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Software-based decision-support: a basis for the development of a predictive system for sustainable management of haemonchosis in small ruminants

By

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PROTOCOL APPROVAL

This study was approved as PROTOCOL V 023/05 by the Research Committee and the Animal Use and Care Committee of the University of Pretoria, under the Belgian Co-Operation Grant, Number AG 534.

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THESIS SUMMARY

Data generated by five years of FAMACHA© clinical evaluation trials on one farm, and two years of trials on a second farm in South Africa, where targeted selective treatment was applied to treat haemonchosis in sheep, was used as a basis to explore new computational epidemiological methods to analyse the results of the trials. The research flowed from the earlier work of Dr. J.A. van Wyk and co-workers at the Faculty of Veterinary Science, University of Pretoria, who did much to develop, introduce, and validate the FAMACHA© system in South Africa and elsewhere in the world.

Clinical haemonchosis was common during the summer rainfall season, and was found to increase in severity during January and February of each year. Sensitivity analysis of FAMACHA© data indicated that on the first farm (Farm 1) investigated, many of the animals that were clinically non-diseased were in fact anaemic, but due to misclassification, these animals were not detected. This was not the case on the second farm (Farm 2), where most animals that were clinically diseased according to FAMACHA© were found to be truly anaemic. The high prevalence of misclassification on Farm 1 has important implications for monitoring and chemotherapy of haemonchosis. The results indicated that under the conditions where the data were generated, the FAMACHA© system is sensitive enough, and adequately specific, to detect anaemic sheep despite misclassification.

The application of Receiver Operating Characteristic curve analysis to the FAMACHA© method to select FAMACHA© categories for treatment, was in agreement with the findings that misclassification on Farm 1 would of necessity require that different treatment thresholds would need to be implemented to achieve the same test sensitivity as on Farm 2. Although the use of the Receiver Operating Characteristic method requires the use of dedicated software to generate results, especially if large data sets are analysed, it was found to be an accurate and valid way of indicating FAMACHA© threshold categories for treatment on both farms, for a desired sensitivity.

A previously published multiple regression model was modified to incorporate stochasticity in the FAMACHA© proportions and the body mass of sheep, in order to simulate probable worm count. The fluctuations in simulated worm count adequately reflected the changing epidemiological situation of haemonchosis as indicated by temporal histograms of differential FAMACHA© proportions in flocks. The model was most sensitive to changes in

FAMACHA© proportions in the sample, followed by increasing variability in body mass as a worm season progressed. Furthermore, for a given class of animal, a range of probable haemoglobin values could be associated with a preselected threshold worm burden. The model was sensitive to blanket drenching events, as a lower intensity of infection was predicted immediately after blanket drenching in all samples. It followed that model indications could be used probabilistically, to indicate minimum haemoglobin levels that would need to be sustained in order to prevent overwhelming worm burdens in a given class of animal.

The penultimate chapter of the thesis is concerned with alternative methods of evaluation of rainfall as a risk factor for haemonchosis. Three different periods of rainfall, in relation to FAMACHA© sampling events, were evaluated in terms of entropy, or spread, and tested for strength of association with simulated flock haemoglobin values by regression analysis. Shannon's entropy was used as an indicator of rainfall variability. Findings indicated a negative, and significant, correlation between rainfall entropy and flock haemoglobin level. On the strength of the association, a simulation model was proposed, which could theoretically indicate a probable range for expected flock haemoglobin level in a subsequent two-week period following FAMACHA© evaluation, provided that rainfall entropy is known.

This work attempts to bridge the gap between implementation of the FAMACHA© system, and the investigation of several vital issues that would need to be addressed in the development of a wider ranging anthelmintic treatment decision-support system to delay anthelmintic resistance.

The application of important quantitative methods, such as two-graph Receiver Operating Characteristic analysis, Monte Carlo simulation, and Shannon's entropy to the FAMACHA© system, have provided new perspectives from which to develop an integrated computerized decision-support system. The thesis strongly supports the continued use of the FAMACHA© system in its present form, but the work has emphasised several key issues, such as misclassification, the need to develop decision-support systems that are useable in real time at farm level as opposed to regional level, and that the FAMACHA© system can and should be used as a basis for further development of decision-support software.

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.

CHAPTER 2

Blueprint for a software-based decision-support system for countering anthelmintic resistance at farm level

2.1 Introduction

As often occurrs with new technology, the FAMACHA© system, which was devised for sustainable worm management, is only slowly being adopted by farmers. It has been suggested that an important reason for this unwillingness is due in part to the complexity of integration of the FAMACHA© method with epidemiological factors, and partially to disbelief that resistance would ever become a problem, granted the dozens of different anthelmintics on the market. Reluctance to adopt the FAMACHA© system could be a reflection of the labour effort involved, as even where labour is not expensive, farmers have many demands on their time. The alternatives to the simple drenching programmes of the past are not only more difficult to manage, but are also more labour intensive. The problem is complicated further by a progressive global shortage of persons with the necessary experience to train farmers in the new methods.

It is suggested that only decision-support software will be able optimally to integrate the range of factors such as rainfall, temperature, host age and reproductive status, pasture type, pasture infectivity and anthelmintic formulation for sustainable worm management. The computer model being proposed is to be based almost entirely on periodic retrospective analysis of clinical data accumulating during a given worm season and is to consist almost entirely of tactical, targeted selective treatment, in contrast to conventional strategic drenching of all animals.

In 1985 Van Wyk warned that, unless anthelmintics were used more sustainably, it was possible that resistance would affect all available compounds to the extent that no effective remedies would remain for worm control. To this, Van Wyk (1990) added that while the above was regarded even by some helminthologists as alarmist, it seemed possible that resistance could escalate to include compounds from all the anthelmintic groups in individual worm populations. In 1997 the first case was reported where the total population of a worm species was resistant to compounds from all of the anthelmintic activity groups available for use against gastrointestinal nematodes in sheep and cattle at the time, namely the

benzimidazoles, imidazothiazoles, macrocyclic lactones, organophosphates and the salicylanilides/substituted nitrophenols, as well as the first reported cases of resistance to nitroxynil and disophenol (Van Wyk, Malan & Randles 1997b). Although resistance was referred to as "rampant" at the time (Van Wyk, Malan & Bath 1997a), it was still borderline in the case of some of the compounds. Subsequently it has escalated continuously, recently causing Van Wyk (2006) to comment that we had entered the final phase where nothing remained on some farms with which to control worms at a level commensurate with profitable animal production.

Van Wyk *et al*. (1999) recorded some populations of *H. contortus* that were less than 40 % susceptible to all four of the compounds used in their surveys in South Africa, namely rafoxanide, albendazole, levamisole and ivermectin, to represent the four different activity groups on the market at the time. Today even moxidectin, arguably the last major compound to reach the market, is already seriously affected by resistance (Leathwick 1995; Thomaz-Soccol, De Souza, Sotomaior, Castro, Milczewski, Mocelin, Pessoa & Silva 2004; Rhodes, Leathwick, Pomroy, West, Jackson, Lawrence, Moffat & Waghorn 2006). In some cases there has been practically complete failure even when moxidectin was administered together with other compounds (Table 2.1 – C.S. Sotomaior, personal communication 2006).

TABLE 2.1 Worm populations found in a survey in Paraná State, Brazil, to be less than 80 % susceptible to moxidectin drenched either alone or together with other compounds (C. S. Sotomaior, personal communication 2006)

Worm populations	Moxidectin	Moxidectin plus other*
Less than 80 % susceptible	21	6
Less than 30 % susceptible	6	0
Mean	48 %	55 %
Range	0-74 %	30-77 %

*Moxidectin and closantel and/or moxidectin and nitroxynil

While previously only marginally affected by drug resistance, the worms of cattle are following suit (Hosking, Watson & Leathwick 1996; McKenna 1996; Rhodes *et al*. 2006), leading to a warning by Waller (1997, 2003) that cattle worms had already reached levels of anthelmintic resistance similar to those at which the worms of small ruminants had been a decade earlier. Coronado, Escalona, Henriquez, Mujica & Suarez (2003) recorded total failure of ivermectin against a population of *Cooperia* sp. infection in cattle in Venezuela.

The number of resistant bovine worm populations is rising rapidly, as indicated by resistance to ivermectin on 55 % of 69 farms surveyed in Argentina (Caracostantogolo, Castaño, Cutullé, Cetrá, Lamberti, Olaechea, Ruiz, Schapiro, Martinez, Balbiani & Castro 2005).

2.2 Refugia for sustainable worm management

Parasites which escape any given control measure, for example worms on pasture when their hosts are drenched, are said to be in refugia (Martin, Le Jambre & Claxton 1981; Martin 1989; Van Wyk 2001). One of the most effective ways of obtaining relatively large numbers of worms in refugia is to selectively treat only clinically affected animals, i.e. a system of targeted selective treatment. This may seem to defeat the purpose of anthelmintic treatment for worm control. However, worm burdens are markedly overdispersed within a given flock or herd (Barger 1985), to the extent that in most outbreaks of helminthosis only a minority of animals are unable to withstand the effect of worm infection without anthelmintic treatment. Previously, this phenomenon could not be utilised in practice, since individuals with high *Haemonchus* burdens could only be identified for treatment with the aid of laboratory faecal worm egg count testing which is not feasible, since every animal needs to be tested at relatively short intervals at the height of the worm season. For instance, with severe *H. contortus* challenge of sheep, Malan *et al*. (2001) recorded a drop of up to seven percentage points in haematocrit within seven days. In other words, a sheep which was apparently still coping well with mild anaemia could develop terminal anaemia within less than a fortnight.

It was only after a South African team had devised a system of clinical grading and classification of the anaemia caused by haemonchosis that targeted selective treatment became practical. From the initial results of Malan & Van Wyk (1992) the FAMACHA© system of targeted selective treatment was developed (Bath *et al*. 1996, 2001; Van Wyk & Bath 2002). Highly significant levels of correlation have been demonstrated between the results of clinical FAMACHA© classification and microhaematocrit determination, the laboratory gold standard used to determine an animal's true anaemia status (Van Wyk & Bath 2002). By treating only individuals that are not coping with worm challenge at any given time, the unselected portion of the worm population is given a genetic advantage since, given that highly effective anthelmintics are used, the small numbers of offspring from the survivors of the chemicals in the treated animals are genetically overwhelmed by susceptible worms.

The FAMACHA© method is of course applicable only to haematophagous worm species such as *H. contortus* and, although not specifically tested, probably also to the various hookworms and *Fasciola* spp.

2.3 Optimal application of targeted selective treatment is complex

As pointed out by Van Wyk, Hoste, Kaplan & Besier (2006), the modern methods with potential for countering anthelmintic resistance are unfortunately such that targeted selective treatment and sustainable integrated parasite management are considerably more complex to apply and require greater labour inputs than conventional drenching programmes which could be followed by the farmer, but which also led to the present situation with regards to anthelmintic resistance.

2.3.1 Worm infection not "all-or-nothing"; worm burdens are important

With conventional exclusive reliance on drenching programmes, only a relatively small number of well-spaced treatments was required for good worm control. In contrast, with any system of sustainable integrated parasite management, it needs to be taken into consideration that practically all grazing ruminants are infected with worms practically all of the time (Gordon 1981). The implication for helminths is that, in order to manage worms on a sustainable basis, farmers and their advisors now need to accurately evaluate the relative risk of selection for resistance with use of various formulations of a given anthelmintic, in relation to a variety of factors, such as a move to "clean" pasture, as discussed above and listed in Table 2.2.

2.3.2 Extension services progressively depleted

The complexity of integrated parasite management systems developed over the past decade is compounded by the global decline in numbers of parasitologists and extension personnel with the necessary field experience required for effective technology transfer to farmers, both in developed and developing countries (Van Wyk 2003; Van Wyk *et al*. 2006).

2.4 Can software-based decision-support offer a solution?

Van Wyk and others (Van Wyk 2003, 2006; Van Wyk *et al*. 2006) propose that the solution to the above problems lies in computer based decision-support systems. What is required for regions where there are no well-versed experts, is an automated decision-support system that is so specific that it will lead the farmer in decisions such as whether or not to treat on day X on farm Y when animals are handled, how many of each class of animal to treat; which compounds to use/not to use, the interval before the animals concerned need to be examined again, and how to optimally to integrate all factors with relevance to the farm and conditions at the time.

Without expert knowledge about matters such as the susceptibility of worm populations to available anthelmintics, the relative susceptibility of the class of animal involved to worm challenge, the level of worm infection in the animals, the likelihood of escalating worm challenge during the rest of the worm season, and the relationships between active ingredients and the formulation characteristics of various products, it is virtually impossible to obtain *sustainable* worm management at farm level.

There is no feasible way that farmers can be trained collectively to the level where they will be able, without expert advice, to independently integrate all relevant factors into optimum sustainable worm management decisions at a given time. However, the expert can conceivably be partially replaced by an automated decision-support system.

Various worm management models have been proposed in the past, such as those by Gettinby (1989), Echevarria, Gettinby & Hazelwood (1993), Smith & Grenfell (1994) and Learmount, Taylor, Smith & Morgan (2006), amongst others, which have widely different objectives, and very few models are based on selective treatment of animals. Many parasite models, effective as they are in the hands of experienced statisticians, involve intractable mathematics and/or probability theory. Smith & Grenfell (1994) differentiated between generic models that were simple formulations applicable to whole classes of parasites, and specific models which were more complex and addressed questions regarding particular species. Furthermore, many of these models are also rich in the biological detail of the parasites, addressing issues such as larval survival rate, initial pasture larval contamination, parasite fecundity, and others, all of which require extensive, on-going laboratory sample analysis. The primary objective of many of these models is to predict the timing of the peak worm challenge for a given season, in order to recommend prophylactic or strategic

drenching of all animals at a given time, to prevent overwhelming worm infection. Given effective compounds, this is usually highly effective and thus popular with farmers, but it selects severely for worm resistance, especially if the animals are moved to “safe” or “clean” pastures after treatment (Martin 1989; Van Wyk 2001).

Since there were no economically feasible on-farm methods for evaluating the state of worm challenge on which to base decisions on management, it is understandable that only models such as the above were practicable. However, the fact that methods for clinical evaluation of helminthoses, such as the FAMACHA© system, have become available for on-farm use by the farmer, merits re-evaluation of such models. It is suggested that both of what appear to be the main limiting factors for optimal application of targeted selective treatment in sustainable integrated parasite management, namely high labour requirements and complexity, can be addressed through decision-support modelling. In this work, a site specific Monte Carlo simulation model is presented, based on the selective treatment of animals deemed to be anaemic due to haemonchosis. The model differs for the most part from previous models in that it is a published regression model that generates risk predictions for haemonchosis by simulating probable worm burdens in groups of sampled sheep.

2.5 Factors to consider in a computer-based decision-support system

As pointed out by Van Wyk (2006) it is essential for farmers to appreciate that optimum, not maximum, production is the prerequisite for sustainable integrated parasite management. In general, if any animal production system is so far removed from nature that it is not possible to maintain profitable animal production without heavy and total dependence on chemicals, then a change to a more sustainable farming system should seriously be considered (Malan, Horak, De Vos & Van Wyk 1997).

Table 2.2 contains a summary list of factors which would need to be taken into consideration in decisions on worm management, and in Fig. 2.1 interactions for the same are illustrated. From this it is clear that a great many factors interact with one another, and for optimal sustainable integrated parasite management, they should be incorporated into the decision-support process.

Although simulation models are of necessity an over-simplication of the host-parasite system, there has to be a trade-off in terms of how much detail is added to a model in

relation to its practical application. For example, can it realistically be used by personnel at farm level for decisions by farmers as to treatment options for given situations of risk of disease? In what format will it be available for use on the farm? How are the results interpreted? The simulation model presented in this work is based on data of the anaemia level and mean body mass of groups of sampled sheep, incorporated into a linear regression model, and simulated by random sampling from the pre-determined statistical distributions of flock haemoglobin level and body mass. The model is simple to apply, and importantly, the results of model simulation have a straightforward interpretation. The model is based on a published linear regression model (Roberts & Swan 1982), which estimates the worm count of sheep by incorporating haemoglobin value and body mass. The preliminary indications are that the simulation model makes biological sense, in that much of what is seen in the field of the progression of haemonchosis over a given worm season by application of the FAMACHA© method can be explained by the two principal inputs of the model, namely haemoglobin value and body mass. It is envisaged that the type of model described here, or a modification of it, would be an integral component in an integrated decision-support system.

TABLE 2.2 Information required for modelling with decision-support software, for *Haemonchus contortus* infection.

(1) FARM LOCATION	Province, District, Grid Reference		
(2) DATE OF EVALUATION	Year, Month, Day		
(3) RAINFALL	(a) Long-term		
	(b) Present		
(4) TEMPERATURE (MIN/MAX)	(a) Long-term		
	(b) Present		
(8) ANIMAL HUSBANDRY	(a) Animal production	(i) Stud	*((i)) BLUP*
			((ii)) Not BLUP
		(ii) Commercial	
		(iii) Resource-limited	
	(b) Animal production type	(i) Breed own lambs	
		(ii) Lambs bought in	
	(c) Principal product	(i) Wool	
		(ii) Meat	
		(iii) Mixed	
(9) PASTURES	(a) Natural	Slope	*((i)) Valley*
			((ii)) Hills/Mountains
		Acocks classification (Acocks 1988)	
		Grazing history	*((i)) Present*
			((ii)) Next paddock
	(b) Improved	Crop species	*((i)) Irrigated*
			((ii)) Not irrigated
(7) ANIMAL HOSTS	(a) Breed		
	(b) Sex		
	(c) Class & Age		
	(d) Identified or not	(i) Identified	*((i)) Indiv. Records*
		(ii) Not identified	*((ii)) Not indiv. Records*
	(e) History	(i) Worm challenge	
		(i) Vaccination	*((i)) Vaccine types*
			((ii)) Dates
	(f) "Drench-all-and-move"?	(i) Yes - After what interim	
		(ii) No	
(5) WORMS	(a) Dominant spp.	(i) Annual cycling	
	(b) Resistance history	(i) Yes - Which anthelmintics affected	
		(ii) No	
(6) DIAGNOSTICS	(a) FAMACHA©	(i) Individual records	
		(ii) Histograms of flock results	
	(b) Haematocrits		
	(c) Worm egg counts	(i) Individual	
		(ii) Composite	
	(d) Host weights & growth		
(10) ANTHELMINTICS ON MARKET	(a) Types	(i) Trade names	
		(ii) Formulations	*((i)) Oral*
			((ii)) Injectable
			((iii)) Bolus
		(iii) Residual efficacy	
(11) MODELLING and TESTING	(a) Data required	(i) FAMACHA©	
		(ii) Haematocrit	
		(iii) Condition Scoring	
		(iv) Worm egg counts	
		(v) Host weights and growth	
		(vi) Climate per farm	
		(vii) Best Linear Unbiased Prediction (BLUP) analysis	

2.5.1 Farm location

In countries such as South Africa, and also in Australia with widely differing climatic zones from a Mediterranean-type climate to subtropics, and where numerous, widely disseminated field trials have been conducted, farm location gives a good indication of the principal worm species to be expected on a given farm (Horak 1981a). It can also be an aid for judging the likelihood of the presence of anthelmintic resistance on the farm. For instance, in communal farming regions in South Africa, where resource-poor farmers predominate, there is generally less anthelmintic resistance than on commercial farms (Van Wyk *et al*. 1999; Vatta & Lindberg 2006).

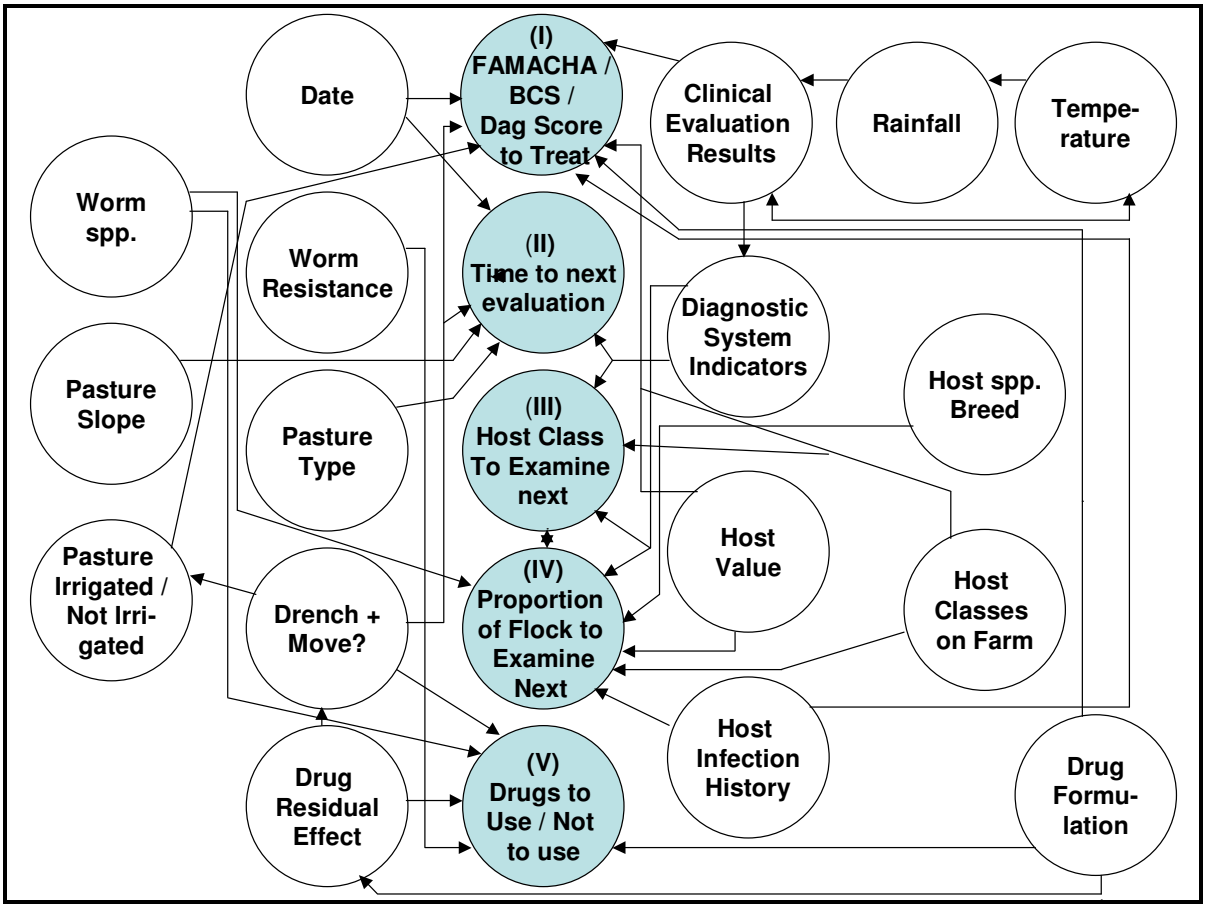


FIG. 2.1 Schematic presentation of factors to consider in arriving at decisions, their interactions with one another, and the envisaged outputs, represented by the shaded circles (I) to (V) for *Haemonchus contortus* infection in sheep.

2.5.2 Rainfall and other sources of moisture

Moisture is one of the main ecological requirements for worms to be able to develop in faecal pellets or pats and translate to pasture. In an attempt to streamline an epidemiological approach to worm management and decisions on anthelmintic treatment, so-called bioclimatographs were produced by earlier workers to delineate combinations of rainfall and temperature conducive to development of different worm species (Gordon 1981). These can at best only be a rough guide to the levels of worm challenge to be expected, since they cannot be used for identifying individual animals which are not coping with worm challenge. Without this, knowledge of conditions which favour worm development can have only limited applicability.

Conventionally, most papers on helminth epidemiology list only total monthly rainfall, and do not give an indication of the daily amount and character thereof in relation to subsequent worm proliferation. However, there is likely to be a threshold of amount of rainfall over time that will be required before the faecal pats or pellets in which infective larvae are contained, will soften sufficiently for the larvae to escape onto pasture and thus be available to their hosts. Consider, for instance, a total of 25 mm of rainfall in a given month. If this amount were to fall as a single shower over a period of one to a few hours it may conceivably be sufficient to soften somewhat moist faecal pats or pellets, but not those exposed to dry conditions for some weeks. Alternatively, if the same amount of rain were to fall over a matter of minutes, run-off may be so fast that the faeces are not softened sufficiently for translation, and on a slope much of the faecal load may conceivably be washed away into streams or distant pools and the worm larvae thus be lost to the hosts from which they originated. On the other hand, if the rain were to fall in four equal showers which are spaced evenly over the course of a month at 6 mm per week for four weeks, it seems unlikely that the faeces would become sufficiently softened to set entrapped larvae free.

It is proposed that the amount and character of rainfall be studied in relation to levels of worm infection, to get an indication of the threshold amount/distribution of precipitation required for larvae to be able to escape from faecal deposits. Rainfall can, for instance, be classified according to periodic amounts, as well as dispersion and character in relation to observed helminth epidemiology. This is discussed in Chapter 6 where the Shannon entropy model is applied to rainfall data, in order to determine an index of the spread and evenness of rainfall during inter-sample periods. It also seems likely that satellite imagery will be of

help as an indication of rainfall amount and effect, and thus of the extent of worm development to be expected from time to time, as well as the proportions of animals that should be treated in any targeted selective treatment worm management system.

2.5.3 Temperature

Temperature is one of the principal determinants of the seasonal cycling of different species of helminths (Veglia 1915; Gordon 1981). At lower temperatures larval development within eggs either ceases or is considerably delayed, or the eggs may die (Rose 1970). However, rising temperature during spring and summer progressively speeds up development until worm eggs hatch in an intensive wave, the so-called concertina effect (Rose 1970). In general, once the worm eggs have hatched and the emerging larvae have developed to L_3, the latter are less susceptible to extremes of temperature than are the undeveloped eggs.

2.5.4 Animal hosts

Host species and breed

There are important differences between both the species and the breed of the host in relation to worm infection (Baker, Mwamachi, Audho, Aduda & Thorpe 1999). If two host species such as sheep and horses are alternated on pasture or run together on the same pasture, they tend to control each other's helminths by ingesting larvae, which cannot propagate themselves in the "wrong" host and thus die.

Host reproductive status

In the initial trials designed to test the principle of clinical evaluation of the anaemia of haemonchosis according to the colour of the ocular mucous membranes it was shown that while 83 % of dry, non-pregnant ewes were able to withstand severe worm challenge unaided, only 70 % of heavily pregnant and 45 % of lactating ewes in the same flock were able to do so (Malan *et al*. 2001). This phenomenon has important implications not only for decision-support modelling, but also for labour saving in targeted selective treatment application.

