicated that the mean worm burden, as calculated deterministically, is not a good indication of central tendency as the risk of disease increases, due to the lack of variability in the deterministic model. This underscores the fact that simulation can expose underlying trends in the data which can be used for decision-making. These findings are not entirely intuitive, since, if the model were to be interpreted in a purely deterministic manner, the final interpretation of the risk of disease would lack the resolution provided by probabilistic sampling. A more intuitive prediction of the model, however, was the fact that as more FAMACHA© classes were encountered in a group of sampled group of animals, the higher the intensity of infection and thus the higher the predicted risk of disease. The underlying principle is that quantitative risk assessment models, even though they may be an over-simplification and in most cases incomplete, are useful as tools to evaluate relationships between risk and those factors which are subsequently used to ameliorate risk (Lindqvist & Westöö 2000).

In Chapter 6, rainfall data for Farm 1 was processed with the Shannon entropy model (Shannon 1948), which allowed not only the total rainfall between evaluation events, as has been done with much conventional work with haemonchosis, but also the amount of rainfall per day, to be processed into a single entropy value. The total amount of rainfall was described in terms of its contribution to the risk of disease, by incorporating the entropy, or spread, of rainfall during specified inter-sample periods, into the Shannon entropy model. The issue of spread of rainfall was addressed by Viljoen (1964) who wrote that "Under the influence of heavy rains…distributed over 19 days in January, ideal conditions were created for the free-living larvae". More recently, McCulloch, Kuhn & Dalbock (1984) wrote that daily, weekly and monthly rainfall figures, although easy to record, gave no real indication of pasture "wetness", since one heavy thunder-shower could produce the total recorded rainfall for a month while overhead conditions remained "bright and shiny" for some time after the fact. McCulloch *et al.* (1984) also described "dangerous 8-week rainfall periods", in which reference was made to an 8-week rainfall period where at least 5 weeks exhibited the estimated minimum 4-week rainfall requirement for elevated pasture infectivity for the property where trials took place.

The fact that there was generally good agreement between the processed entropy values of rainfall and simulated haemoglobin levels in groups of sampled sheep, would suggest that rainfall data processed with the entropy model could be a useful, if not absolute, indicator of the risk of disease. This is at least partly because it is not only the total rainfall that may increase pasture infectivity, but also how that rainfall is available in the micro-environmental conditions necessary for maintaining high pasture infectivity. If the available rainfall is spread over a relatively long time period such as several days with discrete rainfall events, then it would be reasonable to assume that micro-environmental conditions would favour a higher overall moisture retention in herbage, and thus lead to a more prolonged period of pasture infectivity. These probabilities are reflected in the Shannon entropy model as high or low entropy values of rainfall. It is also reasonable to assume that high rainfall entropy will also directly affect ambient micro-environmental temperature conditions, since the continual cloud cover needed to maintain high rainfall entropy would decrease desiccation and ultra-violet exposure of larvae. The effect of larval desiccation during periods of high light intensity and temperatures, especially during late spring and summer in the southern hemisphere, has been well documented (Parnell 1963). The main conclusions and future perspectives of the analyses are discussed in Chapter 7.