In early targeted selective treatment trials and field investigations, all classes of sheep were treated similarly as regards intervals between clinical evaluations and FAMACHA© classes to treat (Malan *et al*. 2001; Van Wyk *et al*. 2001; Van Wyk & Bath 2002). Instead, the sharp

differences in susceptibility of the different classes hold the potential that intervals between clinical evaluations be set according to the most susceptible classes in flocks. Labour can then be considerably reduced by examining only these animals until such time as there are indications of mounting worm challenge, at which time other classes can be included. Such an approach can progressively contribute considerably to labour-saving in field use of targeted selective treatment, which is a serious limitation at present.

Host age

The conjunctiva of the lower eyelid, which is the correct spot to view for FAMACHA© evaluation, is considerably more difficult to inspect in suckling lambs than in adult sheep and goats (J.A. van Wyk, personal communication 2005). Farmers are reluctant to handle such young lambs at the relatively short intervals required at the peak of the *Haemonchus* season. Thus, allowance needs to be made for this in recommendations and also in any intended model. A possible solution is to treat all suckling lambs when severe worm challenge is likely, or else to examine only a few of the lambs and to treat all if the proportion of FAMACHA© category 1 animals is lower than, say, 80 % or 90 %. Such treatment of lambs should have little effect on selection for anthelmintic resistance, on condition that their mothers are not treated at the same time and can therefore maintain worms in refugia. To some extent there is support for this type of approach from New Zealand. Despite five drench treatments at 28-day intervals where all but 10 % of lambs (the heaviest lambs were left undrenched) were drenched in a trial, Leathwick, Waghorn, Miller, Atkinson, Haack & Oliver (2006) found no significant differences in live weight gains between drenched and undrenched lambs after the last two treatments. However, they also reported that either leaving a proportion of the lambs undrenched or drenching lambs only when faecal worm egg counts exceeded a threshold level failed to create a measurable pool of unselected larvae for some parasite species.

History of a given flock

Irrespective of the results of FAMACHA© evaluation at a critical time, such as immediately pre-winter in relatively wet summer rainfall regions where the nutritional value of pasture becomes severely reduced in winter, decisions on which clinical categories of animals to treat must take the relative level of worm challenge experienced by the flock in the current worm season into consideration in the case of targeted selective treatment. The level of

worm infection in late autumn and winter in summer rainfall areas will generally be considerably higher than with most systems of conventional programmed drenching, and worm burdens which are well tolerated in summer could then seriously affect animals when the pasture deteriorates nutritionally. The history of infection is also of importance in decisions concerning clinical categories of FAMACHA© to treat, for instance when animals are moved to "clean" grazing before being treated.

Stud or commercial flock

In general, stud sheep and goats are worth more than commercial animals, and this has to be allowed for when formulating recommendations on questions of risk. When balancing risk with labour saving in relation to intervals between clinical evaluations of a given flock, this needs to be considered. Stud breeders and the management of experimental farms are also more inclined to drench their animals excessively, with the result that the worm populations on such farms are more likely to be resistant than on most other farms (Van Wyk, Van Schalkwyk, Bath, Gerber & Alves 1991). For such farming systems it will therefore be more important in the required decision-support software to emphasise worm resistance testing than otherwise. Stud animals are also invariably individually identified, with the result that it is easier to track and subsequently cull overly susceptible individuals than in commercial flocks, where animals are often not individually identified. On the other hand, with planned global introduction of "farm-to-fork" identification of farm animal products this difference is likely to disappear in the near to medium future.

2.5.5 Pastures

Pastures play a crucial role in the epidemiology of gastrointestinal helminth infections, and a variety of factors will have to be taken into account in models aimed at an automated decision-support system.

Number of paddocks

The more paddocks there are available on a farm, the more scope there is for planning movements of animals to minimise the levels of worm challenge in a given worm season, provided that record keeping of animals frequenting each paddock over time is implemented. For instance, given a multitude of paddocks and availability of both sheep and cattle, in the case of the so-called "50-50" grazing system (Kirkman & Moore 1995) where

half the available grazing lies fallow for up to a year, sheep can be moved repeatedly "ahead" of accumulating worm numbers. The paddocks vacated by sheep can then be used to graze cattle or horses, to allow longer periods of time before the sheep return to the pastures they infected previously (Moore & Van Wyk 1997). In this way most classes of sheep will be able to manage with almost no drenching, especially if targeted selective treatment is practised, without compromising profitability as regards optimum utilisation of pastures (Michel 1976, 1985).

Pasture type - current paddock and planned movement to the next paddock

Improved pastures have a greater carrying capacity than natural pastures, particularly if irrigated, providing optimised conditions for a substantial build-up of worms and serious challenge to susceptible hosts. Michel (1976, 1985) does point out, however, that higher concentrations of hosts on pasture do not necessarily lead to higher worm challenge, given optimum utilisation of pasture. In the latter case animals are withdrawn when the available herbage has been utilised to the level where animal production levels off, and the animals return only once sufficient re-growth has occurred for optimum animal production. Thus, numbers of animals on a given pasture can be expected to be reciprocal with grazing period and pasture contamination with worms can optimally be expected to be unaffected by grazing pressure.

Pasture herbage species

Within reason, the more edible bushes, shrubs and trees there are on pasture and the more animals browse, the lower the build-up of worm infection that is to be expected. In South Africa, for instance, Horak, Knight & Williams (1991) and Horak, MacIvor & Greeff (2001) reported negligible numbers of worms recovered from Angora goats with ample browsing on "Valley Bushveld" pasture in the Eastern Cape Province, a region where the climate is otherwise conducive to heavy worm infection in both small ruminants and cattle that do not have access to edible bushes and trees for browsing (Horak 2003; Horak *et al*. 1991, 2001; Horak, Evans & Purnell 2004). Additionally, some tannin-rich plants such as *Lespedeza cuneata* have antiparasitic properties, but the most commonly observed effect of bioactive forages is depressed worm egg counts (Hoste, Jackson, Athanasiadou, Thamsborg & Hoskin 2006). Other effects, such as reduced nematode numbers and parasite fecundity, may be of value in sustainable integrated parasite management if these can be shown to be

consistently obtainable when bioactive forages are made available to sheep.

Pasture grazing history

Season, type and amount of rainfall, periods of grazing and class of animals involved all have an important bearing on the role pasture will play in the levels of worm infection experienced by grazers and hence the potential to result in overwhelming levels of worm infection in grazing animals. For instance, pastures grazed by weaner lambs in autumn in a Mediterranean-type climate will constitute a considerable risk for susceptible classes of sheep in the ensuing spring and early summer (Michel 1976). On the other hand, the opposite is true in the so-called 50-50 grazing system of Kirkman & Moore (1995) and Moore & Van Wyk (1997) in South Africa, where all the animals on a given farm are concentrated on half of the available pasture for up to a full year, with the other half lying unutilised in the interim, and is therefore almost worm-free when animals are returned to it after the rest period. If animals are drenched directly before they are moved to this pasture, severe selection for drug resistance is likely to occur since the pasture will be populated primarily by the offspring of worms which withstood the drench. Note, however, that in the tropics, in contrast, removal of animals from pasture for as short a time as a month will also lead to practically “clean” pasture (Barger 1994).

2.5.6 Worm species and anthelmintic resistance

Dominant worm species and their susceptibility to anthelmintics will dictate the measures required for avoiding losses, and an intimate knowledge of these factors will be a pre-requisite for any system of worm management. Provision will have to be made for winter rainfall regions such as the eastern and southern Cape in South Africa (Horak 2003; Horak *et al*. 1991, 2001, 2004). In relatively arid regions with erratic and highly variable amounts of summer rainfall per year, such as the Kalahari semi-desert in southern Africa, outbreaks of haemonchosis are uncommon (J.A. van Wyk, personal communication 2005), but tend to occur unexpectedly from time to time when good rain falls.

2.5.7 Diagnostic methods

As noted above, targeted selective treatment requires that animals be examined repeatedly during the worm season. In the case of *Haemonchus* sp., evaluation of relatively large numbers of animals needs to be done at short intervals during the peak worm season to

prevent losses of excessively susceptible individuals. This automatically excludes any known laboratory test on the ground of impracticality and leaves only methods of clinical evaluation such as FAMACHA©. No single method of clinical evaluation is sufficient on its own for use with mixed worm infections, since methods of clinical evaluation are needed for both bloodsucking and non-bloodsucking worms such as *Teladorsagia* and *Trichostrongylus*. For this reason provision must be made in the system for a variety of methods, such as FAMACHA© and weight-change based evaluation.

2.5.8 Anthelmintics to use or avoid

Correct use of anthelmintics for obtaining an optimal balance between worm management and minimal selection for drug resistance is dependent on a variety of factors. It is difficult to convince farmers to buy anthelmintics according to active ingredient rather than on trade name. In South Africa even the listing of the activity group by number on the label of every registered anthelmintic has apparently not overcome this problem. Furthermore, each farmer breeds his or her own "brand" of resistance according to the drugs used over time and the circumstances in which they are used. Formulations for a given compound may differ considerably, for example benzimidazole drenches compared to slow-release boluses.

While short-acting formulations like some benzimidazoles may be ideal for a specific set of circumstances, sustained release formulations of these compounds may be contra-indicated. For instance, it is unlikely that the ultra long-acting moxidectin which was introduced to the market recently, with the claim of being "highly effective" for a period of three months against *H. contortus* in Australia and four months in South Africa, can be used without selecting severely for resistant worms. Although the best short-term option from the point of view of the farmer may be to use a persistent anthelmintic that achieves a substantial reduction in worm burden, this strategy is not sustainable because a high proportion of the worms accumulating in the sheep are resistant (Le Jambre, Dobson, Lenane & Barnes 1999). Hence it will be necessary for the envisaged decision-support model for each different situation to list by both trade name and active ingredient all the registered drugs which are recommended, excluding others regarded as unsuited to the occasion.

2.5.9 Treatment in relation to movement of animals to other pastures

Conventionally, animals are drenched strategically at the time of a move to rested pasture (Theiler 1912; Michel 1976). If the move occurs soon after anthelmintic treatment and the animals do not become re-infected with unselected worms in refugia before the move, severe selection for anthelmintic resistance takes place if the pasture to which they are moved is uninfected. Even though this is well known, it is difficult to formulate recommendations for avoiding this possibility. Because of the large differences in residual action between different formulations of a given compound it is not possible to recommend, for different compounds or formulations the general time periods that must be allowed between drenching and the intended move. For this reason Molento, Van Wyk & Coles (2004b) propose that in the case of a move to safe or "clean" pasture the animals are not dewormed before, but only after the move, with the time before treatment on the new pasture to be dictated by the level of infection of the animals at the time they are moved.

2.6 Modelling approach

The envisaged decision-support software model will need to address a variety of questions for optimal worm management (Table 2.2), with a complex system of interactions between them (Fig. 2.1). An important consideration is that presently available methods for application of targeted selective treatment are relatively labour intensive in addition to being complex. In the light of the large variety of interacting factors that need to be considered in an automated decision-support system provision must be made in the model for use of both specific data and also expert opinion until sufficient data has been generated.

2.6.1 Retrospective analysis of clinical evaluation data

The nature of methods used for identifying stragglers in a given flock for drenching leads to a multiplicity of data sets by the time worm challenge becomes serious in any worm season. For instance, in the case of the FAMACHA© system, expressing the proportions of the various categories in the form of a histogram graph makes it possible to follow the progressive adverse effect of mounting worm challenge (Fig. 2.2). With retrospective analysis of the results, drenching can be adjusted to include more or fewer FAMACHA© categories to ensure that the animals are not overwhelmed. At the same time allowance can be made for the optimum number of undrenched animals to produce sufficient numbers of free-living helminth stages in refugia for sustainable management. This is addressed in

Chapter 3 with sensitivity analysis, and in Chapter 4, where Receiver Operating Characteristic analysis was applied to FAMACHA© trial data. As an example, while the general recommendation has thus far been to routinely treat all sheep and goats evaluated to be in FAMACHA© categories 3–5, this can be adjusted at the height of the worm season to include animals in FAMACHA© category 2, or, if this adjustment does not succeed in reversing the tendency to increasing severity of helminthosis, all animals can be treated.

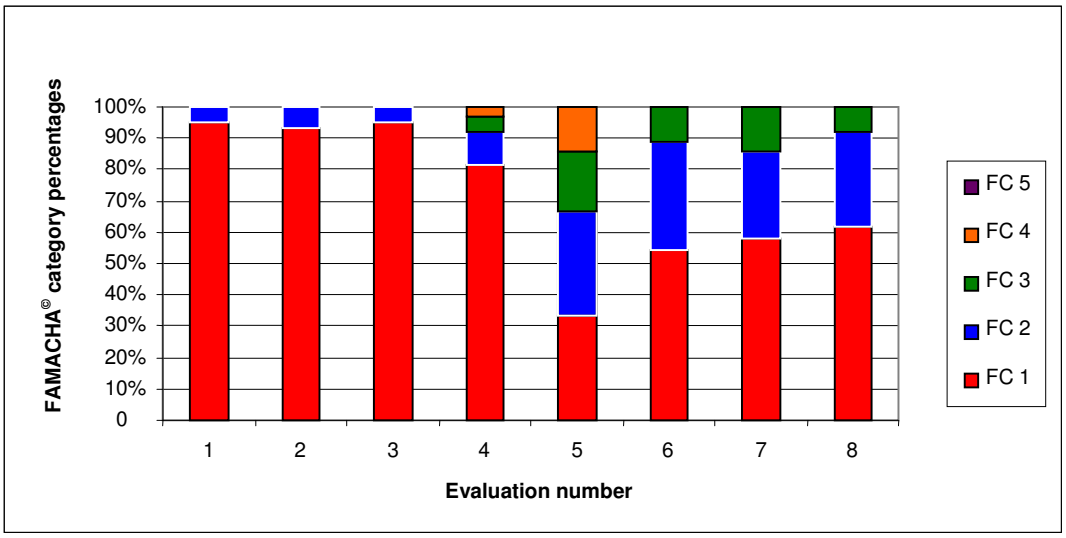


FIG. 2.2. Example of FAMACHA© results for eight evaluations over a *Haemonchus* season in South Africa for a group of 130 ewes; FAMACHA© category 5 not present (FC = FAMACHA©).

2.6.2 Model framework

Labour saving would be one of the aims of the intended model. As discussed above, it would require, from retrospective analysis of clinical evaluation data, to recommend the minimum levels of drenching which will prevent excessive losses in the production of the animals concerned, while allowing the optimum numbers of worms in refugia. Labour saving can be achieved, amongst others, by estimating the maximum safe interval between clinical evaluations according to season and risk factors, such as rainfall and temperature, during the period immediately preceding the point of the decision, and the minimum proportions of animals to be examined per flock (Table 2.2).

2.7 Effective technology transfer

2.7.1 Technology transfer previously ineffective

Unabated escalation of anthelmintic resistance, despite the availability of effective countermeasures (Van Wyk *et al*. 2001; Rhodes *et al*. 2006) indicates that efforts at technology transfer to both farmers and scientists are possibly inadequate. Despite an emphasis on various methods of integrated parasite management instead of dependence only on drenching for worm control, the majority of farmers still depend almost exclusively on chemical control, with the excuse that losses in production could result with adoption of available alternatives. While it was clearly shown in the 1980s that strategic drenching at a stage when there are few free-living worm stages in refugia selects severely for anthelmintic resistance (Martin *et al*. 1981; Martin 1989), this was largely ignored until the beginning of the present century (Van Wyk 2001). Even today, a variety of advertisements concerning worm management ignore the concepts of refugia and sustainable integrated parasite management, and propagate drug sales at the cost of sustainability of worm management (Van Wyk 2003).

The most important reasons for the failure of technology transfer are a combination of complexity of alternatives to the simple drenching programme and a shortage of the necessary extension personnel. There have also been many different anthelmintics available to the consumer, leading to the problem that both farmers and their advisors have refused to give credulity to early warnings on anthelmintic resistance. While the possibility of resistance of worm populations to all available compounds was predicted more than twenty years ago (Van Wyk 1985), this was regarded by many as alarmistic (Van Wyk 1990). It is apparently only when there are signs of total failure of anthelmintics on their own farms that most farmers are prepared to consider simple drenching programmes (Van Wyk, Malan & Randles 1997b). The fault does not lie entirely with the farmer, however. Worm management is more complex than for most diseases against which there are effective vaccines and farmers need to be given large amounts of basic information on worm management to be able to “translate” on-farm observations and results into optimum sustainable worm management.

2.7.2 New approach required

The use of software-based decision-support which is specific to time and place and contains recommendations on issues such as drugs to use, categories of animals to treat, and intervals between clinical evaluations, could be effective for technology transfer to individual farmers (Van Wyk 2003, 2006). With this approach it should not be necessary to train farmers or their advisors to the level of being able themselves to evaluate the complexities of the optimum period between treatment and movement of animals in accordance with the drug formulation used, as this can in theory be managed by dedicated software.

Sufficient relevant data has been generated in most countries to form the basis for development of the suggested software and the technology for on-farm application is already available even for regions with practically no infrastructure, other than access to electricity. Most commercial farmers use computers for farm and stock management on a daily basis, and satellite transmission can be employed for resource-poor communal farmers where landlines are absent or not dependable. A relatively inexpensive option for the latter farmers is to provide their veterinary and agricultural extension advisors with the necessary equipment and, as suggested above, make provision in the software for decisions based on expert opinion utilising estimates of factors such as rainfall and temperature where specific data is not available. The computer-generated decisions could then be communicated to communal farmers via cell (mobile) phone.

In the light of ubiquitous poverty it may seem unlikely that the per capita use of cellphones in Africa appears to be the highest in the world. However, cellphone use is generally higher in developing, compared to developed, countries (J. Britz, University of Wisconsin-Milwaukee, personal communication 2005). For instance, by 2001 mobile subscriber numbers in Africa had overtaken those of fixed lines, the first region of the world to achieve this. In addition, the effect of GSM (“Group Special Mobile”, or wireless) technology is twice as big in developing nations as in their developed neighbours (Anonymous 2005). A special consideration is that farming activities in many, if not most, resource-poor farming communities are centred around easily accessible farmers’ committees. In other instances communication between these farmers and their advisors can proceed by whatever means used at present.

The technology required for the satellite access suggested above is inexpensive in relation to the considerable potential of such a decision-support system for specific, accurate technology transfer. Broadband Global Area Network (BGAN) in South Africa recently quoted US$7 per Mb of data downloaded and a monthly charge of US$30. While these tariffs are expensive if large amounts of data are to be downloaded, only relatively small volumes of downloading will conceivably be required for instituting a worm management system via satellite, since once the software is loaded, it is likely that little else will be required until it needs to be updated. In other words, we need to move on from software that provides general information by region, to specifics tailored not only to the individual farmer, but more precisely to day-day decisions on worm management by that farmer.

2.8 Discussion

For both the resource-poor and the commercial farmer there is an urgent need for decision-support software, to a large extent to neutralise the sales pressure of drug manufacturers and their representatives. For the commercial farmer it is a matter of attempting to retain efficiency of at least some of the modern-day anthelmintics. For the resource-poor the prime consideration is to avoid many of the mistakes made in the commercial farming sector. Developing farmers are inclined to buy animals from commercial farmers to improve their stock, in which case excessive drenching can be expected to decrease the frequency of occurrence of susceptible genes and allow only the ones conveying drug resistance to pass.

The principal advantage of using clinical methods of analysis such as FAMACHA© is that without the need for laboratory intervention, a series of results are obtained on-farm by the farmer himself as a given worm season progresses, on which decisions can be based for risk-evaluated sustainable integrated parasite management. Through retrospective analysis of results in a simplistic way, to depict consecutive FAMACHA© results in series of bar graphs, it is possible to visually follow changing ratios between the various FAMACHA© categories as an indication of developing anaemia (Fig. 2.2). This is a fundamental deviation from conventional models for worm control, which have thus far practically all been based on predicting, at the start of a given worm season, the levels of worm infection to be expected, and then applying anthelmintics prophylactically in a way which did not make provision for a build-up of worms in refugia before chemical prophylaxis was instituted. In contrast, when targeted selective treatment is applied as suggested, untreated animals provide growing pools of helminths in refugia. Even if all the animals need to be given a treatment in mid-

worm season, the pools of larvae in refugia are available for re-infection, as long as long-acting formulations of anthelmintics are avoided (Van Wyk 2001).

It is clear from Table 2.2 that the inclusion of the vaccination history of the animals concerned could allow an automated decision-support system to be broadened to include other aspects such as external parasites and infectious diseases in general. As a first step, however, the presently envisaged software is aimed primarily at internal parasites in small ruminants.

2.9 Conclusion

Anthelmintic resistance is so advanced at present that drastic counter-measures are required, especially as regards present methods of technology transfer. Every effort should be made to have alternatives in place, should new anthelmintics perhaps become available in future, in an effort to prevent a recurrence of the failure of the macrocyclic lactones, to which resistance was recorded within a period of three years of the first member of the group having been introduced to the market for sheep (Carmichael, Visser, Schneider & Soll 1987; Van Wyk & Malan 1988).

Thus far anthelmintic resistance has largely been confined to the worms of small ruminants and horses, for which the global market for anthelmintics is too small to counter the high financial risk of development of drugs in unrelated new activity groups (McKellar 1994). However, it is to be expected that what appears to be an increase in anthelmintic resistance in the worms of cattle (Caracostantogolo *et al*. 2005; Rhodes *et al*. 2006) will have the effect of stimulating drug firms to resume development of unrelated new compounds. It is essential to have methods for preventing a repetition of the mistakes of the past as regards anthelmintic usage in place before any such new compounds reach the market.

CHAPTER 3

Validation of the FAMACHA© eye colour chart on two South African sheep farms under commercial farming conditions

3.1 Introduction

Multiple anthelmintic resistance in the highly pathogenic nematode parasite *Haemonchus contortus* is a severe problem on commercial sheep farms in South Africa, and has the potential to become just as problematic on communal farms in the country. The proportion of the parasite population that escapes drug selection is at present thought to be the most important factor in influencing the rate of development of resistance (Van Wyk 2001; Leathwick *et al.* 2006). It has been largely in response to this that targeted selective treatment systems, notably FAMACHA©, were developed. Although much has been done to validate the FAMACHA© system in South Africa, it is still important that the method be tested for its operating characteristics in terms of sensitivity, specificity and predictive values under farming conditions on an ongoing basis. Once the sensitivity and specificity of a test are known, then a corrected estimate of the true prevalence of disease can be estimated. It is important to know the probability that an animal classified as test positive is truly positive and alternatively the probability that an animal classified as test negative is truly negative. These two probabilities are the predictive values of the test and they depend on sensitivity, specificity and prevalence (Thrusfield 2001). The standard 2 × 2 table method (Thrusfield 2001) was used in this investigation, to calculate the above-mentioned properties. When using the FAMACHA© system, disease management of a flock depends on accurate identification of diseased individuals, to include these individuals in the proportion of the flock that is to be treated. The FAMACHA© system has in this respect been successfully used as a stratification method, to classify individual animals requiring treatment (Bath *et al.* 2001).

The FAMACHA© system reduces the uncertainty about the state of haemonchosis in individual sheep, and can therefore be regarded as a diagnostic test (Greiner & Gardner 2000). The clinical performance of a diagnostic test can be described in terms of its diagnostic accuracy, which represents the ability of the test to correctly classify test subjects into clinically relevant subgroups (Zweig & Campbell 1993). However, as the FAMACHA©

system is based on a rating method (Hanley & McNeil 1982) with the different FAMACHA© categories from 1–5 representing the increasing probability of an abnormal test result, the results of FAMACHA© classification of a sample of sheep are required to be dichotomised into two groups, each representing the infected and uninfected proportions of the flock. This is an artificial distinction, since, due to the extra-binomial nature of the variation in worm burden infection in flocks, almost all animals are infected, but due to overdispersion, the minority of the animals harbour the highest individual number of worms (Barger 1985; Wilson, Grenfell & Shaw 1996; Herbert & Isham 2000). While diagnostic tests are subject in terms of sensitivity and specificity to arbitrary definitions (Begg 1987), the application of the FAMACHA© system provides reasonable scope to adjust for this arbitrariness because it has five categories that could potentially provide five different views of the infection status of a flock.

One limitation to the 2 × 2 table method of estimating sensitivity and specificity is that there is usually a single pre-determined criterion, referred to as a cut-off point, to indicate a true positive test result (Linden 2006). As an example, all animals in a sample could be classified as test positive, or FAMACHA© categories 2–5 could be regarded test positive with FAMACHA© category 1 test negative, FAMACHA© categories 3–5 test positive and FAMACHA© categories 1 and 2 test negative, etc. Although these dichotomized FAMACHA© test results are still arbitrarily chosen, assessment of the epidemiological risk of infection will have a strong influence on FAMACHA© categories selected as thresholds of infection status. Thus, the “resolution” of the FAMACHA© system can easily be adjusted to estimate test measures of sensitivity and specificity against a given haematocrit cut-off value.

Overdispersed worm burdens are related to a number of factors due to the fact that some animals may habitually graze in an area of pasture with higher levels of larvae, differences in immunity status of animals due to age, sex, nutrition, parturition, or previous exposure and genetic differences in animals’ ability to tolerate or expel worms. This results in extra-binomial variation, necessitating the use of a negative binomial model, rather than a poisson model. Additionally, the immune response caused by macroparasites such as *H. contortus* may depend on the number of parasites in a particular host (Bishop & Stear 2003). Barger (1985) described the negative binomial distribution of trichostrongylid nematodes in grazing lambs (n = 104), and indicated that the distribution of worm burdens in a flock would have an effect on anthelmintic treatment. Anderson & May (1982) estimated that, in helminth-infected humans, selective treatment of the most heavily infected 8 % of the population would reduce

the mean worm burden of the population by 50 % if worms were highly overdispersed, but added that the advantages of targeted treatment in terms of costs no longer incurred would have to be offset against the costs of identifying the most heavily infected individuals. It was largely also in response to the latter problem, that the FAMACHA© system was developed.

A validation study of the FAMACHA© system was conducted on a variety of commercial sheep farms in South Africa (Van Wyk & Bath 2002). Two of these farms, for which extensive data sets were generated, were selected for the present investigations. Over a period of five years, the system was tested in Merino sheep on the first farm (Farm 1; 26°40'53"S, 30°16'47"E) under routine farming conditions. The colours of the conjunctivae of sheep were scored on a 1–5 scale using the FAMACHA© chart, and blood samples were periodically collected from each animal for haematocrit determination. Only sheep that were classified into FAMACHA© categories 3, 4 and 5 were treated with anthelmintics, with approximately 260 sheep being re-evaluated at each sampling event. The farmer himself was mainly responsible for FAMACHA© scoring on Farm 1. Data for both FAMACHA© scores and haematocrit were evaluated using different criteria for anaemia. Firstly, FAMACHA© eye scores of 3, 4 and 5 and haematocrit values of ≤22 %, ≤19 %, and ≤15 % were separately considered to be anaemic. Sensitivity and specificity were maximised at a haematocrit cut-off value of ≤15 % at 0.83 and 0.85 respectively, but this haematocrit value is thought to be too low to be safe under conditions of selective anthelmintic treatment where animals are only treated when they are deemed to be anaemic. In contrast, sensitivity increased to 0.93 when eye scores of 2, 3, 4, and 5 were considered anaemic at the safer and more realistic haematocrit cut-off value of ≤19 %, but the predictive value of a positive was low indicating that many non-anaemic animals would be treated. Considerable classification bias was detected in scoring for FAMACHA© categories 1–4 on this farm, leading to the recommendation that animals in FAMACHA© category 2 should have been treated in addition to animals in FAMACHA© categories 3–5, in order to increase sensitivity and prevent the likelihood of non-treatment of sheep with a haematocrit of ≤19 %.

A second validation study was conducted on data from Farm 2 (27°23'48"S, 29°22'24"E), a commercial Merino farm, over a period of two *Haemonchus* seasons, under the so-called "Field Ram Club" system. Haematocrit determinations were done on all the rams each year, both at the beginning and at the height of the *Haemonchus* season, with approximately 200 rams being re-evaluated at each sampling event. Rams were only treated if their haematocrits were ≤15 %. In the interim every ram judged to be in FAMACHA© category 4

or 5 was bled for haematocrit determination, and only rams with haematocrit values of 15 % or lower were dewormed with effective anthelmintics. The results from Farm 2 indicated that the accuracy of anaemia estimation was higher than that of Farm 1, and that for identical haematocrit cut-off values and proportions of the sampled flock considered to be diseased, sensitivity, i.e. the conditional probability that a sampled animal has a positive test result, was always higher for Farm 2. This meant that on Farm 2, any animal defined as anaemic by the pre-determined cut-off value for the haemaocrit, had a higher probability than for Farm 1 of being detected as anaemic and treated. Sheep on Farm 2 were generally less anaemic, despite being treated at a lower FAMACHA© threshold than sheep on Farm 1, but these sheep were more accurately "scored" by the farmer into FAMACHA© categories, and were also evaluated much more frequently during periods of peak worm challenge.

3.2 Materials and methods

3.2.1 Origin of data and FAMACHA© test procedures

The data that was analysed consisted of anaemia status as evaluated by FAMACHA© score, and haematocrit values, originating from naturally infected sheep on the two farms (Fig. 3.1). The farms are situated in the summer rainfall region of South Africa. Merino sheep are predominant in this area, and *H. contortus* is the dominant nematode parasite. Climatically, the region is a part of the temperate eastern plateau, at an altitude of approximately 1 500m above sea-level, with cool, rainy summers and cold, dry winters. Over a five year period on Farm 1, from November 2000 until April 2005, two classes of animals were introduced annually into a series of FAMACHA© trials, namely replacement rams (RAMREP) and replacement ewes (EWEREP), each individually identified with a uniquely numbered ear tag. The two groups of sheep were farmed under extensive conditions, in separate flocks, according to sex. Each flock was grazed at intervals of approximately 3–5 weeks through a series of different paddocks according to available herbage. The number of sheep on the farm during the study period varied between 1 200 and 1 800, but only 130–200 sheep of each class were sampled at each FAMACHA© evaluation in the various trials per class and year. At the start of each of the five annual trials, each sheep was scored into a FAMACHA© category, its body weight and condition score determined, and it was dewormed. This was followed by a period during which only animals clinically judged to be in FAMACHA© categories 3–5 were dewormed. However, once general "severe worm challenge" was evident, usually in January or February of each year, all sheep were again dewormed. Then,

until the end of each trial, only the animals in FAMACHA© categories 3–5 were treated as before. From November to April the following year, sheep were mostly evaluated at intervals of 3–4 weeks, but in some instances the evaluation intervals were longer, at up to five weeks. A total of 7–11 sampling events took place per worm season. At the time of the "severe worm challenge", haematocrit determinations were done on all the sheep. In addition, during the 2000/2001 season, haematocrit determinations were done both initially at the start of the trials in November, and during the height of the worm season, in January or February.

On Farm 2, data collected from rams over a period of two seasons, 1998/1999 and 1999/2000, was evaluated. In this system, young rams, usually 6 months old at the start of the trial, belonging to club members were compared in the field over a period of 10–11 months. The rams were grazed on common pasture. At the end of each trial, the rams were evaluated for weight gain and wool production, and the best performing rams were sold at auction. During the course of each trial, a haematocrit determination was done for each animal judged to have had a FAMACHA© score of 4 or 5, and it was only treated if the haematocrit was ≤15 %. However, on five different occasions over the two-year trial period, the haematocrit of every ram was determined, in addition to its FAMACHA© score, for calibration purposes. This was the principal difference between the two data sets, as, even though rams on Farm 2 were scored according to FAMACHA©, only individuals in FAMACHA© categories 4 and 5 were treated, and then only if their haematocrits were ≤15 %. During the 1999 season worm challenge became intense from January on Farm 2, and FAMACHA© evaluation and sampling of individual animals for haematocrit determination and drenching was carried out more frequently, at which point the animals were evaluated weekly during the peak worm season in February instead of fortnightly as before. During the 1999/2000 season, lower levels of infection were experienced, and the increased frequency of evaluation was not repeated.

FIG. 3.1 Map of South Africa indicating position of Farm 1 (red square) and Farm 2 (blue square). Refer to text for geographical co-ordinates.

3.2.2 Statistical analysis

Data from the RAMREP and EWEREP classes on Farm 1 were pooled for comparing the accuracy of the clinical FAMACHA© scores with the haematocrit value used to determine the true presence or absence of anaemia in the trial animals, similar to the method used by Vatta *et al*. (2001) and Kaplan *et al.* (2004). For the observed haematocrit values of FAMACHA© categories 1–5, the mean, median, 5th percentile, 95th percentile, and standard deviation were calculated and tabulated against their ordinated FAMACHA© scores, using Excel spreadsheets. Two-way frequency tables were constructed, and sensitivity, specificity, predictive value of a positive and predictive value of a negative were calculated for the data. FAMACHA© scores of 3, 4 and 5 were considered to be test positive and FAMACHA© scores of 1 and 2 were considered to be test negative.

For the purposes of determination of test sensitivity and specificity on Farm 1, three different haematocrit cut-off values were considered to be anaemic. Accordingly, values were considered anaemic if ≤22 %, ≤19 % and ≤15 %, and the above test parameters were calculated separately for these three values. The haematocrit value of ≤22 % was chosen as it is the upper limit of FAMACHA© category 3, which was used on this farm as a treatment threshold. True positives were defined as sheep that were anaemic with haematocrits of ≤22 %, ≤19 %, or ≤15 % and FAMACHA© scores of 3, 4 or 5. Sheep were defined as false positives if they were not anaemic, with haematocrits of >22 %, >19 %, or >15 % but with FAMACHA© scores of 3, 4 or 5. False negatives were defined as sheep that were anaemic with FAMACHA© scores of 1 and 2 while true negatives were defined as sheep that were not anaemic but with FAMACHA© scores of 1 and 2.

A further analysis was conducted with FAMACHA© categories 2, 3, 4 and 5 considered to be test positive and FAMACHA© category 1 considered to be test negative. For this part of the analysis, haematocrit values were considered anaemic if ≤22 % or ≤19 %. Test operating characteristics were calculated as described above.

Data for Farm 2 were analysed in a similar way to Farm 1 but haematocrit values were considered anaemic only if ≤22 % or ≤19 % and only individuals in FAMACHA© 3–5 were considered to be test positive for comparison between the two farms.

3.3 Results

Farm 1

The results of Farm 1 indicated that the percentages of sheep that would be correctly treated with haematocrit cut-off values of ≤22 %, ≤19 % and ≤15 % when FAMACHA© categories 3–5 were treated were 68.3 %, 82.8 % and 65.6 %, respectively (Table 3.1a–c). The sensitivity of the FAMACHA© system to identify sheep that are anaemic with haematocrit cut-off values of ≤22, ≤19 and ≤15 when only animals that were in FAMACHA© categories 3, 4 and 5 were treated, was low for all haematocrit cut-off values (Table 3.2), with the highest sensitivity being obtained for a cut-off of ≤15 %. The specificity of the FAMACHA© method on the other hand, was highest for a haematocrit cut-off value of ≤22 %, at 96 %. The haematocrit cut-off value of ≤22 % was chosen as it is the upper haematocrit limit of FAMACHA© category 3, and would thus include treatment of FAMACHA© categories 4 and 5, in addition to FAMACHA© category 3. However,

haematocrit cut-off values of ≤19 % and ≤15 %, as described by Kaplan *et al.* (2004) were also evaluated. Sensitivity increased as the haematocrit cut-off value decreased (Table 3.2), but the predictive value of a positive decreased. Thus, using FAMACHA© categories 3–5 inclusive (with FAMACHA© category 3 as a threshold), and a haematocrit cut-off of ≤22 %, only 40 % of animals that were anaemic would have been treated due to the large number of false negatives. The proportion of animals correctly treated was highest for a haematocrit cut-off of ≤19 %, at 82.8 % (Table 3.1b), but only 58 % of sheep with a haematocrit of ≤19 % would have been detected (Table 3.2), as the majority of animals correctly left untreated would have been true negatives.

TABLE 3.1a Farm 1. Haematocrit cut-off value is ≤22 %. Results of two-way frequency tables of haematocrit by FAMACHA© score. Percentage of total is given in parentheses, for sheep with assigned ranges in haematocrit values which are based on drenching of sheep with FAMACHA© scores of 3, 4 and 5.

Haematocrit value	False negatives	False positives	Treatment correct	Total
≤22 %	201 (29.7)	-	133 (19.7)	334 (49.5)
>22 %	-	13 (1.9)	328 (48.6)	341 (50.5)
Total	201 (29.7)	13 (1.9)	461 (68.3)	675 (100)

TABLE 3.1b Farm 1. Haematocrit cut-off value is ≤19 %. Results of two-way frequency tables of haematocrit by FAMACHA© score. Percentage of total is given in parentheses, for sheep with assigned ranges in haematocrit values which are based on drenching of sheep with FAMACHA© scores of 3, 4 and 5.

Haematocrit value	False negatives	False positives	Treatment correct	Total
≤19 %	79 (11.7)	-	109 (16.1)	188 (27.8)
>19 %	-	37 (5.5)	450 (66.6)	487 (72.1)
Total	79 (11.7)	37 (5.5)	559 (82.8)	675 (100)

TABLE 3.1c Farm 1. Haematocrit cut-off value is ≤15 %. Results of two-way frequency tables of haematocrit by FAMACHA© score. Percentage of total is given in parentheses, for sheep with assigned ranges in haematocrit values which are based on drenching of sheep with FAMACHA© scores of 3, 4 and 5.

Haematocrit value	False negatives	False positives	Treatment correct	Total
≤15 %	11 (1.62)	-	56 (8.3)	67 (10)
>15 %	-	90 (13)	518 (76.7)	608 (90)
Total	11 (1.62)	90 (13)	574 (65.6)	675 (100)

TABLE 3.2 Farm 1. Sensitivity (Se), specificity (Sp), positive predictive value (Pv+), negative predictive value (Pv-), and prevalence (P) for trial data for given haematocrit cut-off values and treatment of sheep in FAMACHA© categories 3–5. The value for prevalence was calculated from standard two-way frequency tables.

Haematocrit value	Se	Sp	Pv +	Pv -	P	Confidence interval (95 %)
≤22 %	0.40	0.96	0.91	0.62	0.49	(0.458 – 0.532)
≤19 %	0.58	0.92	0.75	0.85	0.27	(0.245 – 0.312)
≤15 %	0.83	0.85	0.38	0.98	0.10	(0.089 – 0.111)

In contrast, when FAMACHA© scores of 2 – 5 (inclusive), and haematocrit cut-off values of ≤22 % and ≤19 % were considered anaemic (Table 3.3a and b), sensitivity was highest when a haematocrit value of ≤19 % was considered anaemic, at 93 % (Table 3.4). Thus, if all sheep in FAMACHA© categories 2–5 were treated, 93 % of sheep with a haematocrit of ≤19 % would have been detected, due to the small number of false negatives (Table 3.3b). The total percentage of correctly treated animals, i.e. true positives + true negatives, would have been 64 %, but this would have been due to the relatively high proportion of false positives (Table 3.3b).

The FAMACHA© scores vs. assigned and observed median haematocrit values are given in Table 3.5. Observed mean and median haematocrit values were lower than assigned mean values, indicating bias, or misclassification, on the part of the evaluators (Table 3.5). For example, the assigned minimum haematocrit value of FAMACHA© category 1 is given to be above 28 %, but the observed median for animals classed as being in this category was 23 %, and the assigned median haematocrit value of FAMACHA© category 2 (range 23–27 %) is given as 25 %, yet a relatively low value of 19.5 % was observed from the data. Similarly, the given median haematocrit value for FAMACHA© category 3 is 20 %, with an observed median value of 15 %, and the given and observed median haematocrit values of FAMACHA© category 4 are 15 % and 11 % respectively. The proportion of haematocrit values falling within assigned ranges are given in Table 3.6.

TABLE 3.3a Farm 1. Haematocrit cut-off value is ≤22 %. Results of two-way frequency tables of haematocrit by FAMACHA© score. Percentage of total is given in parentheses, for sheep with assigned ranges in haematocrit values, which are based on drenching of sheep with FAMACHA© scores of 2, 3, 4 and 5.

Haematocrit value	False negatives	False positives	Treatment correct	Total
≤22 %	56 (8.3)	-	278 (41.2)	334 (49.5)
>22 %	-	124 (18.4)	217 (32.1)	341 (50.5)
Total	56 (8.3)	124 (18.4)	495 (73.3)	675 (100)

TABLE 3.3b Farm 1. Haematocrit cut-off value is ≤19 %. Results of two-way frequency tables of haematocrit by FAMACHA© score. Percentage of total is given in parentheses, for sheep with assigned ranges in haematocrit values, which are based on drenching of sheep with FAMACHA© scores of 2, 3, 4 and 5.

Haematocrit value	False negatives	False positives	Treatment correct	Total
≤19 %	13 (1.9)	-	176 (26.1)	189 (28.0)
>19 %	-	227 (33.6)	259 (38.4)	486 (72.0)
Total	13 (1.9)	227 (33.6)	435 (64.4)	675 (100)

TABLE 3.4 Farm 1. Sensitivity (Se), specificity (Sp), positive predictive value (Pv+), negative predictive value (Pv-), and prevalence (P) for trial data for given haematocrit cut-off values and treatment of sheep in FAMACHA© categories 2–5. The value for prevalence was calculated from standard two-way frequency tables.

Haematocrit value	Se	Sp	Pv +	Pv -	P	Confidence interval (95 %)
≤22 %	0.83	0.63	0.69	0.79	0.48	(0.442 – 0.510)
≤19 %	0.93	0.53	0.43	0.95	0.28	(0.246 – 0.314)

TABLE 3.5 Farm1. FAMACHA© score vs. haematocrit: assigned values, observed values and percentiles (n = 675)

FAMACHA© score	Assigned median value of haematocrit range (%)	Observed median haematocrit value (trial data) (%)	Percentage below assigned median for observed haematocrits	Fifth percentile of observed haematocrit value	Ninety-fifth percentile of observed haematocrit value
1	30	23	23 %	19.7	30.5
2	25	19.5	22 %	15.9	27.2
3	20	15	25 %	10.6	23.9
4	15	11	26 %	6.5	18.7
5	10	10.5	-	8.6	11.5

TABLE 3.6 Farm 1. FAMACHA© categories, sample size, assigned haematocrit range and percentage of observed haematocrit values within the assigned range.

FAMACHA© category	n	Assigned haematocrit range of FAMACHA© category*	Percentage of observed haematocrit values within assigned range
1	273	≥28 %	18.8 %
2	258	23 – 27 %	27.9 %
3	126	18 – 22 %	37.5 %
4	16	13 –17 %	44 %
5	3	≤12 %	100 %

* Van Wyk &Bath (2002)

For the intermediate FAMACHA© categories 2, 3 and 4, only 27.9 %, 37.5 % and 44 % of observed haematocrit values respectively, fell within the given limits. For FAMACHA© category 1 only 18.8 % of haematocrit values were above the lower limit of 28 % for the category, while for FAMACHA© category 5, 100 % of the observed haematocrit values were below the upper limit of 12 % but note that there were only three sheep in the latter category. There was thus an increase in the accuracy of FAMACHA© classification from FAMACHA© category 1, which had the lowest overall accuracy of classification, to FAMACHA© category 5, which had the highest accuracy (Table 3.6).

Farm 2

The proportions of false negatives, false positives and correctly treated rams for this farm for individuals treated only if classified as being in FAMACHA© 4 and 5, or with a haematocrit of ≤15 %, are given in Table 3.7a and b. For a positive diagnosis of sheep in FAMACHA© categories 3–5, 86 % of sheep would have been correctly treated at a haematocrit cut-off of ≤22 % (Table 3.7a) while 88 % would have been correctly treated at a haematocrit cut-off of ≤19 % (Table 3.7b). Sensitivity, specificity, positive predictive value, negative predictive value and prevalence data are listed in Table 3.8. FAMACHA© scores versus assigned and observed median haematocrit values for Farm 2 are given in Table 3.9 and the percentage

of observed haematocrit values falling within the assigned ranges are shown in table 3.10. Sensitivity was highest for a cut-off of ≤19 % at 0.80, while specificity was highest for a cut-off of ≤22 % (Table 3.8). The observed median haematocrit values were much closer to their assigned values than was the case on Farm 1 (Table 3.9). The accuracy of FAMACHA© classification was highest for FAMACHA© category 1, with 78 % of observed haematocrit values falling within the assigned range (Table 3.10).

TABLE 3.7a Farm 2. Haematocrit cut-off value is ≤22 %. Results of two-way frequency tables of haematocrit by FAMACHA© score. Percentage of total is given in parentheses for rams with assigned ranges in haematocrit values, which are based on drenching of sheep with haematocrit ≤15 %. FAMACHA© categories 1–2 were considered test negative.

Haematocrit value	False negatives	False positives	Treatment correct	Total
≤22 %	73 (9)	-	130 (16.1)	203 (25.2)
>22 %	-	39 (4.8)	564 (70.1)	603 (75.0)
Total	73 (9)	39 (4.8)	694 (86.0)	806 (100)

TABLE 3.7b Farm 2. Haematocrit cut-off value is ≤19 %. Results of two-way frequency tables of haematocrit by FAMACHA© score. Percentage of total is given in parentheses, for rams with assigned ranges in haematocrit values, which are based on drenching of sheep with haematocrit ≤15 %. FAMACHA© categories 1–2 were considered test negative.

Haematocrit value	False negatives	False positives	Treatment correct	Total
≤19 %	22 (2.7)	-	93 (11.5)	115 (14.2)
>19 %	-	76 (9.4)	615 (76.3)	691 (85.7)
Total	22 (2.7)	76 (9.4)	708 (87.8)	806 (100)

TABLE 3.8. Farm 2. Sensitivity (Se), specificity (Sp), predictive value of a positive (Pv+), predictive value of a negative (Pv-) and prevalence (P) for trial data for given haematocrit cut-off values and proposed treatment of sheep in FAMACHA© categories 3–5. The value for prevalence was calculated from standard two-way frequency tables. FAMACHA© categories 1–2 were considered test negative.

Haematocrit value	Se	Sp	Pv +	Pv -	P	Confidence interval (95 %)
≤22 %	0.64	0.93	0.77	0.88	0.25	(0.227 – 0.273)
≤19 %	0.80	0.89	0.55	0.96	0.14	(0.116 – 0.164)

TABLE 3.9 Farm 2. FAMACHA© score of rams vs. haematocrit: assigned values, observed values and percentiles (n = 806). FAMACHA© category 5 not represented.

FAMACHA© score	Assigned median value of haematocrit range (%)	Observed median haematocrit value (trial data) (%)	Fifth percentile of observed haematocrit values	Ninety-fifth percentile of observed haematocrit values
1	30	33	23.7	40.8
2	25	26	17.4	36.3
3	20	19.5	12.6	28.3
4	15	16.5	12.5	21.2
5	10	-	-	-

TABLE 3.10. Farm 2. FAMACHA© categories, sample size, assigned haematocrit range and percentage of observed haematocrit values within the assigned range for rams. FAMACHA© category 5 not represented.

FAMACHA category	n	Assigned haematocrit range of FAMACHA© category	Percentage of observed haematocrit values within assigned range
1	365	≥28 %	78 %
2	272	23 – 27 %	40 %
3	134	18 – 22 %	39 %
4	35	13 –17 %	57 %
5	-	≤12 %	-

3.4 Discussion

The work presented here is based primarily on the clinical evaluation data collected from two farms in South Africa. For this reason it was essential to evaluate the accuracy of the clinical data in relation to the haematocrit values used for validating the on-farm use of the FAMACHA© system. In this work, the FAMACHA© diagnostic test was compared with its associated haematocrit values for the two farms where the data were gathered, and sensitivity, specificity, positive predictive values, and negative predictive values were calculated for different haematocrit cut-off values. In order to establish the sensitivity and specificity of a diagnostic test, it is important to decide which test values, or range of values, will be used to indicate a test positive individual (Thrusfield 2001). If there is a significant penalty, such as death, or severe production loss, for failing to detect a test positive individual, then it is important that test sensitivity should be maximised. Within the context of the FAMACHA© system of targeted selective treatment, this would mean that it is essential to have a test that has a high probability of correctly classifying individuals which are truly anaemic and require treatment to avoid death. This is of crucial importance both for ethical reasons and for the economic success of farmers applying the FAMACHA© system. Lowering of test sensitivity will progressively lead to increased numbers of false negatives, which are then not identified as sheep that need treatment, leading to production losses.

Farm 1

One potential drawback of maximising test sensitivity with decreasing prevalence of infection is that a large number of false positive animals are treated. However, this is relatively unimportant in relation to either selection for worm resistance or to financial implications. For instance, despite the relative inaccuracy of FAMACHA© classification on Farm 1, a maximum of 49.5 % of the animals would have been treated, which would have included true positives and false positives under all test criteria (Table 3.1a and Table 3.3a). This compares favourably with blanket treatment systems, where all animals are continually treated both before and during a given worm season. In this series of trials, only sheep scored into FAMACHA© categories 3, 4 and 5 were treated, apart from the blanket drenching events described, and when a realistic haematocrit of ≤19 % was used as a cut-off, only 58 % of sheep that were anaemic were treated (Table 3.2). If a lower haematocrit of ≤15 % were to be used as a cut-off, 83 % of sheep that were truly anaemic would have been treated, but this could potentially be catastrophic to the producer, since the remaining 17 % of sheep with a haematocrit of an already low value of ≤15 % would be in danger of succumbing to haemonchosis. It has been shown that a haematocrit drop of 7 percentage points could occur in as many days, leading to rapid death from terminal anaemia (Malan *et a*l. 2001), and for this reason, a haematocrit cutoff of ≤15 % would be unrealistic for Farm 1 in the present case. A haematocrit cut-off value of ≤19 % would therefore carry less risk under this treatment option. However, if sheep in FAMACHA© category 2 were treated in addition to FAMACHA© categories 3, 4 and 5 in this series of trials, and with a haematocrit cut-off of ≤19 %, then 93 % of sheep that were anaemic would have been detected and treated (Table 3.4). This represents a dramatic improvement over the actual situation where only 58 % of anaemic sheep with a haematocrit of ≤19 % were detected and treated. Even though 33.6 % of the total would have been treated as false positives if FAMACHA© categories 2–5 were treated (Table 3.3b), the total proportion of the animals recommended for treatment would still only have comprised a maximum of only 59 % of the flock. This would almost certainly maintain a sufficient level of refugia for large-scale reduction in selection for anthelmintic resistance while maintaining an acceptable level of parasite control for the producer.

The results from Farm 1 indicate that a high degree of misclassification occurred on the farm (Tables 3.5 and 3.6). Several reasons have been advocated for FAMACHA© misclassification. Among these are (i) wrong interpretation due to complacency and over-

confidence in estimating anaemia score without reference to the FAMACHA© card for calibration, (ii) infrequent examination, for example during the worm off-season from May to October, with resultant loss of prowess and (iii) infrequent replacement of the FAMACHA© card, the colours of which are prone to fade with age or if exposed to direct sunlight for prolonged periods.

Although it is not clear which of the above reasons or combinations of reasons are most likely to be responsible for the observed misclassification, it is clear that the observed median haematocrit values for FAMACHA© categories 1–4 were consistently lower than the expected values (Table 3.5), and that for all but FAMACHA© category 5, only a small fraction of the observed haematocrit values fell within the expected range (Table 3.6). Of all sheep represented, only 98 individuals (14.5 %) were truly in FAMACHA© category 1, leading to the conclusion that the flock was always more anaemic than what was being indicated by FAMACHA©. One possible reason for the low numbers of "healthy" sheep in FAMACHA© category 1 could be that the farmer, even during the peak of the worm season, averaged 21 days between FAMACHA© evaluations, while intervals of seven days are prescribed at the peak of the worm season. This probably resulted in the flock being much more anaemic than he was actually aware of, since the cumulative effect of worm challenge was being masked by FAMACHA© misclassification. The occurrence of a truly non-anaemic sheep in FAMACHA© category 1 during a sample would thus have been a rare event. However, as indicated in Table 3.5, it is evident that if consideration is given to the fact that these were the results of clinical evaluation compared to the laboratory determined haematocrit values over a period of five years, the percentage of deviation from the median values per FAMACHA© category of 1–4 were within the relatively narrow range of 22–26 % (Table 3.5). This indicates that although the FAMACHA© evaluations were relatively constant over the five years, they were at too low a haematocrit level throughout. The sole exception was FAMACHA© category 5, but there were only 3 sheep in this category.

The consistency of the FAMACHA© evaluation on Farm 1 was further supported by Best Linear Unbiased Prediction (BLUP) heritability analysis performed on the data collected at the height of the worm challenge during the FAMACHA© trials on the farm, made possible by the complete genealogy data that were available for the sheep in the trials (Van Wyk & Bath 2002). Every year over the trial period, almost identical heritabilities were recorded for both FAMACHA© score and haematocrit, every time at a level slightly higher than that of the heritability of the faecal worm egg counts done at the same time (Van Wyk & Bath 2002).

Albers *et al*. (1987) reported that host resistance to *H. contortus* infection as measured on the basis of faecal worm egg counts and haematocrit is a moderately heritable trait, and Barger & Dash (1987) demonstrated that, when individuals are evaluated for faecal worm egg counts and haematocrit, the same individuals tend to have the lowest haematocrit and the highest faecal worm egg counts at each evaluation. It thus seems likely that the consistent differences between the clinical FAMACHA© test and its associated haematocrit values could have been rectified by re-training evaluators at an early stage, had this been detected early enough. It is an indication that the ideal would be to evaluate the success of the FAMACHA© evaluation when a person has been applying the system for a few months after the initial training. Furthermore, it emphasizes the necessity of at least basic training of FAMACHA© evaluation and supports the decision not to allow dispersal of the FAMACHA© system without adequate training (Van Wyk & Bath 2002).

The most important finding of this study for Farm 1 is that when dosing only FAMACHA© categories 3, 4 and 5, sensitivity was highest with a haematocrit cut-off of ≤15 % (Table 3.2), and that even then it was only 83 %. A better sensitivity would have resulted if FAMACHA© categories 2, 3, 4 and 5 were treated, with a haematocrit cut-off of ≤19 %, because a sheep with a haematocrit of this value is not in immediate danger of dying unless conditions of severe pasture contamination or nutritional challenge are present. Although Kaplan *et al*. (2004) do not discuss the issue of misclassification, it would appear from their results that their observed median haematocrit values after evaluation of 847 sheep were considerably higher than assigned median values, as evidenced by box and whisper plots demonstrating the relationship between haematocrit value and FAMACHA© scores in sheep. However, data from their study was collected from a total of 39 farms in the southern United States, and involved a large number of different evaluators as well as different breeds and ages of sheep. This is in contrast to the results of the present study on Farm 1 over a five-year period, where animals were scored by the same person, and where observed median haematocrit values were lower than expected (Table 3.5). Since validation trials are continuing on the farm, it is also imperative that as a first step to correcting misclassification, the farmer is at least informed that FAMACHA© category 2 should be included in the drench as well, until the error can be rectified. Calibration of the FAMACHA© scoring procedure on the farm should then be carried out to point out anomalies in his classification process, and re-familiarization with FAMACHA© should be carried out.

Farm 2

The results for Farm 2, where only sheep scored as FAMACHA© 4 or 5, or if their haematocrits were ≤15 % were treated, indicated that application of the FAMACHA© scoring process was more accurate than on Farm 1 (Table 3.9). These sheep were scored mainly by one investigator, with the exception of the first three evaluations in the first year of trials, when FAMACHA© classifications were the combined observations of himself and 1–3 other persons. The lowest accuracy of FAMACHA© classification was obtained for FAMACHA© category 3 on this farm, where 39 % of sheep that were scored into FAMACHA© category 3 had haematocrit values in the assigned range of 18–22 % (Table 3.10), compared to 78 % for FAMACHA© category 1, and the 40 % that were correct for FAMACHA© category 2. A relatively high proportion of sheep scored as being in FAMACHA© category 4 (57 %) was correctly classified compared to Farm 1 (Table 3.6). On Farm 2, FAMACHA© category 5 was not represented in any of the samples. A factor which may have played a role in comparing the two farms is that of all the sheep sampled for haematocrit determination in addition to FAMACHA© scoring on Farm 2, 401 individuals (50 %) were truly in FAMACHA© category 1, with a haematocrit of ≥28 %, compared to only 98 individuals, or 14.5 % on Farm 1. The general level of anaemia was thus lower for sheep on Farm 2 than for Farm 1, as evidenced by these figures. This could further indicate that the higher accuracy of FAMACHA© classification, in addition to much more regular examination of the flock, was the reason that sheep in FAMACHA© category 5 were not encountered on this farm. Epidemiological differences between the two farms, however, would have been important in their own right. Salvage treatments, where blanket drenching of all sheep in a sample was undertaken, was not required on Farm 2 as was the case on Farm 1, despite the fact that a much lower threshold of treatment, i.e. a haematocrit of ≤15 %, was used on Farm 2. Sensitivity on Farm 2 for a haematocrit cut-off of ≤19 % was 80 % if sheep in FAMACHA© categories 3–5 were considered to be test positive (Table 3.8), which represents an improvement of 22 % (i.e. 80 % - 58 %) over the sensitivity obtained on Farm 1 (Table 3.2) for the same set of parameters. Under these conditions, a total of only 21 % of the flock would have been treated if all sheep in FAMACHA© categories 3–5 were treated on Farm 2. If all animals in FAMACHA© 2 were also regarded as diseased, then sensitivity would have increased to 98 % for a haematocrit cut-off of ≤19 %, but specificity would have been low at 52 %, and still only 55 % of the flock would have been treated due to the perpetually high proportion of the flock in the “healthy” FAMACHA© categories 1 and 2.

Since there were no sheep in FAMACHA© category 5 on Farm 2, and also because of the much lower prevalence of disease for equivalent cut-off values and proportions of animals considered to be diseased, a general recommendation for Farm 2 to treat only sheep in FAMACHA© categories 3–5 would have allowed a high level of safety from overwhelming haemonchosis, while still leaving a large proportion of the flock untreated. If this had been done, it is likely that the labour inputs required for FAMACHA© application could have been reduced by enabling increased intervals between evaluations. The recommendation made for Farm 1, in contrast, was that all animals in FAMACHA© categories 2–5 should be treated, and if this drenching regime had been applied on Farm 2, considerable numbers of false positive sheep would have been unnecessarily have been drenched.

3.5 Conclusion

The present results suggest that, as long as the sensitivity of the diagnosis is high enough to avoid non-treatment of a proportion of truly anaemic sheep, production losses should be minimised. This is important, as with the FAMACHA© system, non-treatment of a false negative animal could lead to death, whereas it is acceptable to treat false positive sheep, as long as a considerable proportion of the flock is left untreated (Van Wyk 2001, 2002). The fact that FAMACHA© has a resolution of five different categories, allows wide scope to adjust the sensitivity of diagnosis, and as seen in this study on Farm 1, immediate corrective action can be implemented by simply adjusting the treatment to include the “next up” FAMACHA© category of sheep, without necessarily leading to “excessive drenching” as regards the sustainability of the worm management programme. Correct classification is preferable to corrective action, but the implication is that calibration should take place at least annually on farms where the FAMACHA© system is in use.

The present analyses add further confirmation to previous inputs into validation of FAMACHA© as part of the present paradigm towards employment of targeted selective treatment for sustainable helminth control, as reviewed by Van Wyk & Bath (2002). Similar analyses to those reported here have been conducted by Vatta *et al.* (2001) and Kaplan *et al*. (2004), and all have demonstrated the practicability of on-farm application of FAMACHA© by farmers, without the need for routine laboratory intervention. The results of this study suggest that (i) the sensitivity of the FAMACHA© diagnostic system should be evaluated at more regular intervals to avoid production losses due to misclassification bias; (ii) that calibration of the FAMACHA© scoring process in terms of training is essential, and (iii) that

animals should be examined at least weekly during periods of the highest worm challenge as with previous recommendations (Van Wyk & Bath 2002).

CHAPTER 4

Use of Receiver Operating Characteristic curves for selection of treatment thresholds using the FAMACHA© diagnostic system for anaemia in sheep naturally infected with *Haemonchus contortus*

4.1 Introduction

The purpose of this part of the study was to further compare the diagnostic accuracy of FAMACHA© classification on Farms 1 and 2 in South Africa using Receiver Operating Characteristic curve analysis. The principles and practical application of Receiver Operating Characteristic curve analysis for diagnostic tests have been reviewed by Greiner & Gardner (2000). Receiver Operating Characteristic plots provide an index of accuracy for a diagnostic test by demonstrating the limits of a test's ability to discriminate between states of health such as diseased/not diseased or infected/not infected, over the range of operating conditions for the test (Zweig & Campbell 1993).

Receiver Operating Characteristic curve analysis was used to compare the discriminating ability of the FAMACHA© diagnostic test on the above two farms by selecting two cut-off values for the haematocrit reference test, namely ≤22 % and ≤19 %, to indicate the true presence or absence of disease in four separate sets of analyses. The area under the Receiver Operating Characteristic curve (area under the curve) was calculated for the two haematocrit cut-off values and for data from both farms using STATA (Stata Statistical Software: Release 8.0. College Station, TX: StataCorp LP). Sheep on each farm were regarded as diseased if the haematocrit was ≤22 % or ≤19 %. The cut-off value of ≤22 % was chosen because it is the upper limit for FAMACHA© category 3, and this category is recommended as a treatment threshold during the application of the FAMACHA© system. The cut-off value of ≤19 % was included in the analysis to provide an additional view of the data, and to determine whether there was a difference between the two cut-off values in the diagnostic power of the FAMACHA© method. In Chapter 3, the statistical techniques of sensitivity and specificity, positive and negative likelihood ratios and prevalence of infection were used to define the accuracy of the FAMACHA© method on the two farms. In the present study a distinction is made between the term "cut-off" which is used to classify the true disease status of an animal according to a preselected haematocrit value into true

diseased/non-diseased, and "cut point", which refers to dichotomized FAMACHA© test results. The term "cut point" is also used in the STATA statistical software to designate the FAMACHA© categories as rating points (Hanley & McNeil 1982), used to calculate the area under the curve index values. For example, if all individuals in FAMACHA© categories 2–5 are considered to be test positive, then the cut point is 2, and if all individuals in FAMACHA© categories 3–5 are considered test positive, the cut point is 3, etc. The diagnostic sensitivity and specificity of a test are a function of the cut points of the test, and Receiver Operating Characteristic analysis assesses the diagnostic performance of the system in terms of sensitivity and 1 minus specificity for each possible cut point of the test. A further refinement of Receiver Operating Characteristic analysis, the two-graph Receiver Operating Characteristic curve method (Greiner, Sohr & Göbel 1995; Beck, Gašpar, Mihaljević, Marinculić, Stojčević & Brstilo 2005; Greiner, Pfeiffer & Smith 2000), was used to optimise the selection of FAMACHA© cut points for anthelmintic treatment. This method was used to plot the sensitivity and specificity as a function of the FAMACHA© cut point to maximise sensitivity under the assumptions that the disease occurs at a relatively high prevalence, as almost every animal is infected, but due to over-dispersion of worm burdens (Wilson *et al.* 1996; Herbert & Isham 2000) a few individuals harbour the majority of the parasites, and that non-detection of a truly diseased animal for treatment has a more serious consequence than the incorrect treatment of a non-diseased animal.

The aim of this work was to determine whether there would be differences in the discriminatory performance in the application of the FAMACHA© method for the two pre-determined haematocrit cut-off values and for FAMACHA© cut points of 2 and 3 for the two farms, and to select optimum FAMACHA© cut points for treatment by taking into account the relative consequences of false negative and false positive test results.

4.2 Materials and methods

4.2.1 Origin of data and FAMACHA© test procedures

The data analysed in this work consisted of anaemia status as evaluated by FAMACHA© scores and haematocrit values, collected from naturally infected sheep on the two farms in the summer rainfall area of South Africa. The origin of the data and FAMACHA© test procedures have been described in detail in Chapter 3.

4.2.2 Receiver Operating Characteristic analysis

The area under the Receiver Operating Characteristic curve, which is a summary statistic of the overall diagnostic accuracy and thus the discriminatory power of each diagnostic test (Greiner *et al*. 2000), was non-parametrically calculated for each haematocrit cut-off value and for data from each farm, using STATA. The same software was used to calculate sensitivity, specificity and likelihood ratios using the rating method (Hanley & McNiel 1982). In the present study the results of the haematocrit determination were used as the "gold standard" to validate the FAMACHA© method for two levels of infection on the two farms, namely either FAMACHA© categories 2–5 regarded as test positive, or FAMACHA© categories 3–5 regarded as test positive. The points required to produce the Receiver Operating Characteristic curve were obtained by successively considering increasingly broader categories of abnormal test results, for example by considering FAMACHA© category 5 alone as abnormal, then FAMACHA© category 5 plus 4, then FAMACHA© category 5 plus 4 plus 3, etc. Two-graph Receiver Operating Characteristic analysis was then used to select cut points for treatment for a pre-defined sensitivity of 0.90, i.e. the cut point was selected to ensure that a minimum of 90 % of animals defined as truly diseased by the FAMACHA© cut points would be detected and treated. In all analyses, the selected haematocrit cut-off values of ≤22 % and ≤19 % were used as the reference variable, while the proportional observed FAMACHA© scores were used as the classification variable in the STATA software.

4.3 Results

The performance of FAMACHA© as measured by the area under the curve for both farms is illustrated in Figs. 4.1a and 4.1b. The results of the rating method to obtain the points for the Receiver Operating Characteristic curve for Farm 1 are given in Tables 4.1 and 4.2, and for Farm 2 in Tables 4.4 and 4.5. The area under the curve index values and standard errors for Farm 1 are given in Table 4.3 and for Farm 2 in Table 4.6. The area under the curve was closest to 1 at 0.90 (0.877–0.920) on Farm 2 for a haematocrit cut-off of ≤19 % (Table 4.6 and Fig. 4.1b). The smallest area under the curve index value was obtained for a haematocrit cut-off of ≤22 % on Farm 1 (0.790), while the highest was obtained for Farm 2 for a haematocrit cut-off of ≤19 % (0.901). The results of the two-graph Receiver Operating Characteristic analysis for both farms are given in Figs. 4.2a–d. For farm 1, the FAMACHA© cut points for a sensitivity of 0.9 were FAMACHA© category 2 and category 3 for haematocrit

cut-offs of ≤22 % and ≤19 %, respectively (Fig. 4.2a and b), and for Farm 2 the FAMACHA© cut points for a sensitivity of 0.9 were FAMACHA© 3 for both haematocrit cut-offs (Fig. 4.2c and d).

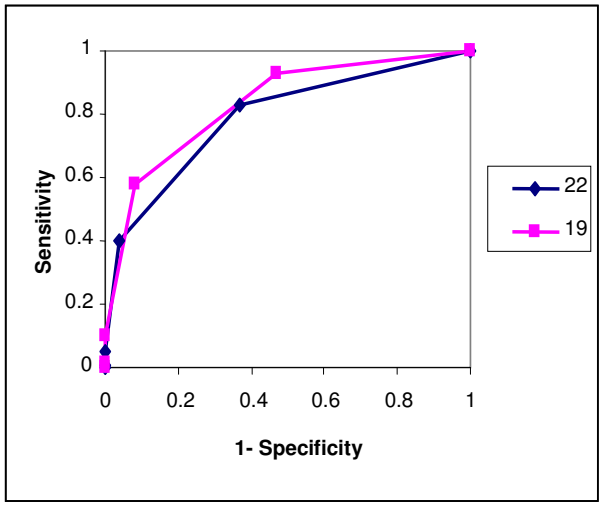


FIG. 4.1a Farm 1. Receiver Operating Characteristic curves. The area under the curve for a haematocrit cut-off of ≤22 % is 0.790, and for ≤19 % it is 0.835

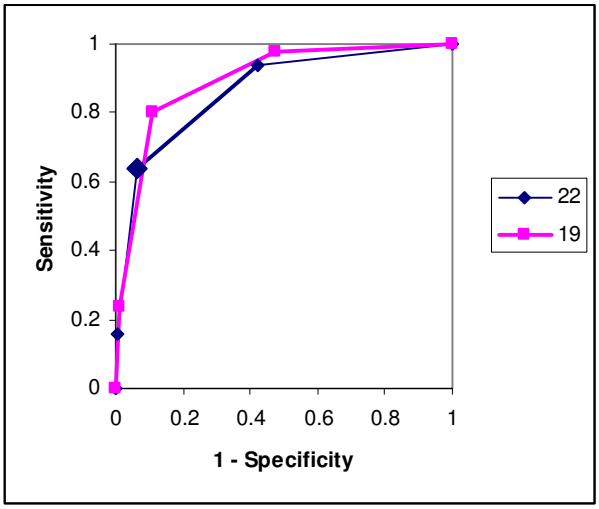


FIG. 4.1b Farm 2. Receiver Operating Characteristic curves. The area under the curve for a haematocrit cut-off of ≤22 % is 0.867, and for ≤19 % it is 0.901

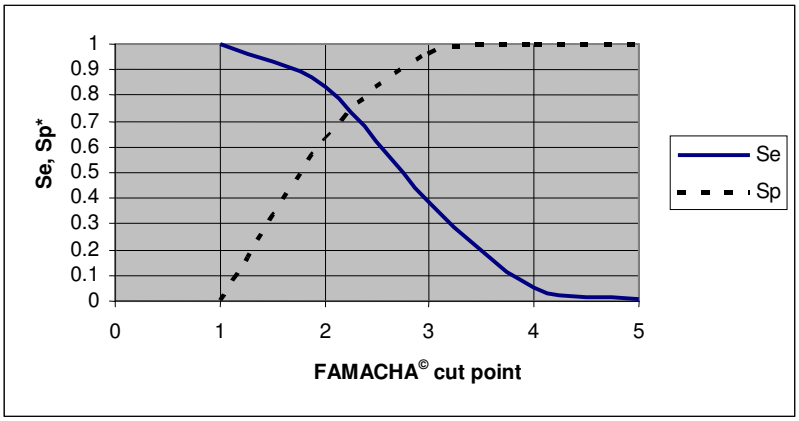


FIG. 4.2a Farm 1. Two-graph Receiver Operating Characteristic plot for a haematocrit cut-off of ≤22 %. *Se = sensitivity, Sp = specificity.

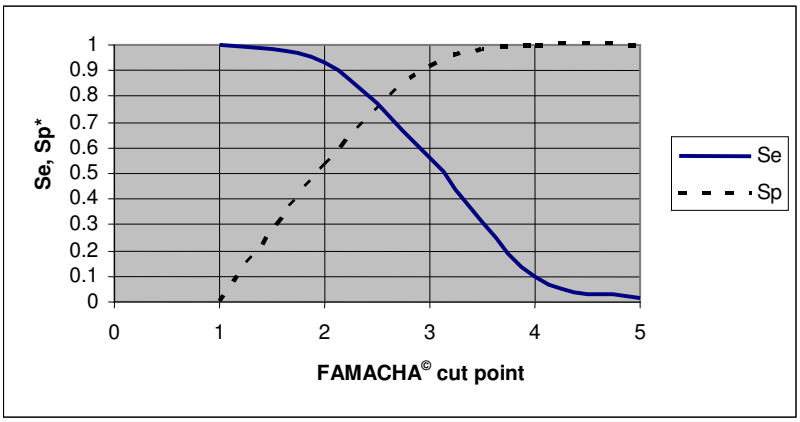


FIG. 4.2b Farm 1. Two-graph Receiver Operating Characteristic plot for a haematocrit cut-off of ≤19 %. *Se = sensitivity, Se = specificity.

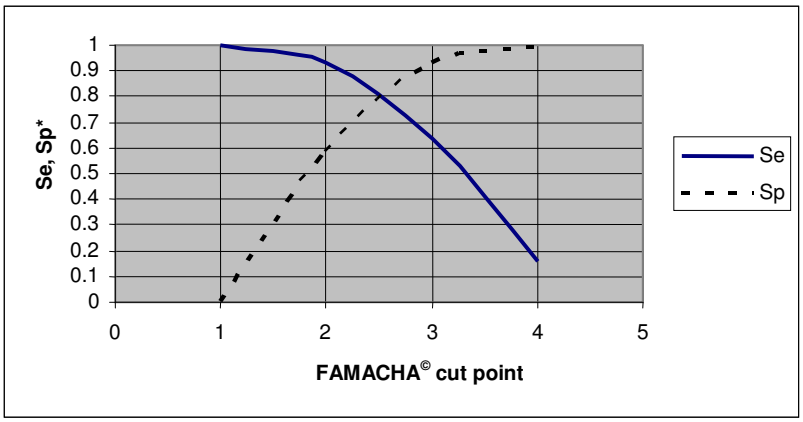


FIG. 4.2c Farm 2. Two-graph Receiver Operating Characteristic plot for a haematocrit cut-off of ≤22 %. FAMACHA© category 5 not represented. *Se = sensitivity, Sp = specificity.

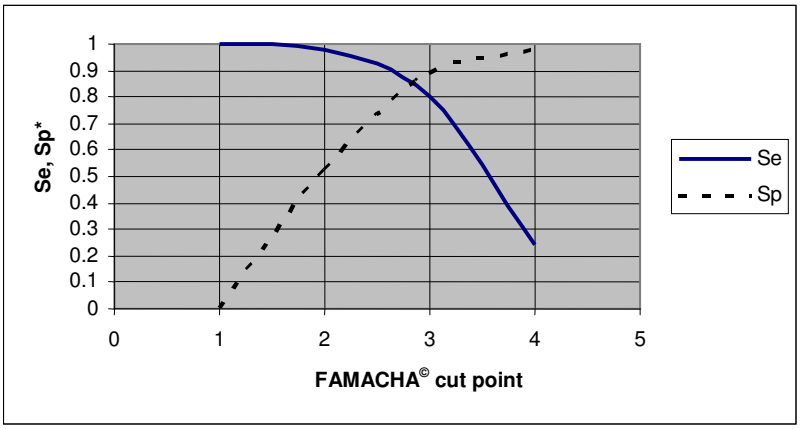


FIG. 4.2d Farm 2. Two-graph Receiver Operating Characteristic plot for a haematocrit cut-off of ≤19 %. FAMACHA© category 5 not represented. *Se = sensitivity, Sp = specificity.

TABLE 4.1 Farm 1. Haematocrit cut-off is ≤22 %. Results of the rating method for FAMACHA© cut point, sensitivity, specificity, percentage of sheep correctly classified, and likelihood ratios (LR).

FAMACHA© Cut point	Sensitivity	Specificity	Classified correctly	LR+	LR-
(≥1)	100.00 %	0.00 %	49.41 %	1.0000	-
(≥2)	83.18 %	63.05 %	73.00 %	2.2512	0.2667
(≥3)	39.94 %	96.19 %	68.40 %	10.4766	0.6244
(≥4)	5.71 %	100.00 %	53.41 %	0.9429	-
(≥5)	0.90 %	100.00 %	51.04 %	0.9910	-
(>5)	0.00 %	100.00 %	50.59 %	1.0000	-

TABLE 4.2 Farm 1. Haematocrit cut-off is ≤19 %. Results of the rating method for FAMACHA© cut point, sensitivity, specificity, percentage of sheep correctly classified and likelihood ratios (LR).

FAMACHA© Cut point	Sensitivity	Specificity	Classified correctly	LR+	LR-
(≥1)	100.00 %	0.00 %	27.89 %	1.0000	-
(≥2)	93.09 %	53.09 %	64.24 %	1.9842	0.1303
(≥3)	57.98 %	92.39 %	82.79 %	7.6156	0.4548
(≥4)	10.11 %	100.00 %	74.93 %	0.8989	-
(≥5)	1.60 %	100.00 %	72.55 %	0.9840	-
(>5)	0.00 %	100.00 %	72.11 %	1.0000	-

TABLE 4.3 Farm 1. Haematocrit cut-off, area under Receiver Operating Characteristic curve, standard error (SE) of the area under the curve and confidence limits (CL) of the area under the curve.

Haematocrit cut-off	n	Area under the curve	SE	Lower 95 % CL	Upper 95 % CL
≤22 %	674	0.7902	0.0159	0.75811	0.82092
≤19 %	674	0.8353	0.0161	0.80511	0.86254

TABLE 4.4 Farm 2. Haematocrit cut-off is ≤22 %. Results of the rating method for FAMACHA© cut point, sensitivity, specificity, percentage of sheep correctly classified and likelihood ratios (LR). FAMACHA© category 5 not represented.

FAMACHA© Cut point	Sensitivity	Specificity	Classified correctly	LR+	LR-
(≥1)	100.00 %	0.00 %	25.19 %	1.0000	-
(≥2)	93.60 %	58.37 %	67.25 %	2.2485	0.1097
(≥3)	64.04 %	93.53 %	86.10 %	9.9015	0.3845
(≥4)	16.26 %	99.67 %	78.66 %	49.0127	0.8402
(>4)	0.00 %	100.00 %	74.81 %	1.0000	-

TABLE 4.5 Farm 2. Haematocrit cut-off is ≤19 %. Results of the rating method for FAMACHA© cut point, sensitivity, specificity, percentage of sheep correctly classified and likelihood ratios (LR). FAMACHA© category 5 not represented.

FAMACHA© Cut point	Sensitivity	Specificity	Classified correctly	LR+	LR-
(≥1)	100.00 %	0.00 %	14.27 %	1.0000	-
(≥2)	98.26 %	52.53 %	59.06 %	2.0701	0.0331
(≥3)	80.87 %	89.00 %	87.84 %	7.3527	0.2149
(≥4)	24.35 %	98.99 %	88.34 %	24.0348	0.7643
(>4)	0.00 %	100.00 %	85.73 %	1.0000	-

TABLE 4.6 Farm 2. Haematocrit cut-off, area under Receiver Operating Characteristic curve, standard error (SE) of the area under the curve and confidence limits (CL) of the area under the curve.

Haematocrit cut-off	n	Area under the curve	SE	Lower 95 % CL	Upper 95 % CL
22 %	806	0.8671	0.0141	0.84185	0.88991
19 %	806	0.9012	0.0135	0.87799	0.92051

4.4 Discussion

An important part of the validation process of the FAMACHA© system has been to investigate the accuracy of the clinical evaluation in relation to the haematocrit gold standard used (Van Wyk *et al*. 2001a; Kaplan *et al.* 2004). The Receiver Operating Characteristic curve, as used in the analysis of the sensitivity of the FAMACHA© system, is a graphical representation of the trade-off between the rates of false negatives and false positives for every possible cut point, and thus represents the trade-offs between sensitivity (Se) and specificity (Sp). Conventionally, the Receiver Operating Characteristic curve plot depicts 1 minus specificity (1-Sp) on the X-axis and sensitivity (Se) on the Y-axis. The area under the Receiver Operating Characteristic curve is a quantitative measure of how rapidly the curve rises to the upper left corner of the plot. The closer the area is to 1, the better the diagnostic test, since, if an area of 1 were to be obtained for the area under the curve, it would imply that the test is perfectly able to discriminate between healthy and diseased individuals, as Se and Sp would both be 100 %. If, on the other hand, the area under the curve is 0.5 or

close to 0.5, then the test would have the same discriminatory power as the binomial probability obtained by tossing a coin to determine the infection status of an animal. In practice, useful diagnostic tests will have an area between these two extremes, that is between perfect discrimination (area under the curve = 1) and no discrimination (area under the curve = 0.5) (Greiner *et al*. 2000).

In this work the rating method was used to calculate the area under the curve (Hanley & McNeil 1982). The first rows in Tables 4.1 and 4.2, and Tables 4.4 and 4.5 represent the extreme case where all individuals are classified as test positive, or alternatively stated, that all individuals in FAMACHA© categories 1–5 are regarded as test positive. This would lead to a situation where there would be a 100 % true positive rate among the truly diseased, and implies that Se = 1, but that there would also be a 100 % false positive rate among individuals which are truly non-diseased with Sp = 0. The last row in the above mentioned tables represents the other extreme where all individuals are classified as test negative, but in this case, there is a 0 % true positive rate among the truly diseased (Se = 0) and a 0 % false positive rate among the truly non-diseased (Sp = 1). At a practical level, if an individual is always regarded as test positive or test negative, it would mean that test results are ignored or no testing has taken place. An example of the first instance where all individuals are always regarded as test positive is commonly encountered in conventional blanket drenching systems when all individuals are treated at any given time. The diagnostic Se and Sp are always a function of the selected cut point of the test (Greiner *et al*. 2000).

The smallest area under the curve was calculated for Farm 1 data (0.79), for a haematocrit cut-off of ≤22 %, while the largest was for Farm 2 (0.90) for a haematocrit cut-off of ≤19 %. All area under curve values obtained in this study were much larger than 0.5, indicating that the discriminating power for the FAMACHA© test was good for both of the haematocrit cut-off values for FAMACHA© data from both farms. On Farm 1 the area under the curve for both haematocrit cut-off values (Table 4.3) was lower than for the same cut-off values on Farm 2 (Table 4.6). However, the accuracy of FAMACHA© classification was also lower on Farm 1 than on Farm 2. All calculated under-curve areas fell within the moderately accurate range of 0.7<area under the curve<0.9 (Greiner *et al*. 2000), while the area under the curve for Farm 2 was highly accurate at a value of 0.9 (Table 4.6) for a cut-off of ≤19 %. The smallest area under the curve was for Farm 1 (0.79), and this value is higher than the accepted value for a moderately accurate test. Thus, the smallest calculated probability in this study, that a randomly drawn individual that is truly diseased as defined by the

haematocrit cut-off value has a higher haematocrit value than a randomly drawn non-diseased individual is 0.79 (Table 4.3; Farm 1), while the highest probability was 0.9 (Table 4.6; Farm 2). Both of the haematocrit values used as cut-offs in this study therefore yielded a moderate to high probability of discriminating between diseased and non-diseased individuals, as defined by their respective under-curve areas.

Kaplan *et al*. (2004), in the southern United States, reported an Se of 100 % in sheep when FAMACHA© categories 3, 4 and 5 were considered test positive with a haematocrit cut-off of ≤15 %. However, they also found that Se decreased to 92 % if the haematocrit cut-off was increased to ≤19 %. Bath *et al.* (2001) found that in goats farmed under resource-poor conditions in South Africa an Se of 80 % was obtained when FAMACHA© categories 3–5 were considered test positive, and recommended that the treatment threshold under these conditions should be FAMACHA© category 3.

In the present work a low proportion (1.9 %) of false negatives for Farm 1 was obtained for drenching sheep in FAMACHA© categories 2–5, with a haematocrit cut-off of ≤19 % (Table 3.3b; Chapter 3). These results are clearly supported by the two-graph Receiver Operating Characteristic plots for Farm 1 (Fig. 4.2a and b), where it can be read directly off the plot that 0.9<Se<1 for a FAMACHA© cut point of 2 and a haematocrit cut-off of ≤22 %, and for a haematocrit cut-off of ≤19 % the same Se could be obtained for a FAMACHA© cut point of 3. Taking into account the findings in Chapter 3, where it was recommended that all sheep in FAMACHA© categories 2–5 be treated, the results of the two-graph Receiver Operating Characteristic analysis for Farm 1 indicate that an Se of at least 90 % would be obtained for a haematocrit cut-off of ≤22 %. Thus, in the diagnostic context of the use of the FAMACHA© system, it would be realistic to specify an Se of at least 90 % in order to ensure that at least 90 % of truly diseased individuals are detected and treated for the defined cut-off values. The selected Se value of 90 % could have been higher, but it was selected as a realistic value for the sheep on the farms concerned. The Se value, however, will vary according to the FAMACHA© cut points chosen as treatment thresholds, as well as according to the haematocrit cut-off values selected as the reference variables for the test. The maximum accuracy for a given haematocrit cut-off value can be read directly off the two-graph Receiver Operating Characteristic graphs in Fig. 4.2 a–d and following this approach, the FAMACHA© cut point value where the two curves cross for all analyses is at FAMACHA© 3, indicating that all sheep in FAMACHA© categories 3–5 should be treated under the assumption that maximum accuracy should be used to select FAMACHA© cut points (Fig.

4.2 a–d). However, simply maximizing the accuracy, defined as the point where the Se and Sp curves intersect (Greiner & Gardner 2000) does not reflect the epidemiological risk situation for FAMACHA© implementation. The relative consequences of false negative test results are potentially much more serious than those for false positive diagnoses due to the selective nature of FAMACHA© treatment (Bath *et al*. 2001).

The main goal for FAMACHA© implementation should be to maximise Se while still leaving a large proportion of the flock undrenched to maintain a proportion of the parasite population in refugia (Van Wyk 2001). For Farm 1 Se was maximised at the pre-selected value of 0.9 at a FAMACHA© cut point of 2 and a haematocrit cut-off of ≤22 % (Fig. 4.2a), while for a haematocrit cut-off of ≤19 %, an Se of 0.9 was achieved at a FAMACHA© cut point of 3 (Fig. 4.2b). However, because the accuracy of FAMACHA© diagnosis was low on Farm 1 it is recommended that unless the primary problem of misclassification can be overcome through re-training of the evaluator on the farm, a FAMACHA© cut point of 2 be selected together with a haematocrit cut-off of ≤22 %. This will ensure that at the more conservative cut-off of ≤22 %, 90 % of sheep with a haematocrit of ≤22 % will be detected as diseased and treated, while the total proportion of the animals recommended for treatment would still only comprise a maximum of (278+124)/675, or 59 % of the flock. This can be observed from Table 3.3a, where there were 278 true positives and 124 false positives among the 402 individuals classified into FAMACHA© categories 2–5, and furthermore, only 56/675 or 8 % were false negatives for a haematocrit cut-off of ≤22 % if FAMACHA© categories 2–5 were to be treated. These findings are further supported by the likelihood ratio of a positive test result (LR+), which is a prevalence-independent combined measure of Se and Sp that is a representation of the odds of the pre-test and post-test probability of disease, which in turn is conditional to a positive test result (Greiner & Gardner 2000). For Farm 1 the LR+ was higher for a haematocrit cut-off of ≤22 % and a FAMACHA© cut point of 2 (Table 4.1) at 2.25, than for a haematocrit cut-off of ≤19 % where the LR+ was 1.98 for the same FAMACHA© cut point (Table 4.2). This also means that the total area under the Receiver Operating Characteristic curve for a FAMACHA© cut point of 2 is larger for a haematocrit cut-off of ≤22 % than for a cut-off of ≤19 %, with a resulting higher probability of discrimination between diseased and non-diseased individuals. These two-graph Receiver Operating Characteristic results support the findings in Chapter 3, but the increased refinement of two-graph Receiver Operating Characteristic analysis indicated that, because of the high proportion of misclassified sheep on Farm 1, a FAMACHA© cut point of 2 is the

most appropriate treatment threshold.

For Farm 2 Se was maximised at the pre-selected value of 0.9 for a FAMACHA© cut point of 3 for both haematocrit cut-off values of ≤22 % and ≤19 % (Figs. 4.2c and d). However, because of the higher accuracy of FAMACHA© evaluation on Farm 2 compared to Farm 1, the proportion of sheep classified correctly according to FAMACHA© on Farm 2 was much higher than that achieved on Farm 1 (Chapter 3). The results of the two-graph Receiver Operating Characteristic analyses for Farm 2 indicated that a FAMACHA© cut point of 3 could be therefore be recommended for both haematocrit cut-off values of ≤22 % and ≤19 % (Fig. 4.2c and d). The LR+ for Farm 2 was 9.9 and 7.3 for the haematocrit cut-offs of ≤22 % and ≤19 %, respectively, for a FAMACHA© cut point of 3 (Tables 4.4 and 4.5). This value is much higher than the LR+ for a FAMACHA© cut point of 2, at 2.2 and 2.07, for the haematocrit cut-offs of ≤22 % and ≤19 %, respectively, indicating a higher cumulative area under the curve for a FAMACHA© cut point of 3 than for a cut point of 2 on Farm 2, and therefore also a higher degree of discrimination between diseased and non-diseased individuals. Further support is also provided for the recommendation that a FAMACHA© cut point of 3 would be safe to implement on Farm 2.

4.5 Conclusion

The calculation of the area under the curve for the FAMACHA© system for haematocrit cut-off values of ≤22 % and ≤19 % indicated that the diagnostic accuracy of the FAMACHA© system was moderate to high, indicating 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 index values ranged from a minimum 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 selection of suitable FAMACHA© cut points as thresholds for anthelmintic treatment, where it is assumed that Se must have a certain minimum selected value, could readily be achieved with two-graph Receiver Operating Characteristic curve analysis. On Farm 1 a FAMACHA© cut point of 2 and a haematocrit cut-off of ≤22 % was found to be epidemiologically most suitable for a selected Se of 90 %. On Farm 2, where the risk of disease was lower, a FAMACHA© cut point of 3 and a haematocrit cut-off of ≤19 % could be safely recommended for a required Se of 90 %.

The results of this work show that implementing the FAMACHA© system with haematocrit cut-off values of ≤22 % and ≤19 % is sensitive and adequately specific and that these values can safely be used to monitor haemonchosis in sheep on the two farms.

CHAPTER 5

A stochastic model to estimate worm burdens and associated risk factors in sheep naturally infected with *Haemonchus contortus*

5.1 Introduction

Risk can be defined as the possibility of loss or injury coupled to the probability of such loss or injury (Singh 2000), and the main components of risk analysis are risk assessment, risk management and risk communication (Thrusfield 2001). Quantitative risk assessment is defined as a mathematical model containing inputs and outputs which are expressed numerically (Murray 2004). The work in the present chapter is concerned with the risk assessment part of risk analysis, as it is concerned with hazard identification, and estimation of the probability and magnitude of the risk of haemonchosis as indicated by simulating worm counts in sheep infected with *H. contortus*.

Several input parameters may influence the outcome of a given risk assessment, which may be deterministic or stochastic in nature. Deterministically, a single point estimate for each input variable in a biological risk assessment model will lead to a single output estimate, which does not reflect the inherent natural variability contained in the input parameter. With stochastic risk assessment, model input parameters are represented by distribution functions, in which the variability contained in the parameter is approximated (Vose 1998; 2000). The specified input distribution for a parameter thus ensures that for each value of the parameter drawn by Monte Carlo (random) sampling from the input distribution, there will be an associated probability that the parameter will assume this value. Each re-calculation of such a model is called an iteration, and in each iteration a value is randomly drawn from the specified input distribution to give a result for a given iteration. The result of many iterations of the model, where values are randomly selected from a specified input distribution is thus also a distribution, which provides a range of probabilities for the output of the simulation (Vose 2000). In the present investigation, a previously published deterministic linear regression model from the literature (Roberts & Swan 1982) was used as the basis of a stochastic simulation model by allowing model inputs to vary according to the statistical distributions fitted to input parameters by @Risk software (Palisade Corporation). This model was chosen as it uses both haemoglobin and body mass to estimate worm count, and could readily be applied to the data that was available to the author. All simulations of the

regression model were undertaken using Monte Carlo sampling, and each simulation consisted of 10 000 iterations of the model.

5.2 Materials and methods

5.2.1 Origin of data and the model system

The sheep from which the data used to develop the following model originated from part of a series of trials on Farm 1 to estimate the heritability of FAMACHA© classification (Van Wyk & Bath 2002). The data analysed with the model consisted of anaemia status of Merino sheep, as evaluated by FAMACHA© score (Bath *et al*. 1996, 2001), haematocrit values, and body mass data, collected from naturally infected sheep in the summer rainfall area of South Africa. The origin of the data and FAMACHA© testing procedures have been described in detail in Chapter 3. In this chapter it is described how data emanating from Farm 1 was used to simulate worm counts, and thus the risk of haemonchosis, in groups of sampled sheep. The same model was used to simulate worm burdens for two classes of sheep on the farm, namely young replacement ewes (EWEREP), which annually replace aging ewes in the flock, and rams of similar age (RAMREP), with approximately 130 and 200 sheep per sample, respectively. Although ten data sets, which included five consecutive years (2001–2005) of data for the EWEREP class, and five years for the RAMREP class were analysed with the model, the analyses for the RAMREP and EWEREP sheep for the 2001/2002 season were considered as typical for the progression of the disease on Farm 1, and are therefore discussed in detail.

For simplicity, the model outputs are depicted as summary graphs across the range of sampling events. The graphs summarise the changes in output distributions across time by taking five parameters from each distribution – the mean, and two upper and two lower band values. The changes in these five parameters are graphed across the sample output range. The two upper band values represent the 80th and the 95th percentile value of each distribution, while the two lower band values represent the 20th and the 5th percentile value of each distribution. The summary graph thus shows the trends in model output in terms of simulated worm burden, from one sample to the next. The wider the spread of the distribution about the mean, the larger the variability in probable worm count, and thus also the higher the risk of disease (Vose 2000).

5.2.2 Statistical analysis

Data from both classes of sheep were pooled per year to examine the relationship between FAMACHA© scores and haematocrit values on the farm (Chapter 3). The relationship between haematocrit value and the FAMACHA© score for Farm 1 was determined and compared to assigned values (Chapter 3), and @Risk was used to determine the distribution of observed haematocrit values for each FAMACHA© category. For the observed haematocrit values for FAMACHA© categories 1–5, the mean, 5th percentile, 95th percentile, and standard deviation were calculated and tabulated against their ordinated FAMACHA© scores. The mean and standard deviation of the observed haematocrits for each FAMACHA© category, as fitted by the distribution fitting function in @Risk, were used to describe a Normal distribution function from which the mean haemoglobin value of a sample was simulated.

The stochastic model

The multiple regression model of Roberts & Swan (1982) was used to estimate the risk of haemonchosis, based on the mean haemoglobin levels and body mass of sheep. Multiple regression analysis of the original model indicated that log worm count and haemoglobin are predictable from the model, but body mass was not predictable from either log worm count or haemoglobin. The model allows the estimation of the worm burden of an animal by taking its body mass and haemoglobin level into account according to Equation (1):

*Log Worm Count = (Body mass * 0.0168) + (Haemoglobin * -0.20706) +3.8936……..(1)*

A typographical error in the original published model caused the constant term for body mass to be reported incorrectly as 0.0.68, and back substitution of data supplied in the article was used to re-calculate the value for the constant (D. Berkvens, personal communication 2006). The corrected body mass constant was thereafter validated with data included in the original publication of Roberts & Swan (1982). The correct value was calculated to be 0.0168, and when this value was substituted into Equation (1) for a sheep weighing 20kg and a having a haemoglobin level of 10.5 g/dl, a worm count of 111.77 was obtained, compared to an almost identical value of 112 reported by Roberts & Swan (1982). This corrected mass constant was used in all subsequent analyses.

The variables in the model were entered as distribution functions according to those generated by "Bestfit" version 4.5 (Palisade Corporation) in the @Risk software. The distribution for body mass was modelled with a Normal distribution, which was among the best fitting distribution functions fitted to the body mass data by the distribution fitting function in @Risk version 4.5. The Normal distribution is commonly used to describe variability in body mass. In the stochastic model, the Normal distribution for body mass was entered into the model with its arguments, i.e. the mean and the standard deviation of the sampled body mass data. The resulting Normal distribution for body mass is given in Fig. 5.1. In the sample in Fig. 5.1, 90 % of sheep had body mass values between 26.11kg and 45.70kg.

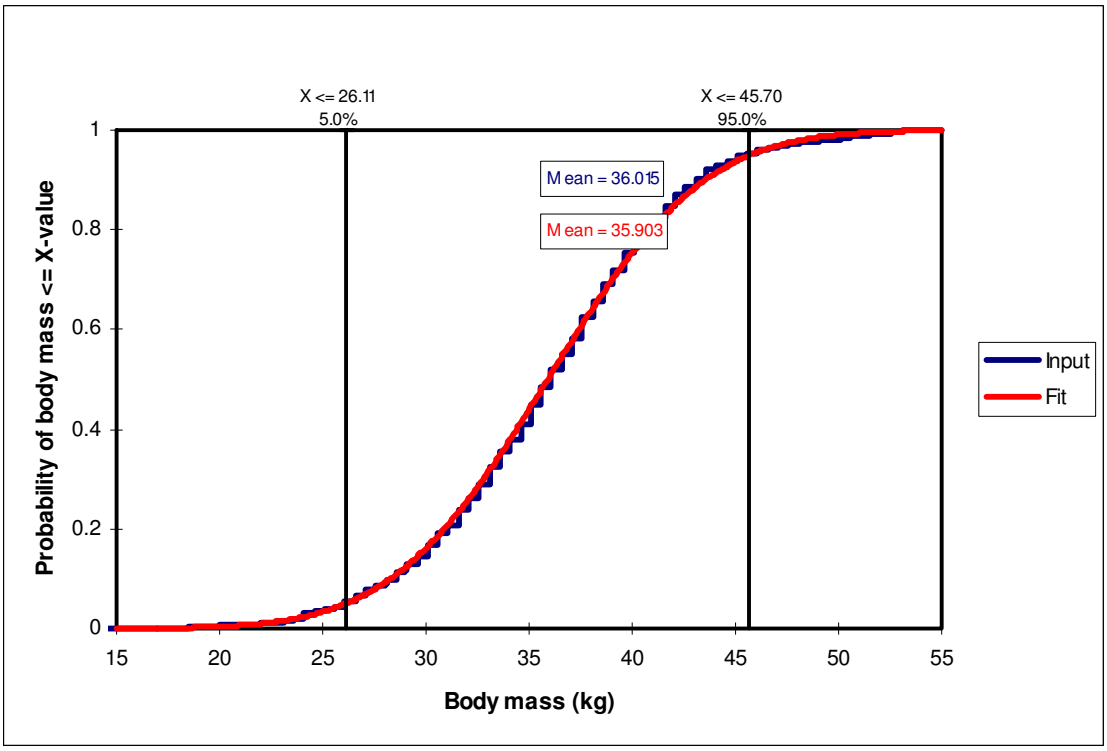


FIG. 5.1 Cumulative distribution function for the body mass of a sample of 179 RAMREP sheep on Farm 1 for the 2000/2001 *Haemonchus* season. The stepped blue line represents the observed body mass values in the sample and the red line represents the @Risk-fitted Normal (35.90,3.65) distribution.

Equation (1), which forms the basis of the model, required that the input blood parameter for anaemia, i.e. the FAMACHA© score, be in the form of blood haemoglobin level expressed in g/dl (Roberts & Swan 1982). In the light of the highly significant correlation between haematocrit and haemoglobin on the one hand (Stockham & Scott 2002), and the

FAMACHA© value with haematocrit on the other (Van Wyk & Bath 2002), the haematocrit values were converted to haemoglobin values. @Risk was used to determine the distribution of haematocrit values for each FAMACHA© category, and haematocrit values for FAMACHA© categories 1–4 were found to be normally distributed within each category. FAMACHA© category 5 (n = 3) had too few data values for @Risk to define a distribution, and the mean and standard deviation for this category were therefore calculated, but were entered into the model in the form of an assumed Normal distribution.

The ordinated FAMACHA© scores were initially converted to haematocrit values by allowing each of the intermediate FAMACHA© categories 2, 3 and 4 to represent their most likely corresponding haematocrit value, defined as the median of the five haematocrit percentage points represented by each category. Although this approach would be appropriate for FAMACHA© classification where the accuracy of anaemia estimation was high, the data used in the model emanated from Farm 1, where misclassification of sheep into FAMACHA© categories occurred (Chapter 3). A Normal distribution function was fitted to the observed haematocrit values for each FAMACHA© category (Table 5.1).

TABLE 5.1 Farm 1. FAMACHA© score vs. haematocrit: assigned mean haematocrit values, fitted mean values, and percentiles and standard deviations of the fitted Normal distribution for haematocrits of 675 sheep of both sexes from 2000-2005.

FAMACHA© score	n	Assigned mean value of haematocrit range	Fitted mean, @Risk Normal distribution (trial data)	Fifth percentile of haematocrit	Ninety-fifth percentile of haematocrit	Standard deviation
1	272	30	25.1	19.7	30.5	3.27
2	258	25	19.5	15.9	27.2	3.45
3	126	20	15	10.6	23.9	4.02
4	16	15	11	6.5	18.7	3.71
5	3	10	10	8.6	11.5	1.03

The mean haematocrit value for FAMACHA© category 2, which represents an assigned haematocrit range of 23 %–27 %, was set to the fitted mean of its observed range at 19.5 %; category 3 (18–22 % assigned haematocrit range) was set to 15 %, and category 4 (13–17 %) was set to 11 %. Category 1, with a haematocrit range of >27 %, and category 5, with a range of <13 %, were set at their fitted mean values of 25 % and 10 % for the data from

Farm 1, respectively (Table 5.1).

The conversion to mean haemoglobin level per FAMACHA© category was initially effected by dividing each fitted mean haematocrit value by 3, since the haemoglobin value is typically one-third of the haematocrit (Hall & Malia 1984; Jain 1993; Stockham & Scott 2002) with the normocytic anaemia due to blood loss which is characteristic of *H. contortus* infections (Owen 1968). However, the mean corpuscular haemoglobin concentration in most blood samples is in the range of 32–36 g/dl (Stockham & Scott 2002). Therefore, the assumption made above that the mean corpuscular haemoglobin concentration is relatively constant at 33.3 g/dl, and that the conversion to haemoglobin content follows a simple linear trend according to the equation

*Haemoglobin (g/dl) = 33.3 * haematocrit %.....(2)*

was modified, to include variability in both the mean corpuscular haemoglobin concentration and the observed haematocrit values. The FAMACHA© categories were therefore dichotomised into two groups with similar upper but differing lower mean corpuscular haemoglobin concentration boundaries for each group. The non-anaemic FAMACHA© categories of 1 and 2 were assigned a lower and upper mean corpuscular haemoglobin concentration value of 32 and 36 g/dl respectively, within the defined normal range, and the more anaemic FAMACHA© categories 3–5 a more depressed lower and normal upper limit of 26 and 36 g/dl respectively, since severely anaemic sheep could reasonably be expected to develop an iron, cobalt and/or copper deficiency that will depress the mean corpuscular haemoglobin concentration to a lower level than the normal range (F. Reyers, personal communication 2006). This range of probable mean corpuscular haemoglobin concentration values was entered into a truncated Uniform (32,36) distribution function for FAMACHA© categories 1 and 2, and a truncated Uniform (26,36) distribution function for FAMACHA© categories 3, 4, and 5. The Uniform distribution was then multiplied by a Normal distribution function based on the fitted mean and standard deviation of the haematocrit data for each FAMACHA© category, to simulate the haemoglobin concentration for the FAMACHA© category. This process is illustrated at the top of Fig. 5.3, where it can be seen that for all animals in FAMACHA© category 1, the mean corpuscular haemoglobin concentration was modelled as Uniform (32,36) and the haematocrit as Normal (25.1,3.27), to give a distribution for the haemoglobin concentration in the relevant FAMACHA© category.

The final distribution for the FAMACHA© variable in each sample was modelled by incorporating the distribution for haemoglobin values for each FAMACHA© category obtained by simulation as described above, into a Discrete distribution function, with the format:

Discrete ({xi},{pi}), i = 1 to n……..(3)

where *x* represents the simulated output distribution for haemoglobin content in each FAMACHA© category present in the sample, and *p* represents the probability of occurrence of the particular category. The Discrete distribution in this instance represents a composite probability distribution of the occurrence of FAMACHA© categories in groups of sampled sheep, incorporating the proportional occurrence of FAMACHA© categories by probabilistic branching (Vose 2000). Any number of points can be specified for the Discrete distribution. Thus, if in a particular sample only two categories of animal were observed, the parameters for the distribution could be set for the proportional occurrence of those two categories; if there were three categories, the third category could be incorporated.

The output of the Discrete distribution function for the haemoglobin value of a sample is given in Fig. 5.2. The histogram in Fig. 5.2 is produced in @Risk by grouping data into several bars or classes and the number of values in any class is the frequency of the class. The approximate probability that the output variable lies within the range of the class is determined by the frequency divided by the total number of values. Note that the number of classes used in a histogram plot will determine the scale of the Y-axis, which means that the wider the bar width, the higher the probability that values will fall within the bar (Vose 2000). A schematic diagram of the simulation model is given in Fig. 5.3. The output of the simulation is obtained by selecting an EXCEL worksheet cell as a simulation output for a given sample and a distribution of possible outcomes is generated for every selected output cell according to variability in the input cells. In the simulation model in Fig. 5.3, the antilogarithm value of the model output was selected to create an output probability distribution for simulated worm burden by Monte Carlo simulation.

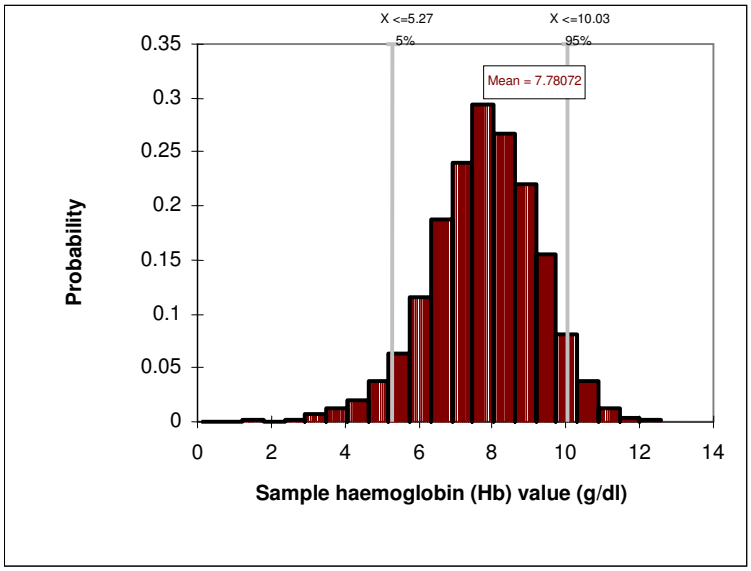


FIG. 5.2 The Discrete distribution for the FAMACHA© variable for a EWEREP sample (n = 133). The mean haemoglobin value was 7.78 g/dl, and 90 % of the simulated haemoglobin values were between 5.27 and 10.03 g/dl.

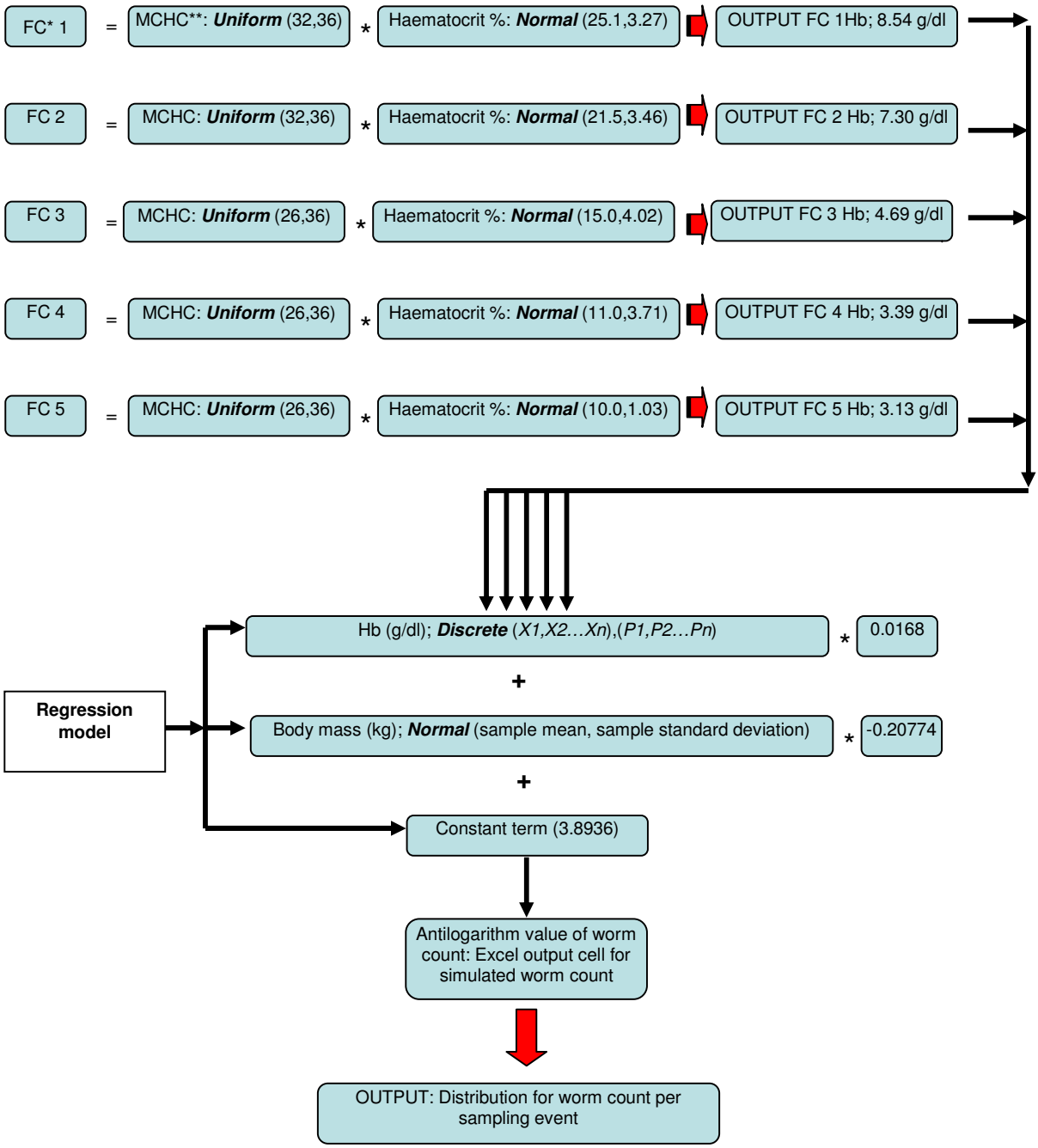


FIG. 5.3 Schematic diagram of the model used to simulate mean worm count of sampled sheep. Fitted statistical distributions are given in bold italicized letters. Bold red arrows indicate Monte Carlo simulated outputs of the model. (*FC = FAMACHA©, ** MCHC = mean corpuscular haemoglobin concentration)

5.3 Results

The model output for the EWEREP class for the 2001/2002 *Haemonchus* season is illustrated in Fig. 5.4a, the percentages of sheep in the different FAMACHA© categories in Fig. 5.4b and a summary of the data input is listed in Table 5.2. Table 5.3 lists the model output in terms of mean worm count, and the 5th and 95th percentile of worm count. Sheep in the EWEREP trial were blanket drenched at the time of the first sampling on 19 November 2001 and again on 7 January 2002. The summary graph, Fig. 5.4a, represents the seasonal trend in the predicted variability of worm burdens, with a predicted major peak of infection in mid-season (January), followed by a lesser peak towards the end of the season (April).

The model output for the RAMREP class is illustrated in Fig. 5.5a, the percentages of sheep in the different FAMACHA© categories in Fig. 5.5b, and the corresponding data of the RAMREP model is given in Tables 5.4 and 5.5. The sheep in the RAMREP trial were blanket drenched on 10 November 2001 and again on 7 January 2002. The seasonal trend in predicted worm counts was similar in both classes of sheep, but the RAMREP class was clinically more apparent as suffering from worm infection as can be seen from the sample on 7 January 2002, where only FAMACHA© categories 1, 2, 3 and 4 were present in the EWEREP class (Table 5.2), whereas FAMACHA© categories 1–5 were present in the RAMREP class (Table 5.4). Additionally, on 7 January 2002, approximately 50 % of the sheep in the EWEREP class were in FAMACHA© category 1, compared to only 5 % of the sheep in the RAMREP class (Figs. 5.4b and 5.5b).

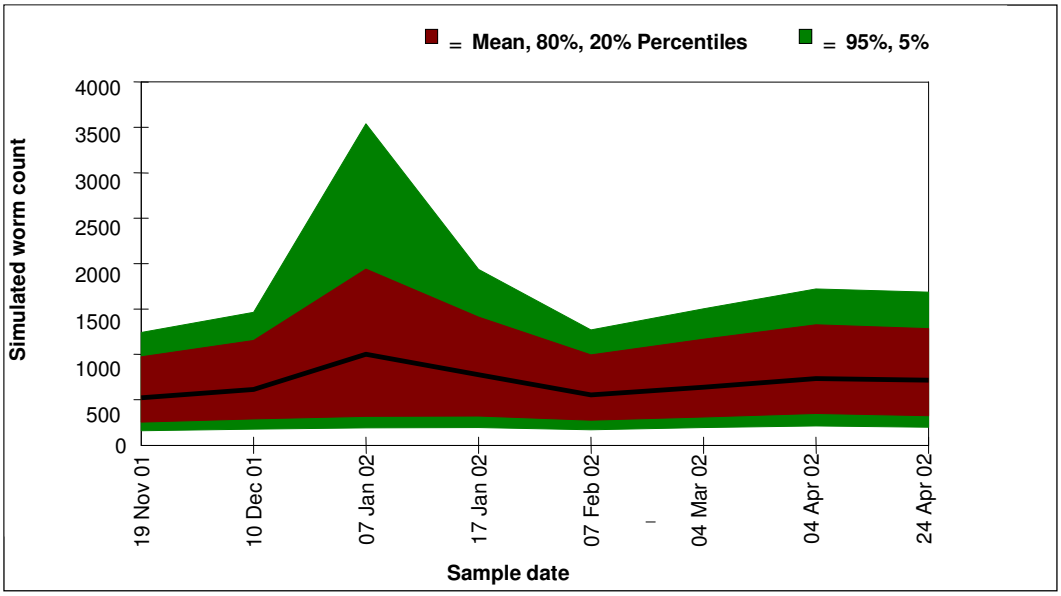


FIG. 5.4a EWEREP (n = 130). Model output for simulated worm count, 2001/2002 season. The black line represents the simulated mean worm count.

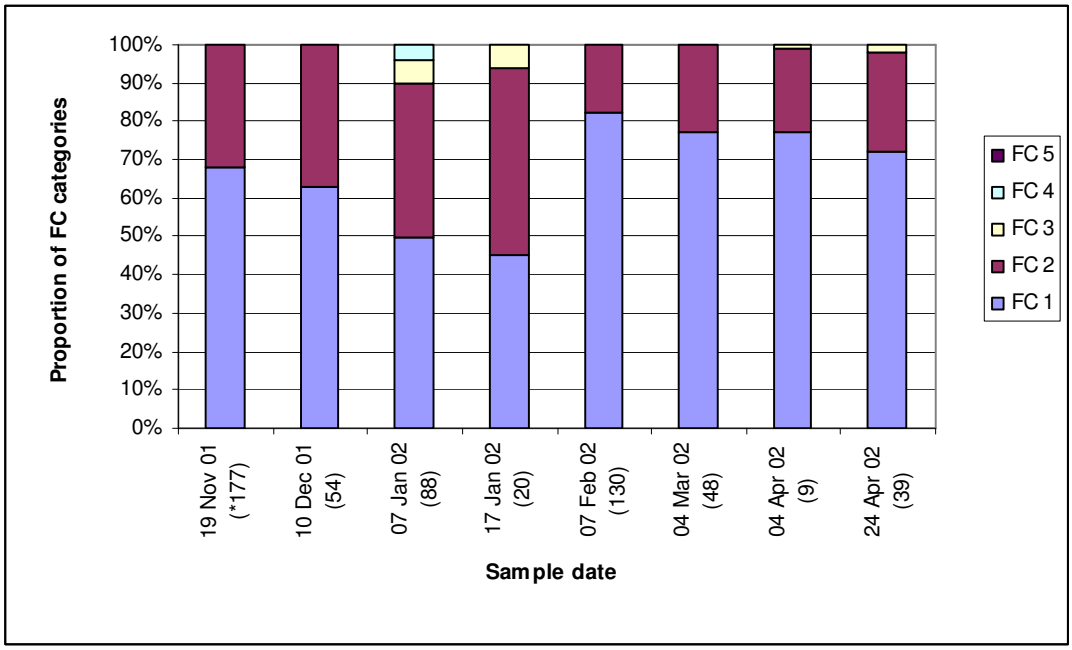


FIG. 5.4b EWEREP: Proportional representation of the FAMACHA© categories per sample. Rainfall between sampling events is given in parentheses, in mm. (*Rainfall for preceding 4 weeks). FC = FAMACHA©.

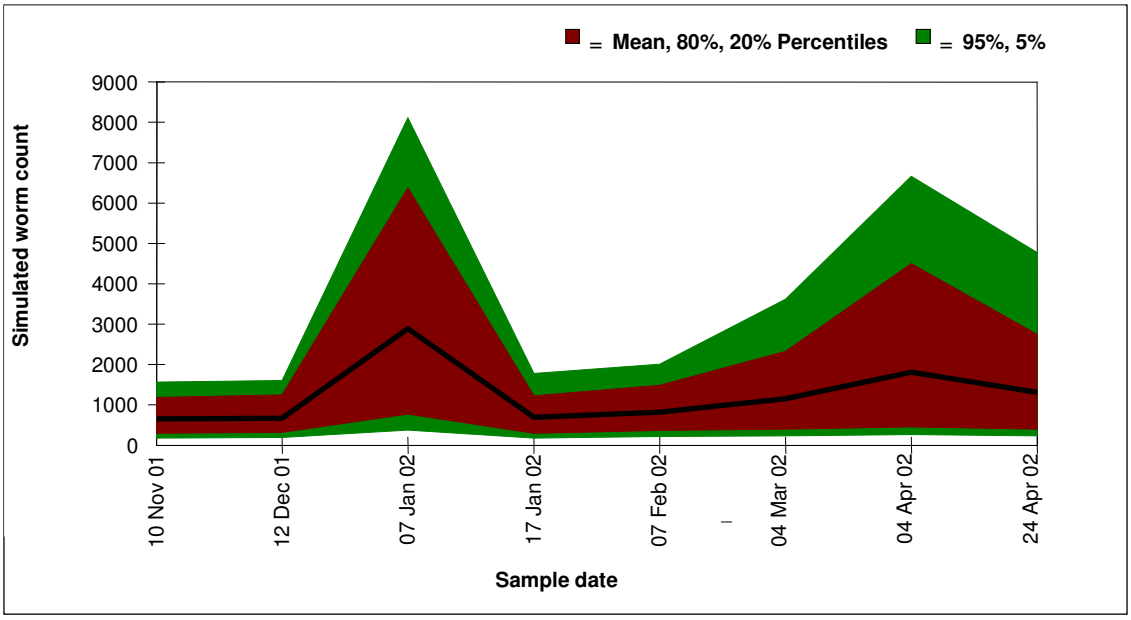


FIG. 5.5a RAMREP (n = 120). Model output for simulated worm count, 2001/2002 season. The black line represents the simulated mean worm count.

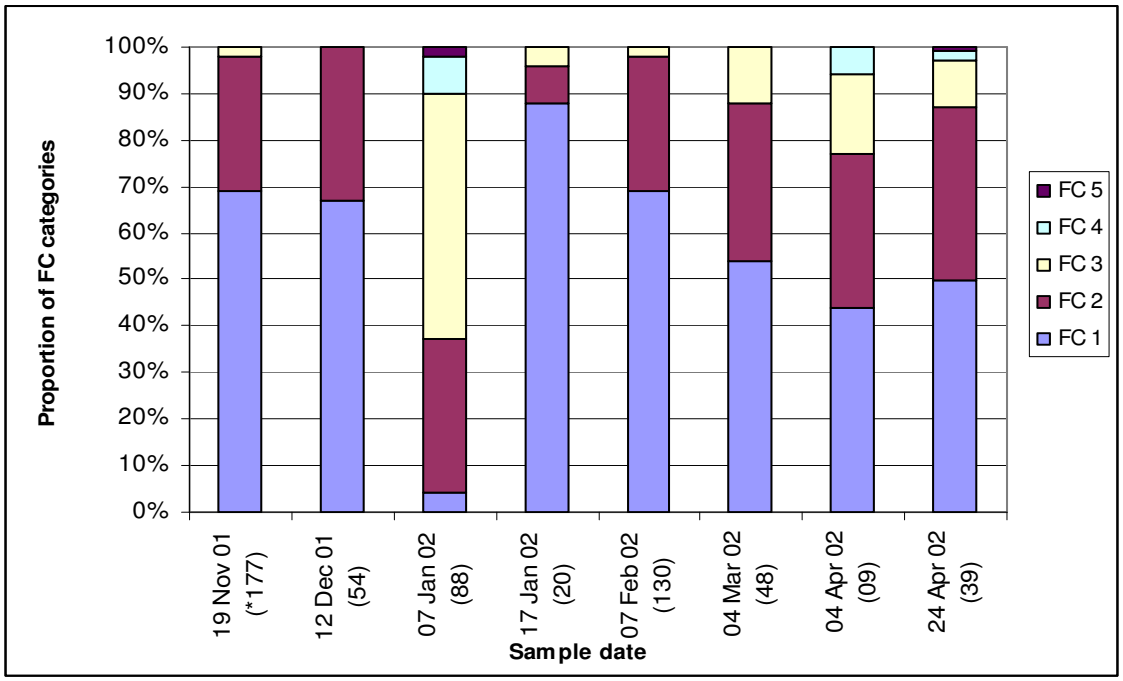


FIG. 5.5b RAMREP: Proportional representation of the FAMACHA© categories per sample. Rainfall between sampling events is given in parentheses, in mm. (*Rainfall for preceding 4 weeks). FC = FAMACHA©.

TABLE 5.2 Table of summarised data input of the EWEREP class, 2001/2002 season into the simulation model.

Sample date	Mean body mass	Standard deviation: body mass	Body mass constant	FAMACHA© categories in sample	Haemoglobin constant	Overall constant
19 Nov	25.7	3.2	0.0168	1,2	-0.20774	3.8936
10 Dec	28.97	3.42	0.0168	1,2	-0.20774	3.8936
07 Jan	28.7	3.38	0.0168	1,2,3,4	-0.20774	3.8936
17 Jan	28.96	3.14	0.0168	1,2,3	-0.20774	3.8936
07 Feb	29.63	3.22	0.0168	1,2	-0.20774	3.8936
04 Mar	32.39	3.36	0.0168	1,2	-0.20774	3.8936
04 Apr	34.84	3.53	0.0168	1,2,3	-0.20774	3.8936
24 Apr	32.36	3.48	0.0168	1,2,3	-0.20774	3.8936

TABLE 5.3 EWEREP class, 2001/2002 season. Fifth, 50th and 95th percentile values of simulated worm count.

Sample date	Fifth percentile: worm count	Fiftieth percentile: worm count	Ninety-fifth percentile: worm count
19 Nov	151	525	1 210
10 Dec	175	615	1 449
07 Jan	188	1 008	3 272
17 Jan	190	778	1 975
07 Feb	169	550	1 239
04 Mar	191	642	1 469
04 Apr	212	727	1 675
24 Apr	196	710	1 693

TABLE 5.4 Table of summarised data input of RAMREP class, 2001/2002 season into the simulation model.

Sample date	Mean body mass	Standard deviation: body mass	Body mass constant	FAMACHA© categories in sample	Haemoglobin constant	Overall constant
10 Nov	30	4.07	0.0168	1,2,3	-0.20774	3.8936
12 Dec	31.4	4.11	0.0168	1,2	-0.20774	3.8936
07 Jan	32.39	4.51	0.0168	1,2,3,4,5	-0.20774	3.8936
17 Jan	31.42	4.99	0.0168	1,2,3	-0.20774	3.8936
07 Feb	35.38	4.23	0.0168	1,2,3	-0.20774	3.8936
04 Mar	35.31	4.68	0.0168	1,2,3	-0.20774	3.8936
04 Apr	36.78	4.55	0.0168	1,2,3,4	-0.20774	3.8936
24 Apr	34.69	4.58	0.0168	1,2,3,4,5	-0.20774	3.8936

TABLE 5.5 RAMREP class, 2001/2002 season. Fifth, 50^{th}, and 95^{th} percentile values of simulated worm count.

Sample date	Fifth percentile: worm count	Fiftieth percentile: worm count	Ninety-fifth percentile: worm count
10 Nov	179	651	1 534
12 Dec	193	662	1 569
07 Jan	378	2 933	8 129
17 Jan	176	681	1 639
07 Feb	221	835	2 019
04 Mar	231	1 131	3 591
04 Apr	266	1 813	6 516
24 Apr	235	1 304	4 722

5.4 Discussion

The main focus of the present model was to use actual data gathered from sampled animals under farming conditions in order to estimate the risk of disease at the time of sampling. A complication of computational models is that with annual diseases such as haemonchosis, identical patterns of disease outbreaks rarely occur, even on farms that are close together (Gettinby 1989). This author further stated that qualitatitive patterns or long-term predictions of disease are of little value at farm level, where weather and management practices can so alter the course of disease that only site specific models can be of any use to estimate the

risk of disease. This was an important reason for the approach in this study to use a model, which simulates levels of worm infection at different points in time at a particular site for tactical intervention, as opposed to forward extrapolation for suggesting strategic prophylactic measures. It is envisaged that the output of the model will be used as an adjunct to the FAMACHA© system, and as such would not be specifically required to address the chronic effects of *Haemonchus* burdens.

The task of the "black box" towards which this study is aimed, will be to integrate the results of the present model with a multitude of other factors discussed in Chapter 2 and also the application of Receiver Operating Characteristic curve analysis, to arrive at a set of outputs, namely specific worm management recommendations at a given time. While the FAMACHA© system is based on and was calibrated according to haematocrit ranges (Van Wyk *et al.* 2001a), it is well established that with the normocytic anaemia (Owen 1968) observed in haemonchosis, there is a high level of correlation between haematocrit and haemoglobin values of sheep. Furthermore, the large variation in mean worm burden of a given flock of sheep over a *Haemonchus* season will more than offset any small degree of variation which may occur between the trends of mean haematocrit and haemoglobin values over this period. Hence it is regarded as valid for application of the Roberts & Swan (1982) model, to convert observed haematocrit percentages to corresponding haemoblobin values.

For both classes of sheep, the model strikingly reflected the changing epidemiological situation as regards *H. contortus* challenge during the course of the worm season, especially in relation to FAMACHA© evaluation (Figs. 5.4 a, b and 5.5 a, b). It is also noteworthy that, while only the results of one of the five years' duration of the trials are presented here, those of the other four are very similar as regards the model reflecting differences in level of *H. contortus* challenge over each worm season.

5.4.1 EWEREP class

In each trial, the risk of haemonchosis was lowest at the start of the season. For instance, sheep were initially dewormed on 19 November 2001, when only FAMACHA© categories 1 and 2 were present in the sample. The risk of disease then increased slowly until the second sample on 10 December 2001, and then sharply to the third sample on 7 January 2002. At which time there was a peak in infection and FAMACHA© categories 1–4 were present. This sharp increase in worm burden, accompanied by a downward shift in the mean haemoglobin

level in the flock, can be partly related to the 177 mm of rain recorded on the farm over a period of 12 days during the four-week period immediately preceding the time of the first sampling event on 19 November 2001. Between 19 November and 10 December, a further 54 mm of rain was recorded over a period of 7 days, followed by 88 mm over a six day period between 10 December 2001 and 7 January 2002. Although there was a general downward trend in the amount of rainfall recorded between the first and the third sample, the initial high amount of rainfall and its spread, which was 177 mm over 12 days, probably laid the foundation for much of the increased risk of disease that was apparent at the time of the third sample.

When the second blanket anthelmintic treatment was administered on 7 January 2002, the proportion of animals in FAMACHA© category 1 was still higher than those in category 2, but FAMACHA© categories 3 and 4 were also present. The effect of the blanket treatment was apparent at the sampling of 17 January 2002 (Fig. 5.4a), when the simulated worm burden decreased sharply, until 07 February 2002, where it again closely approximated the value seen at the first sample date (Fig. 5.4a). The model thus clearly identified both blanket treatments during this season. Simulated worm burdens then continued to rise from the fifth to the seventh samples on 7 February, 4 March, and 4 April 2002, when a second, lesser peak in infection was indicated by the model, concomitant with an increase in the number of FAMACHA© categories per sample. This trend can be explained by relatively high and well-distributed rainfall that fell up to the sixth sample on 04 March 2002, after which less rain fell, followed by a downward trend in simulated mean worm burden after 04 April 2002 (Table 5.3).

From the results of the model it appears that if rainfall, as a major factor in the development of haemonchosis, were to be used as a risk indicator and the risk model applied at some time between the two samples on 10 December 2001 and 7 January 2002, then it would probably have indicated that the incidence of clinical disease was increasing rapidly. The logical step would then have been to drench more liberally, to include sheep in FAMACHA© categories 2–5, as opposed to only treating animals in FAMACHA© categories 3–5. This is in agreement with the findings of the sensitivity and specificity analysis, and also the Receiver Operating Characteristic analysis of Farm 1 data in Chapter 3 and Chapter 4. The latter analysis indicated that, in order to detect and treat 90 % of sheep with a haematocrit of ≤22 % on Farm 1, all sheep in FAMACHA© 2, 3, 4, and 5 should be treated. On the other hand, it should be kept in mind that the FAMACHA© evaluators on Farm 1 were shown in Chapter 4

consistently to have underestimated the true occurrence of anaemia in the trial animals. In other words, primarily animals in FAMACHA© categories 4 and 5 were being treated, while the requirement was for treatment of all sheep in categories 3–5 throughout the year. Had the latter occurred, it seems likely that the levels of worm infection would not have been as high as recorded. If the sheep which were truly in FAMACHA© category 2 had been included and intervals between sampling been shorter, serious worm challenge could perhaps have been averted while still leaving sufficient undrenched sheep, as far as possible to avert selection for anthelmintic resistance. Of paramount importance was the fact that the model functioned well by reflecting fluctuating levels of worm challenge throughout the *Haemonchus* season.

The high level of infection reached on 7 January 2002 was exacerbated by the extended period between samples, which was almost four weeks, with no anthelmintic intervention during this period. The FAMACHA© method requires sheep to be evaluated weekly over a relatively short period during the peak worm season, specifically to avoid this kind of risk (Van Wyk & Bath 2002). It is probable that the magnitude of the large peak in infection on 7 January 2002 could have been reduced using this approach, while at the same time leaving the sheep truly in FAMACHA© categories 1 and 2 untreated, in accordance with the paradigm of selective treatment to maintain sufficient parasites in refugia (Van Wyk 2001).

5.4.2 RAMREP class

The model indicated very similar general trends for RAMREP and EWEREP, although with considerably higher worm burdens for RAMREP. The mid season peak in infection on 7 January 2002 in both classes of sheep, as well as the late season peak in infection, was more severe for RAMREP than for EWEREP classes (Figs. 5.4a and 5.5a). A possible reason for the higher indicated worm burdens in the RAMREP class could be that males of many vertebrate species are known to be more susceptible to parasite infections than females because sex steroids (androgens in males and oestrogens in females) are involved in modulating host immunity (Klein 2000). Sexually mature male vertebrates are often observed to carry higher parasite burdens in the field, due to the fact that sex steroid hormones alter genes that influence susceptibility and resistance to infection (Gauly *et al*. 2002). Androgens are known to reduce immunocompetence in males and sex steroid hormones compromise the effects of disease resistance genes and behaviours, causing males to be more susceptible to parasitic infections (Klein 2000; Gauly *et al*. 2002).

The risk of disease was relatively constant from the first sample on 10 November 2001 to the second on 12 December 2001, after which worm burdens increased sharply until 7 January 2002 (Fig. 5.5a). Simulation with the model indicated low levels of worm infection after blanket anthelmintic treatment at the first sampling occasion on 10 November 2001, and again on 17 January 2002. The sharp increase in simulated worm burden after 12 December 2001 until the first peak of infection on 7 January 2002 can, as in the case of EWEREP, again be attributed to the high and well distributed rainfall, despite the initial blanket treatment on 10 November 2001. After 17 January 2002, the mean and the 95th percentile values of the simulated worm burden increased again, tracking the general upward trend in the amount and spread of rainfall until 4 April 2002. If the model indications are correct, then the RAMREP class was at a higher risk of disease than the EWEREP class throughout the season, even in "low-risk" samples such as the first sample on 10 November 2001, where 70 % of the RAMREP sheep were in FAMACHA© category 1, and the 4th sample on 17 January 2002, where 90 % of sheep sampled were in FAMACHA© category 1 (Fig. 5.5b). This observation is supported by the fact that the model predicted much higher worm burdens for the RAMREP class throughout the season. Rams are usually heavier than ewes, but in the present case the difference in mean body mass was only 2.3 kg on 24 April 2002, although the standard deviation, and thus the variability, of mean body mass was considerably higher for RAMREP than EWEREP. On the other hand, it needs to be kept in mind that, while the two flocks shared pastures in rotation, they never ran together on the same pasture at any given time, with the result that they could have been exposed to different levels of worm challenge. In the other years included in this study, however, the same trend was observed, with RAMREP having higher simulated worm burdens than EWEREP.

For the RAMREP class, all five FAMACHA© categories were present at the last sample, compared to only FAMACHA© 1, 2, and 3 for the EWEREP class. This contributed to the higher simulated worm burdens for the RAMREP class due to more depressed haemoglobin levels in the RAMREP flock.

If the model were to be used deterministically by the input of single point-estimate values rather than distributions, much of the uncertainty pertaining to the variability in worm burdens would be lost, due to the fact that there can only be one output - a single worm count value - for any two single input combinations of haemoglobin and body mass. Thus, the inherent variability in the biological system would not be accounted for. Input parameters

to the model were described as distributions and, consequently, the output of the model for each sample which is simulated is also a distribution of values. Although further work is needed, the preliminary indications are that the model presented here, and the associations between the risk of disease and factors that are associated with it, should allow a rapid initial assessment of the health of a flock when used in conjunction with the main FAMACHA© clinical indicator of haemonchosis. For instance, if it is assumed that a mean worm burden of 1 000 is the maximum tolerable risk level, based on values in Tables 5.6 and 5.7 (the upper limit for chronic infections) (Reinecke 1983) and the midpoint value for moderate infections (Hansen & Perry 1994), and it is desired not to exceed a worm burden of this magnitude, then what haemoglobin level is associated with this worm burden? From a stochastic viewpoint, the additional statement "given the variability in haemoglobin level and body mass in the sample" could be added to the previous sentence.

TABLE 5.6 Relationship between number of *H. contortus*, blood loss and clinical signs of haemonchosis in adult sheep (Reinecke 1983)

Syndrome	***Haemonchus contortus* adults**	**Potential blood loss**	**Clinical signs**
Chronic	100–1 000	5–50 ml/day	Anorexia, but anaemia may not occur
Acute	1 000–10 000	50–200 ml/day	Anaemia, bottle jaw, lethargy
Hyperacute*	10 000–35 000	200–600 ml/day	Anaemia, sudden death

* This syndrome is rare in South Africa; animals die suddenly, often indicated only by severe anaemia and dark faeces

TABLE 5.7 Severity of *H. contortus* infection (Hansen & Perry 1994).

Light	**Moderate**	**Heavy**
1–500	500–1 500	1 500+

A scenario analysis based on the results of the output distributions for both classes of sheep, for the samples with the highest predicted risk of disease on 7 January 2002 (Figs. 5.6a, b), indicated that the minimum haemoglobin level within model iterations resulting in a worm count of ≤1 000 worms for the EWEREP and RAMREP classes was 7.05 g/dl and

7.92 g/dl, respectively. The lower delimiter has been set in @Risk to a value of 1 000 worms in Figs. 5.6a and 5.6b, to determine the percentage of iterations that resulted in ≤1 000 worms, which was found to be 76 % of iterations for the EWEREP class and 27 % for the RAMREP class. These probabilities can be estimated directly from the Y-axis scale in Figs. 5.6a and b for the pre-set threshold of 1 000 worms on the X-axis. Under the assumption that the model is valid, these would be the minimum haemoglobin levels that would have to be maintained for the mean worm counts of the two classes of sheep on the farm to remain under the selected pathogenic threshold of 1 000 worms, indicating that rams had a three-fold higher overall probability of exceeding the threshold of 1 000 worms in the sample. However, the probable mean haemoglobin level for the EWEREP class on 7 January 2002 was 7.6 g/dl, and 5.6 g/dl for the RAMREP class at the same sample date (data not shown). Thus, the EWEREP class, on average, had a higher haemoglobin concentration than was required to maintain the selected worm threshold, implying that on average, the worm burden would be maintained below 1 000, while the RAMREP class had a much lower haemoglobin concentration than was required to maintain an infection threshold of ≤1 000 worms. Furthermore, the model predicted that only about 20 % of the EWEREP class would have had a worm burden of between 1 000 and 3 339, while approximately 68 % of the RAMREP class would have had a worm burden greater than 1 000 but less than 8 459 (Figs. 5.6a and b).

Virtually any infection threshold could be selected, but in practice the threshold selected for disease risk estimation would depend on the susceptibility to worm infection of the class of animal. For instance, in the initial trial for evaluating the use of colour variation of the conjunctivae of sheep for detecting differing levels of anaemia (Malan & Van Wyk 1992, Malan *et al*. 2001), there were marked differences in the drenching requirements of different classes of sheep on common pasture under conditions of severe *Haemonchus* challenge. In their trial, sheep categorized as possibly anaemic according to the colour of the ocular mucous membranes, were treated only once haematocrit determinations returned values of 15 % or less. Of the total of almost 300 ewes on common pasture, only 20 % of dry ewes required one or more anthelmintic treatments over the trial period of five months, compared to 30 % of sheep that were heavily pregnant towards the end of the trial, and 55 % of ewes which had lambed shortly before the trial and had lambs at foot at the peak of the *Haemonchus* season.

It is also apparent from Fig. 5.6a that the probability that an animal in the EWEREP class has more than 3 300 worms is small, and from Fig. 5.6b that an animal in the RAMREP class has more than 8 000 worms is equally small. The simulated upper 5th percentile band value for worm count for EWEREP resulted in ≥3 200 worms, compared to a much higher value of ≥8 100 worms for RAMREP. The probability that an animal sampled from the EWEREP class had ≤1 000 worms was approximately 0.75, while the same probability for the RAMREP class was only 0.27. These probabilities, generated by random sampling from specified input distributions, are typical of the increased usefulness of stochastic models over their deterministic counterparts. A model using random sampling from input distributions could thus be a useful indication of the risk of disease, and the attendant factors that may subsequently be used to ameliorate risk.

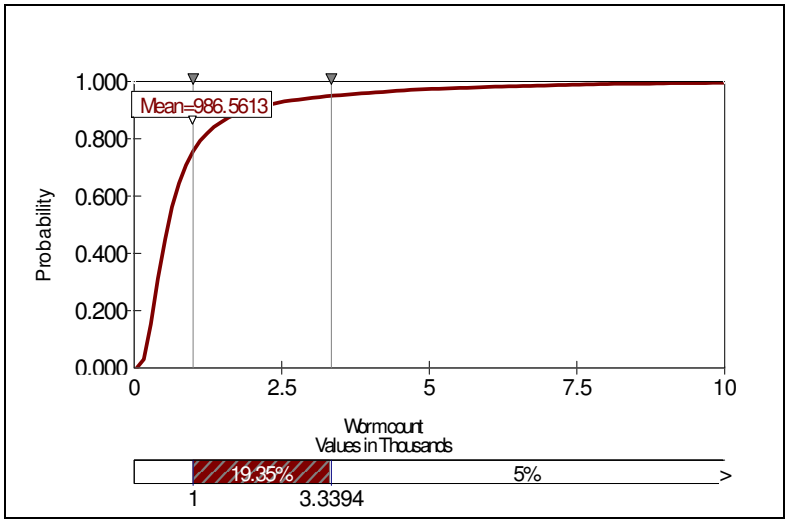


FIG. 5.6a Ascending cumulative output distribution for worm count for the EWEREP class, 7 January 2002. Refer to text.

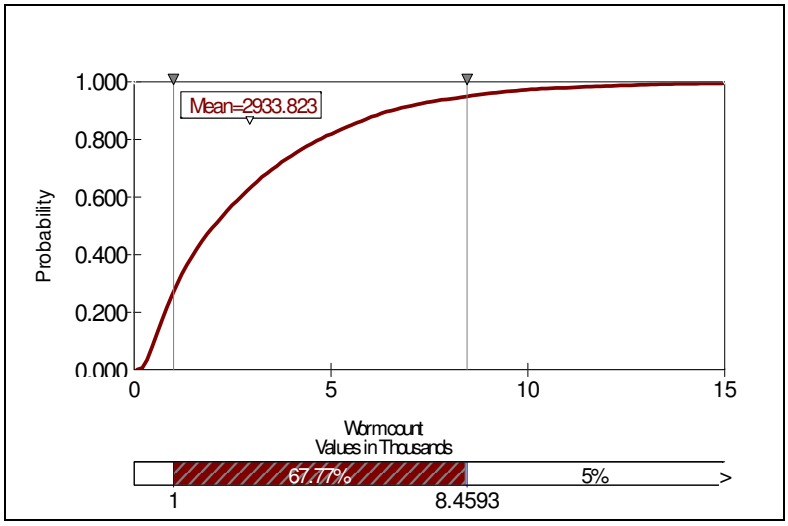


FIG. 5.6b Ascending cumulative output distribution for worm count for the RAMREP class, 7 January 2002. Refer to text.

Furthermore, since each individual animal needs to be evaluated at relatively frequent intervals during the peak worm season to identify and treat the stragglers that are unable to manage unaided, the FAMACHA© system compares favourably in economic and logistical terms with monitoring of faecal worm egg counts. Egg counts in most targeted selective treatment systems are allowed to rise to considerably higher levels than in the conventional strategic drenching approach, to the extent that stragglers are already at risk of production losses and possibly even of dying by the time that they are detected and treated (Van Wyk *et al*. 2001a; Van Wyk & Bath 2002). In the case of *H. contortus* this commonly occurs about a third of the way into the principal worm season. Without intervention, extreme worm burdens can be expected to develop later on in the season, to the extent that even some of the more resistant/resilient individuals will be seriously affected and at risk of severe losses in production. Furthermore, even animals with moderate levels of anaemia can similarly be regarded as at risk, since worm challenge can escalate sharply after a short space of time, with up to a 7 percentage point drop in haematocrit being reported in seven days in exceptional cases (Malan *et al*. 2001).

Within the limits of the trial plan, which included blanket drenching at the peak of worm challenge, the model indications of the simulated risk of disease fitted the generally accepted pattern of the seasonal progression of haemonchosis during most simulations. Thus, the model generally indicated a low risk of disease at the beginning of the season, usually October-December, with a sharp rise in risk of disease after this period, especially if

the period was accompanied by a high preceding amount of well-spread rainfall. From the results of application of the model, it appears that its use should make it possible to estimate the differential risk of disease to different classes of animals at any given point during the worm season, and to act accordingly. More FAMACHA© categories can be drenched if need be under conditions of severe worm challenge, or labour requirements can be reduced by lengthening the periods between evaluations of the animals when the risk of disease is estimated to be low.

A potential disadvantage of the use of the FAMACHA© system generally, and also specifically as it is applied to the present model, is that it will always be important to know the accuracy of FAMACHA© classification on the farm where it is being applied. As was seen in Chapter 3, it is necessary to have an accurate indication of the true mean haematocrit value for a given FAMACHA© category on the farm concerned in order detect and treat truly anaemic animals. However, this should not be a problem, as was the case on Farm 2, where accuracy of FAMACHA© evaluation was high. The model in its present form offers scope to adjust the haematocrit values for a given FAMACHA© category prior to simulation, which is a convenient way to adjust for misclassification. This could be subjectively achieved by the modeller after the fact if there were indications during the FAMACHA© evaluation that a lower than acceptable accuracy was achieved by the evaluators. Alternatively, the system could be calibrated against the FAMACHA© scores obtained from the evaluators at an initial evaluation event by haematocrit determination, and the expected haemoglobin values could then be obtained by simulation as described. This would be a one-off event, as initial calibration should suffice for at least the *Haemonchus* season to follow, or until extension personnel or a veterinarian determined that sufficient grounds exist either to re-calibrate FAMACHA© evaluation or to re-train evaluators.

5.5 Conclusion

The regression model used in this work was found to be valid under the conditions of the farm where the data was collected. The model output was a consistently good fit to the observed trend in FAMACHA© proportions and the attendant variability in body mass of sheep through a given worm season. This was not entirely unexpected, as the model is most sensitive to the haemoglobin value, and thus by extrapolation, the haematocrit, upon which the FAMACHA© system is based. This study has shown that a suitable regression model with stochastic input variables can be used to estimate the risk of disease of sheep in

real-time, and also to be useful in allowing the user to “see”, by scenario and sensitivity analysis, the effect of variability on the risk of disease under high or low risk conditions. The model output is able to account for variability in haemoglobin levels and body mass over time, and it is also site-specific, as its inputs consist of data gathered directly at the site of exposure of sheep to infective larvae, i.e. not only at the level of the individual farm, but also the individual flock and class of animal. Small ruminant husbandry in the South African context is playing an increasingly important socio-economic role in traditional farming systems in small ruminants raised under resource-poor conditions (Vatta & Lindberg 2006). Although the data used in this work was collected under commercial farming conditions, the model could also be applied by extension personnel working in resource-poor communities, using the FAMACHA© scores and body mass of animals in the format presented here to assess the health, and subsequent treatment recommendations under resource poor conditions. Incorporation of the model output, with measurement of spread and amount of rainfall at the point of exposure which is the individual farm, into a decision tree framework for anthelmintic treatment, is a logical next step on the way to the final “black box”. A need was also identified to incorporate rainfall data into an index of “wetness” in order to relate worm burdens not only to absolute rainfall values, but also to how the rain was spread over rainfall events.

CHAPTER 6

Use of Shannon's entropy to process rainfall data as a risk factor in sheep naturally infected with *Haemonchus contortus*

6.1 Introduction

The success rate of free-living larval development in *H. contortus* is largely determined by climatic variables such as temperature and moisture (Donald, Southcott & Dineen 1978; Soulsby 1982; Kao, Leathwick, Roberts & Sutherland 2000). Under optimal environmental conditions of which moisture and temperature are the most important, larvae develop to the infective third stage (L_3) larvae in four to six days, with temperatures below 9ºC resulting in little or no development (Soulsby 1982; Onyiah 1985). Nematode species such as *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* are more resistant to desiccation and also have the ability to develop at lower temperatures than *H. contortus* (O'Connor *et al*. 2006), but summer temperatures in much of the summer rainfall region of South Africa where *H. contortus* is a problem are rarely limiting to its development.

The ovine faecal pellet is relatively small compared to larger and much more moist bovine faecal pats, and the small size, coupled to moisture limitation, is likely to affect the development and survival of the pre-infective stages of the parasite to a greater extent than in bovine faecal pats (Paton, Thomas & Waller 1984). Although rainfall, in absolute terms, has to date been successfully described as an independent dose variable in many *Haemonchus* spp. studies, such as Rossiter (1961), Thomas (1968), Horak & Louw (1977), and Ikeme, Iskander & Chong (1987), it is not only the total amount of rainfall, but also the "spread", or amount of rainfall per event, and the number of rain events, that largely determines the dynamics of larval motility and availability on pastures (Viljoen 1964). Early work in South Africa which addressed the effect of the number of days of rainfall, in addition to the total rainfall on helminth infections, was carried out by Viljoen (1964) and Muller (1964, 1968). More recently McCulloch, Kuhn & Dalbock (1984) described "dangerous 8-week rainfall periods", in which reference was made to a rainfall period where at least 5 of the 8 weeks satisfied a minimum 4-week rainfall requirement that these authors suggested would be responsible for elevated pasture infectivity for the property where the trials took place (Eastern Cape Province, South Africa), but added that, in evaluating "wetness", ground slope was also important in the determination of the minimum 4-week rainfall

requirement.

To evaluate the association between temporal availability of rainfall and the risk of haemonchosis as indicated by flock haemoglobin levels, the rainfall data from Farm 1 was analysed using Shannon's entropy theory model. Shannon (1948) developed the theory of informational entropy as a measure of information or uncertainty. The concept of entropy has often been applied to estimate the spatio-temporal variability of rainfall (Sonuga 1975; Al-Zahrani & Hussein 1998; Singh 2000; Kawachi, Maruyama & Singh 2001; Silva, Cavalcanti & Nascimento 2003; Maruyama, Kawachi & Singh 2005). Although the entropy theory has been extensively used as a quantitative measure of the potential availability of rainfall as a water resource, its application to rainfall as an integral part of disease risk analysis does not seem to have been explored. The aim of the present work was to evaluate the association between the mean haemoglobin level in a group of sheep naturally infected with *H. contortus*, and treated by selective drenching with the FAMACHA© system, with rainfall data processed with the Shannon entropy model by delineating the entropy distribution of rainfall on the farm.

6.1.1 Calculation of rainfall entropy

Shannon's informational entropy (*H'*) is calculated by

$$H' = -\sum_{i=1}^{s} pi(ln)pi \quad \text{.....(1)}$$

where *pi* represents the probability of the occurrence of the i^{th} value of a discreet random variable, which in the present work is the observed daily rainfall apportionment, *ln* = log $base_n$, *s* is the number of events or rainfall days and *H'* is the entropy of the random variable (Kawachi *et al*. 2001; Maruyama *et al*. 2005). Although any base of logarithms may be used (Kent & Coker 1997), natural logarithms were used in the calculation of entropy in the present study. In this study the daily rainfall intensity over a defined period of four weeks, at differing temporal distances from FAMACHA© evaluations of groups of sheep, is considered as a random variable. The relationship between the intensity, or total rainfall over the period, and its probability of occurrence, or frequency, is therefore calculated with the entropy formula. From Equation 1 it is evident that the value for *H'* will be zero when the total rainfall for the stated period is recorded on one day, while *H'* will have a theoretical maximum value if an equal amount of rain falls on every day within the stated period. The closer *H'* is to its

maximum value, the more uniformly spread the rainfall apportionment becomes, and the lower the temporal variability associated with the rainfall will be. The rainfall entropies were calculated in Excel spreadsheets.

6.1.2 Probabilistic interpretation of rainfall

The apportionment of rainfall was considered in a probabilistic sense, as described by Kawachi *et al*. (2001), by dividing total rainfall into a number of trials, each with a probability of success or failure. Rainfall was divided into 1 mm increments, and each millimeter of recorded rainfall was regarded as a trial. Thus, if the total rainfall over a four-week period was 100 mm, then there would be 100 successful trials during the period. Each day over the recorded period has an equal probability of being selected, so that a day on which 15 mm of rain was recorded was regarded as having being selected 15 times, i.e. 15 successes, and days with zero rainfall are not selected and consequently have zero successes. The rainfall series thus generated represents the accumulated occurrence frequencies of daily rainfall from the first up to the s^{th}, or last day of recorded rainfall. Implicit in this is that rainfall data was available in the form of total daily rainfall, and not as the number of rainfall events per day.

6.2 Materials and methods

6.2.1 Rainfall data

Daily rainfall values for Farm 1 were available for the period from 2001–2005, and these values were used to calculate rainfall entropy for the periods studied. Three different periods of inter-sample rainfall were regressed against the mean haemoglobin value of a group of sampled sheep in this study. Firstly, rainfall recorded in the four-week period, up to four weeks before a given sampling event, was processed with the Shannon entropy model, and regressed against the mean sample haemoglobin. Secondly, rainfall recorded during a four-week period up to 14 days before a given sample was processed and regressed against mean sample haemoglobin. Lastly, rainfall recorded in the four-week period directly preceding a sample was processed as described above and regressed against the mean haemoglobin level of a given sample. All regression analyses were conducted using the STATA software package.

6.2.2 Sheep haemoglobin data

Findings in Chapter 5 indicated that the observed haematocrit values for FAMACHA© categories were normally distributed within a category. The mean haemoglobin level in a sample of sheep was simulated using @Risk with a Discrete distribution (Vose 2000), according to the following relationship for mean corpuscular haemoglobin concentration and haematocrit:

*Mean haemoglobin content of sample = Discrete {MCHC (Uniform (minimum, maximum) * Haematocrit (mean, standard deviation), X1, X2,…Xn},{p1, p2,…pn}……..……(2)*

where MCHC is the mean corpuscular haemoglobin concentration, and the haemoglobin level for each FAMACHA© category present has a simulated value of *X* and a probability of occurrence *p*. Variability in mean corpuscular haemoglobin concentration and haematocrit was thus included in the simulation. The haemoglobin concentrations of all FAMACHA© evaluations over the previously described five-year period investigated were simulated in the analysis, but samples where a blanket drench had been administered at the previous FAMACHA© evaluation were excluded from the analysis. Thus, samples where only sheep in FAMACHA© categories 3–5 were treated at the previous evaluation were included in the regression analysis to minimise confounding by blanket drench events. Due to the fact that sheep in both classes were usually sampled on the same day, the simulated haemoglobin values of EWEREP and RAMREP were combined for a sample and a mean haemoglobin value per sample was calculated.

6.3 Results

The results of linear regression analysis of mean sample haemoglobin on rainfall entropy indicated that, for the rainfall recorded in the four-week period up to four weeks before a given sample (previous entropy), no predictable change in haemoglobin content of a sample could be ascertained (R^2 = 0.077, *df* = 24, $p>0.05$), and the relationship was not significant at $p\leq0.05$. Similarly, for the rainfall recorded in the four-week period between samples up to and including the day of the sample (present entropy), no predictable change in haemoglobin content of a sample was evident (R^2 = 0.010, *df* = 24, $p>0.05$). However, a negative association between rainfall and haemoglobin was found for these two analyses. In contrast to the above analyses, findings indicated that the mean sample haemoglobin concentration displayed a predictable change with rainfall entropy recorded during the four-

week period up to 14 days (14-day entropy) before a given sample (R^2 = 0.551, *df* = 24, $p<0.001$), indicating a highly significant negative association between the 14-day rainfall entropy value and the mean haemoglobin level in the sample, with an explained proportion of the total variation in the data of 55 %. The result of linear regression analysis of mean sample haemoglobin content on the 14-day rainfall entropy is illustrated in Fig. 6.1.

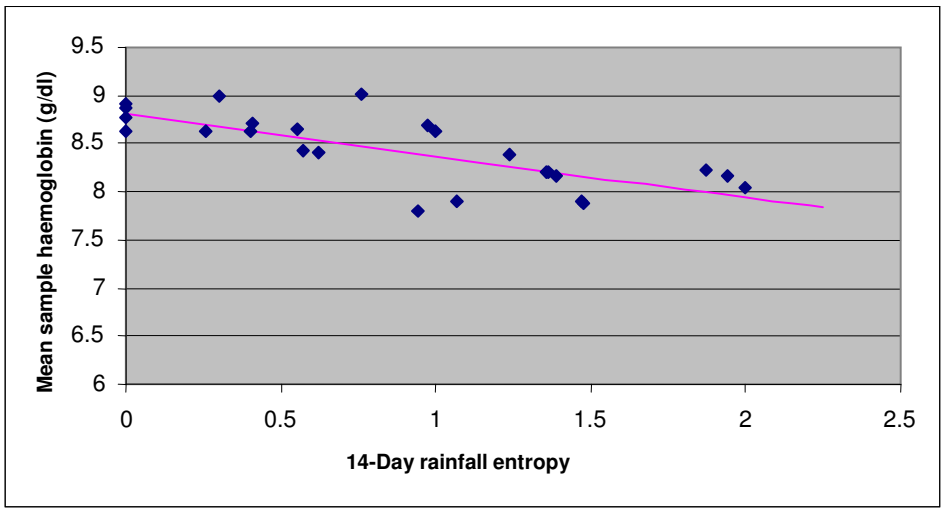


FIG. 6.1. Farm 1. Mean sample haemoglobin level at different 14-day rainfall entropy values, as calculated by the Shannon entropy model. The regression equation is y = -0.433 (entropy) + 8.81.

6.4 Discussion

Although normal faecal moisture may be adequate for development of some *H. contortus* eggs through to the pre-infective larval stage but not the infective stage, sustained availability of moisture is necessary for development to L_3. Furthermore, short, single rainfall events are unlikely to lead to infective pasture, since rapid drying limits moisture availability in the microclimate under high rates of evaporation (O'Connor *et al*. 2007).

In evaluating the above results, it should be kept in mind that that, firstly, only data emanating from one farm over a five-year period was analysed. The rainfall on the farm concerned is much more consistent than that of most of the summer rainfall region of South Africa, which is characterized by more erratic rainfall in terms of amount and frequency of occurrence. What needs to be further evaluated, is the effect of variation in amount and frequency (e.g. short thunder showers vs. the same amount of softer rainfall over a period of numerous hours), and intervals between rainfall events.

The results of this study indicated a significant negative association between daily rainfall data processed with the Shannon entropy model and the mean sample haemoglobin concentration on Farm 1 14 days after a FAMACHA© evaluation, using the 14-day entropy values calculated as described, and while only treating sheep in FAMACHA© categories 3, 4 and 5. It is interesting to note that the regression line in Fig. 6.1 intercepts the Y-axis at a predicted haemoglobin value of 8.81g/dl. This effectively means that on average, under conditions of zero rainfall entropy, the mean flock haemoglobin level should be maintained at 8.8 g/dl of haemoglobin, a figure which translates to a mean haematocrit of approximately 29 %, which is above the minimum assigned haematocrit value of FAMACHA© 1. This was not unexpected, as FAMACHA© category 1 represents a haematocrit range of above 27 %, and represents a FAMACHA© test result that indicates an animal as being classified into the optimum (i.e. definitely normal) FAMACHA© category (Van Wyk *et al*. 2001a). The relatively high explained proportion of the total variation in the data of 55 % (R^2 = 0.551) is also an indication that the Shannon entropy model could be used to index "wetness", due to the well established fact that rainfall is an important risk factor in the maintenance of pasture infectivity. Probabilistically, the quantal response of the flock haemoglobin level to rainfall entropy could also be modelled as:

Haemoglobin level = Normal [-0.433(entropy) + 8.81, 0.251)……(3)

where the value 0.251 represents the standard deviation of the normally distributed error terms of the relationship. An example of a further @Risk simulated probabilistic relationship between haemoglobin and 14-day rainfall entropy for the highest calculated entropy value (*H'* = 2) in the analysis is given in Fig. 6.2. In this figure, it can be seen that for *H'* = 2, 90 % of the flock could be expected to have haemoglobin values that range between 7.53 and 8.35 g/dl approximately 14 days after the current FAMACHA© evaluation on Farm 1, granted that sheep in FAMACHA© categories 3, 4 and 5 are treated. However, the probability of an individual animal being classified into the "healthy" FAMACHA© 1 category, i.e. with a haemoglobin level above approximately 8.5 g/dl diminishes to almost zero as indicated in Fig. 6.2, where there is a probability of almost 1 that a randomly sampled animal would have a haemoglobin level of less than 8.5 g/dl.

A certain degree of caution is required when interpreting this type of relationship, in that the regression model and thus also its stochastic derivative, should only be used within the range of the independent variable for which data are available. This would be particularly important when there is constant and high rainfall, as on Farm 1, and the relationship would

have to be validated for a different, drier geographical area, or even different farms. There were no recognisable periods of drought on the farm. Furthermore, misclassification of animals on Farm 1 where this data originated, meant that fewer animals were treated than necessary at any given FAMACHA© evaluation, decreasing the effect of confounding by anthelmintic treatment (Chapter 3).

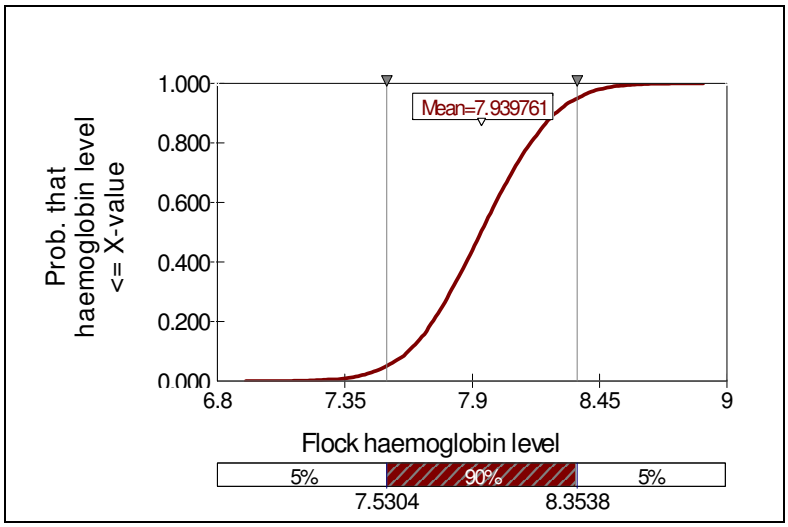
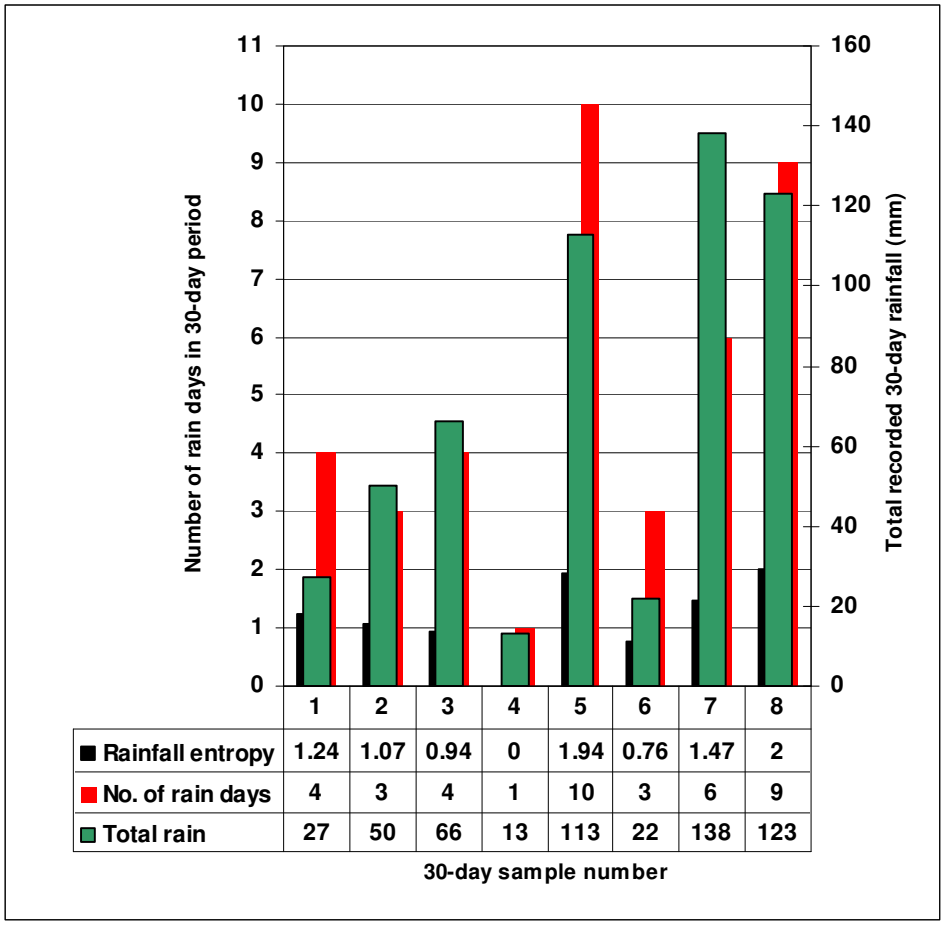


FIG. 6.2. Cumulative distribution function for flock haemoglobin level (g/dl) for a maximum calculated rainfall entropy value (*H*) of 2.

The relationship between rainfall entropy, total recorded rainfall, and number of rain days for eight selected 30-day periods on Farm 1 is illustrated in Fig. 6.3. An example of how entropy incorporates not only the total rainfall but also the number of days over which the rain falls, and thus the spread of rainfall, can be observed by comparing samples 5 and 7 (Fig. 6.3). The total recorded rainfall for sample 7 was 138 mm over a period of 6 days giving an entropy value of 1.47, compared to sample 5 where 113 mm was recorded over a period of 10 days with an entropy of 1.94. Thus, even though there was a higher absolute rainfall in sample 7, sample 5 had a higher entropy value because the recorded rain fell over ten days in sample 5 compared to six days in sample 7.



	1	2	3	4	5	6	7	8
Rainfall entropy	1.24	1.07	0.94	0	1.94	0.76	1.47	2
No. of rain days	4	3	4	1	10	3	6	9
Total rain	27	50	66	13	113	22	138	123

FIG. 6.3. Farm 1. The relationship between rainfall entropy, number of rain days and total recorded rainfall for eight selected 30-day periods.

The fact that there was relatively good agreement between the 14-day rainfall entropy value and the mean haemoglobin concentration in a sample would suggest that rainfall data processed in this way could be a useful, if not absolute, indicator of risk of disease. This is at least partly due to the fact that it is not only the total rainfall that may increase pasture infectivity, but also how rainfall affects the micro-environmental conditions necessary for maintaining high pasture infectivity. If the available rainfall for a given period is spread over a time period of several days with discreet 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 (Krecek, Groeneveld & Maritz 1992). It is also reasonable to assume that a higher entropy value for rainfall will also affect ambient micro-environmental temperature conditions, since the continual cloud cover needed to obtain high rainfall entropy would decrease desiccation and

ultra-violet exposure of larvae.

The effect of high rates 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), adding impetus to the need to evaluate a quantitative method for estimating the effect of not only the total amount of recorded rainfall, but also the manner in which the spread of rainfall affects the risk of disease in the flock. A potential factor which should also be taken into account with this type of analysis is the fact that pockets of high-risk areas such as areas of green pasture could assist the survival of infective larvae if a sufficiently moist microclimate exists despite generally unfavourable environmental conditions (Besier & Dunsmore 1993b). Furthermore, “total recorded rainfall” as discussed in this work is assumed to include other moisture parameters such as dew, which would make up the total measured precipitation.

6.5 Conclusion

Although further work in this regard is needed, the preliminary indications are that rainfall, expressed in terms of entropy, could be further evaluated as a way of quantitatively evaluating pasture wetness as a risk factor in the development of haemonchosis.

CHAPTER 7

General results and conclusion

In this thesis, the epidemiological tools of sensitivity, specificity, and predictive values were used to further validate the FAMACHA© system on the two farms. The results of the application of these techniques not only supported the continued use of the FAMACHA© system, but also detected misclassification bias on Farm 1. Immediate corrective action could be taken by informing the farmer of the problem, and as described in Chapter 3, he was informed that in order to avoid losses, he should treat all sheep in FAMACHA© category 2 in addition to all sheep in FAMACHA© categories 3–5, as he had been doing. This is not ideal for the application of the FAMACHA© method, as it would be preferable to re-train the evaluator, but due to the flexibility of FAMACHA© (i.e. it has a resolution of five categories), the problem could be immediately corrected by temporarily dosing one category "up" to include FAMACHA© category 2. The inaccuracy of FAMACHA© classification on Farm 1 caused a situation where many anaemic sheep, i.e. true positives as described in Chapter 3, escaped treatment due to non-detection. This may have been good from the aspect of decreased selection for parasite resistance, but, although deaths due to confirmed cases of haemonchosis were rare on the farm, it would have caused production losses and consequent loss of income.

In Chapter 4, and apparently for the first time for the FAMACHA© system, use was made of Receiver Operating Characteristic curve analysis, with haematocrit as the reference variable. Although valid results were obtained for the FAMACHA© method on the two farms in Chapter 3, it is important that an equally valid method is used to empirically select relevant FAMACHA© thresholds for treatment, according to the required sensitivity of the test, and the accuracy of FAMACHA© evaluation. For the Receiver Operating Characteristic method, perfect diagnostic accuracy is represented by a probability of 1 and low, moderate or high levels of accuracy are conventionally set at values above a probability of 0.5, a value which represents a diagnostic test with the same binomial probability of detecting a diseased individual as by tossing a fair coin. Two cut-off values for haematocrit were evaluated for Farm 1 and Farm 2, namely ≤22 % and ≤19 %, using Receiver Operating Characteristic curve analysis. The diagnostic accuracy of the two compared cut-offs for Farm 1 was considered to be moderate, since the area under the curve index was 0.79 and 0.83 for each cut-off, respectively. Diagnostic accuracy for Farm 2, however, was better, with the

area under the curve index values of 0.86 and 0.90 for haematocrit cut-offs of ≤22 % and ≤19 %, respectively. As a further step in the evaluation of the FAMACHA© results for the two farms, two-graph Receiver Operating Characteristic analysis was used to select cumulative FAMACHA© threshold categories, referred to as "cut points", for anthelmintic treatment of sheep. Using two-graph Receiver Operating Characteristic curve analysis, sensitivity and specificity were plotted as a function of the FAMACHA© cut point value, and sensitivity, specificity, and likelihood ratios were used as indicators of test accuracy. From the two-graph Receiver Operating Characteristic plot, appropriate cut points can be selected and used as FAMACHA© treatment thresholds. FAMACHA© cut points that achieved a desired sensitivity of at least 0.9 for FAMACHA© evaluations at both haematocrit cut-off values could thus be estimated. The results of this study indicate that Receiver Operating Characteristic analysis is a useful method for determining the diagnostic accuracy of site-specific FAMACHA© evaluation and that two-graph Receiver Operating Characteristic analysis can be used to select treatment thresholds for sheep, for a pre-selected sensitivity.

Although the FAMACHA© system of selective drenching has been extensively validated in South Africa and the United States (Bath *et al*. 2001; Kaplan *et al*. 2004), it soon became apparent that its application would also benefit by being integrated into a formal decision-making process that could be used by the producer to make decisions pertaining to treatment of sheep. A need was identified to develop a software-based decision-support framework within which stakeholders such as farmers, veterinarians and other professionals would be able to make empirically supported decisions about selective chemotherapeutic treatment of flocks, based upon the integration of existing data and knowledge. Thus, FAMACHA© and body weight data emanating from on-farm studies of *H. contortus* infections in sheep was incorporated into a stochastic quantitative risk assessment model (Roberts & Swan 1982) for Farm 1 in Chapter 5, which it is envisaged could form an integral part of practical decision-making for selective anthelmintic treatment of animals. The originally published deterministic model did not account for variability in the input parameters, whereas the probabilistic model did account for this variability. The increased worm burdens predicted by the model towards the end of the season on 04 April for both ewes and rams (Fig. 5.4a and Fig. 5.5a), could have been because of declining pasture regrowth as rainfall decreased, causing a higher number of third-stage larvae (L_3) per unit mass of herbage. Cooler autumn conditions could also have resulted in higher survival rates for L_3.

The model described here differs from deterministic models in that it accounts for variability in the biological data at every step of the modelling process. It is a quantitative stochastic model that has as its primary inputs the direct measurement of haemoglobin level and body mass in a group of sampled sheep, in order to simulate the probable distribution of the worm burden of the sheep. Why simulate worm burden when the FAMACHA© method has a high probability of detecting an animal which needs treatment? The answer to this question lies in the stochastic, or random sampling nature of the model. For instance, a scenario analysis of the model indicated the minimum required haemoglobin levels within iterations that would result in a mean worm burden of ≤1 000 worms for both classes of sheep in the sample; however, this worm burden would be a subjective decision in practice, since it would be selected according to the perceived susceptibility of the class of animal. This type of information is directly applicable to the drenching decision-making process, and furthermore, it is generated by taking variability in the data into account. It is thus a useful way of generating different "what if" scenarios, in that the risk of disease in a given sample of sheep can be estimated for different threshold worm burden values. Because most predictive models are at best representative of only a part of the whole system, and some models contain invalid and untested statements (Dobson 1999), it is important that predictive models should have as few assumptions about the system as possible.

Smith (1997) stated that when modelling the relationship between production losses and parasitism in ruminants, it is not always clear which index of parasitism would be the most suitable to define the relationship, and further that correlations in quantitative relationships may only exist at certain times during the infection cycle. In this respect the FAMACHA© system has much potential, but it is recognised that the type of simulation model presented here is based only on flock haemoglobin level and body weight, and is further expected to play only a supporting role in the proposed "black box" system. The FAMACHA© system was developed in response to the problem of anthelmintic resistance, and is implemented with the explicit understanding that it is not a question of whether or not resistance will develop, but when, and that resistance can at best be delayed with selective drenching.

Computer models relating parasite populations to anthelmintic resistance have effectively contradicted the recommendations of most worm control programmes, such as those to drench all animals, to only use drugs that are maximally efficient, not to under-dose, and to periodically rotate anthelmintic classes. For example, in developing an anthelmintic resistance model, Barnes, Dobson & Barger (1995) found that irrespective of the drug

rotation strategy, resistance to each drug would develop at a similar rate when two drugs are rotated. The latter authors also found that non-treatment of a few animals in order to preserve susceptible worms would delay selection for resistance, but that this strategy could have some associated risk. This situation was observed on Farm 1 in the present work, where the model predicted a high degree of variability in worm burdens at certain sampling dates, and thus increased risk of disease particularly in overly susceptible individuals at these dates, but as stated before, the effect of selective drenching with FAMACHA© coupled with misclassification could have exacerbated the situation.

Paton *et al*. (1984) used a model based on a mathematical representation of the dynamics of the various stages of the life cycle of *Teladorsagia circumcincta* to predict the numbers of infective larvae on an experimental paddock grazed by lambs and ewes, and found that the "moisture status" of the surface layer of the pasture was important to predict the development and survival of pre-infective larvae. Echevarria *et al.* (1993) adapted a computer model originally developed to study resistance to anthelmintics in *T. circimcincta* in sheep flocks in the United Kingdom for use with *H. contortus* in southern Brazil. The model predicted that when the effect of early versus late season treatment was compared, early season treatment would select more rapidly for resistance. These authors used the simulated effect of different temporal drenching regimes, where all animals were drenched, to extrapolate the differential selection of RR, RS and SS alleles which were assumed to occur in Hardy-Weinberg frequencies at the commencement of simulations.

Leathwick, Vlassoff & Barlow (1995) developed a model for nematodiasis in New Zealand lambs, which included the contribution of ewes to nematode epidemiology as well as the genetic parameters required to simulate development of anthelmintic resistance in the nematode population. The latter authors found that undrenched ewes are potentially important as a refuge for susceptible worm genotypes, and further that these types of models could not reproduce the seasonal and site variations that are inherently found in field data sets. Kao *et al*. (2000) used a published model that used three state variables to represent the free-living and parasitic stages of *Trichostrongylus* spp, *H. contortus*, and *T. circumcincta* in sheep. The variables of the model were the mean number of adult nematodes per host, the pasture density of infective larvae, and the mean level of immunity in the host, modelled as cumulative larval challenge. Kao *et al*. (2000) used the above model to calculate the basic reproduction number (Q_0) that represented the expected number of adult offspring from a single female, introduced into a previously unchallenged host

population on clean pasture, as a way to evaluate worm control procedures. Importantly, they stated that using one set of experimentally derived parameters and one adjustable parameter, the model was shown to fit an example data set reasonably well, and further, that a simple model with only a few parameters would be capable of describing the behaviour of a real system. The simulation model described in the present work would not be expected to describe the total host-parasite system as encountered on the farms investigated, but rather as one of the components in a decision-support system that would be used to estimate differences in susceptibility between classes of animals, and also to track changes in the apparent risk of disease as a worm season unfolds.

Learmount *et al*. (2006) developed a computer model to simulate expected egg counts for a variety of inputs including regional weather data, stocking density, initial pasture contamination levels, parasite species proportions, as well as lambing dates, the timing of flock movements and removal of lambs. The end user is provided with a user interface, and by filling out the available data entry fields, their model is able, by integrating the results of published data on parasite biology and control, to predict the timing of expected peaks in egg counts during a given year. The model was developed for the United Kingdom, and was configured to run with the STELLA™ software platform (Costanza, Duplisea & Krautsky 1998). The values used for many of their model parameters were obtained by meta-analysis of published data. This approach is in sharp contrast to the approach with FAMACHA© implementation, however. Because the FAMACHA© system is effectively a test of the anaemia status of animals, and furthermore requires that the animals be evaluated often during the peak worm season, data on anaemia status, body weight, condition score and rainfall are readily and frequently available directly from the point of exposure. Furthermore, the approach taken by Learmount *et al*. (2006) would be difficult to implement under climatic conditions in many places in South Africa, not only because of a lack of comparable data, but also because of extreme differences in climate, not least of which would be the extremely unpredictable rainfall patterns compared with conditions in the United Kingdom.

The hypothesis that the haemoglobin level in a flock is predictable, or at least influenced, by rainfall entropy on Farm 1, was evaluated in Chapter 6. It is clear from the results that there may be sufficient grounds for continuing with this type of analysis, as the results indicated a high probability of estimating the mean haemoglobin level in a group of sampled sheep if rainfall entropy as calculated by the Shannon entropy model is regressed against simulated haemoglobin values. The lowest correlation between rainfall entropy and simulated

haemoglobin was obtained for the immediate four-week period preceding a given sample, followed by the four-week period, up to four weeks before a given sample. The fact that the highest correlation between rainfall entropy and simulated haemoglobin values was obtained for the four-week period up to 14 days (14-day entropy) before a given sample could be explained by the pre-patent period of *H. contortus*, which is about two weeks after ingested L_3 have moulted into fourth-stage larvae (Dunn 1969; Hansen & Perry 1994), and also the effect of larval stages on the host. 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. Thus, almost all non-hypobiotic worms would be actively feeding by the 14th day after having being ingested. Furthermore, the adult worms may be able to survive for a period of several months in a fully susceptible host (Dunn 1969). These results indicate that, on average, the most effective rainfall entropy period to be used to estimate the short-term trend in flock haemoglobin levels, which would also fit the requirements for the seven to ten day maximum recommended interval for FAMACHA© evaluation during periods of peak infection, would be the 14-day rainfall entropy value. An important factor which should be taken into account when interpreting the effect of rainfall entropy on flock haemoglobin levels on Farm 1 is that even though the evaluator had been treating sheep in FAMACHA© categories 3, 4 and 5, misclassification bias meant that on average, only sheep in FAMACHA© categories 4 and 5 were being treated. This can clearly be seen in Table 3.5, where the assigned median haematocrit value for FAMACHA© category 3 was 20 %, while the observed median haematocrit value was 15 %, which is the assigned median haematocrit value of FAMACHA© category 4. The significance of this finding is that of the 1 957 individual FAMACHA© evaluations for the 2001/2002 season for both classes of sheep, only 160 evaluations (8 %) were represented by sheep in FAMACHA© categories 3, 4, or 5. Thus, apart from the blanket drenches administered at the start, and at the height of the season, during all of the remainder of FAMACHA© evaluations, only 8 % of sheep were treated. However, it will always be important to note that any model predictions based strictly on climate, may not necessarily represent the true risk of disease, because as discussed by Dobson (1999) with a *Fasciola* model, moving animals to a high infection-risk area during periods of low predicted risk may considerably and un-seasonally increase the risk of infection. Similar results were described by Besier & Dunsmore (1993b), where L_3 of *H. contortus* often did not develop, or had a low survival

rate, with a mean survival period of five weeks in summer on dry pastures in the winter rainfall climate of the south coast of Western Australia. This was in contrast to L_3 deposited on perennially green pasture plots, where larvae were recovered for up to four months, both in faecal pellets and on pasture. The infection peaks seen in both classes of sheep on 7 January 2002 (Fig. 5.4a and Fig. 5.5a) could be partly attributed to misclassification, since no sheep were treated at the previous evaluation as only FAMACHA© categories 1 and 2 were present at these evaluations (Table 5.2 and Table 5.4).

By evaluating these factors, it should be possible to recommend a temporal distance between evaluations. This process could be included as a part of a potential quantitative risk model as described, into a “black box” decision-support system. Retrospective examination of model output, and comparison with a present (i.e. real-time) prediction, should indicate the trend in risk of disease in the flock, upon which the short-term management recommendation could be based. Such a recommendation should also include a recommendation as to which classes of animals to examine, based on their known or estimated susceptibilities for given rainfall, nutritional status, parturition status, drenching history and age. Haemoglobin values which are associated with selected worm thresholds obtained by simulation would provide useful quantitative information about the immediate risk status of a given class of animal. A given class of sheep could then be sampled and analysed with the quantitative risk model, which could lead to a drenching decision based on an infection threshold for the class of sheep as discussed in Chapter 5. Since the model is structured in a way which allows the risk of disease to be followed through the season, it could also be used to indicate, in conjunction with the results of two-graph Receiver Operating Characteristic analysis, which FAMACHA© categories should be drenched at a given sample for a given suite of risks, and also the drench to be used, assuming that the efficacy of the drench has been tested on the farm for the dominant worm species present.

In Chapter 1 it was stated that the main aim of this work was to evaluate the applicability of techniques such as 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. Regarding the specific contribution of this work to the “black box”, the results obtained in this work could initially be used in the form of a decision tree, starting with the previously determined relationship between rainfall entropy and mean flock haemoglobin level on the property concerned. Assuming that there is indeed a cause-effect relationship between these two variables, then the probable short

term trend in mean flock haemoglobin levels could be estimated according to the type of relationship presented in Fig. 6.1. Based on this information, a decision could be made to carry on to the next step, or not. A FAMACHA© evaluation and body mass determination of the flock or class of animal within the flock at that time would then provide data to run the simulation model, and the proportion of model iterations for a given class of animal that result in worm burdens close to or higher than a selected threshold would indicate which class or flock is most at risk at the time. If the model indicates that a high proportion of iterations results in undesirable worm burdens in what is known to be a susceptible group such as ewes with new-born lambs, then a decision to drench could be made. The FAMACHA© categories to be drenched would then be indicated by the results of Receiver Operating Characteristic curve analysis for the property, for a desired sensitivity of diagnosis. The "black box" model would thus not be entirely quantitative, but would also incorporate qualitative inputs of expert knowledge in terms of selected threshold worm burdens, as well as associated risk factors that have become apparent during model simulation. Future work would include validating various relationships, specifically the relationship between rainfall entropy and flock haemoglobin level, as well as the proportion of iterations in the stochastic model that result in undesirable worm burdens, threshold worm burdens not to be exceeded in different classes of animal, and field validation of the various model parameters described here.

Haemonchosis falls into the category of endemic parasitosis, but epidemics are rare (Perry & Randolph 1999). Economically, however, and especially in the developing world, the greatest effects of parasitic diseases are manifested in production losses. The sudden removal of parasites by anthelmintic treatment may also be commensurate with hysteresis in the host-parasite system, because parasite-induced pathological changes may still be in effect for some time after treatment (Smith 1997). Although the present work is concerned mainly with the biology and epidemiology of the disease, it will always be of primary importance that producers are made aware of the fact that to implement sustainable parasite management, a certain proportion of economic profitability would have to be sacrificed to maintain sustainability.

Although nematode control programs such as "Wormkill" and "Drenchplan" were developed in Australia to slow down the development of anthelmintic resistance in sheep, they were also expected to save up to 25 % of the direct costs of anthelmintic intervention to the farmer by virtue of fewer drug treatments (Waller 1987). Economically, these costs no longer

incurred would have amounted to AUS $5–6 million annually, and the fact that by 1987, up to 90 % of sheep producers in the northern tableland of New South Wales were using the "Wormkill" program bears testimony to the positive response of producers to costs no longer incurred. The "Wormkill" program, based on the use of specific drenches at specified intervals (Dash, Newman & Hall 1985; Dash 1986), was effective and simple, and was widely adopted due to these properties, even though resistance to closantel was eventually ascribed to the program. It will therefore be the challenge of animal husbandry professionals to ensure that sustainability is presented to producers in the most "palatable" way possible, while also ensuring that management recommendations are realistic and achievable in sometimes hostile farming environments.

A potential problem which was identified during the course of the project was the issue of cost. The @Risk software package used for the risk analysis, and thus to simulate the various models, was purchased at an approximate cost of ZAR 17 000.00 (approximately £1 400.00). Clearly, this would represent a considerable investment to producers or animal health professionals. This, however, should not present an insurmountable problem, as alternative statistical packages are available. Although not as "user-friendly" as @Risk, freeware packages such as the "R" computer language (R Foundation, 2005) have the advantage of being freely available on the Internet. A problem which would have to be overcome if this route were to be followed would be that programming skills become increasingly important. However, files exported as text are generally read without problems in "R" (D. Berkvens, personal communication 2007). Integration of "R" with Excel is not as seamless as is the case with @Risk, since the @Risk software functions as an "add in" to Excel. Programming runs may also be lengthy, but the simulating capacity of "R'' is in most respects more powerful than @Risk.

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