

## 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<sub>3</sub>) larvae in four to six days, with temperatures below 9°C 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<sup>®</sup> 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

$$H' = - \sum_{i=1}^s p_i (\ln) p_i \dots \dots \dots (1)$$

where  $p_i$  represents the probability of the occurrence of the  $i^{\text{th}}$  value of a discrete random variable, which in the present work is the observed daily rainfall apportionment,  $\ln = \log$  base  $e$ ,  $s$  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<sup>®</sup> 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  $s^{\text{th}}$ , 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<sup>®</sup> 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:

$$\text{Mean haemoglobin content of sample} = \text{Discrete} \{ \text{MCHC (Uniform (minimum, maximum))} * \text{Haematocrit (mean, standard deviation)}, X_1, X_2, \dots, X_n \}, \{ p_1, p_2, \dots, p_n \} \dots \dots \dots (2)$$

where MCHC is the mean corpuscular haemoglobin concentration, and the haemoglobin level for each FAMACHA<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> evaluation were excluded from the analysis. Thus, samples where only sheep in FAMACHA<sup>®</sup> 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  $p \leq 0.05$ . 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.

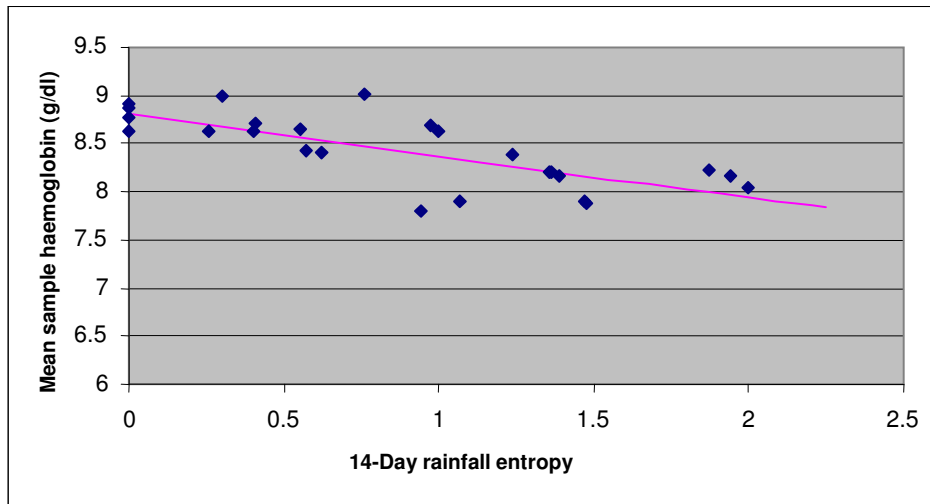


FIG. 6.1. Farm 1. Mean sample haemoglobin level at different 14-day rainfall entropy values, as calculated by the Shannon entropy model. The regression equation is  $y = -0.433 (\text{entropy}) + 8.81$ .

#### 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<sub>3</sub>. 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<sup>®</sup> evaluation, using the 14-day entropy values calculated as described, and while only treating sheep in FAMACHA<sup>®</sup> 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<sup>®</sup> 1. This was not unexpected, as FAMACHA<sup>®</sup> category 1 represents a haematocrit range of above 27 %, and represents a FAMACHA<sup>®</sup> test result that indicates an animal as being classified into the optimum (i.e. definitely normal) FAMACHA<sup>®</sup> 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:

$$\text{Haemoglobin level} = \text{Normal} [-0.433(\text{entropy}) + 8.81, 0.251] \dots \dots \dots (3)$$

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<sup>®</sup> evaluation on Farm 1, granted that sheep in FAMACHA<sup>®</sup> categories 3, 4 and 5 are treated. However, the probability of an individual animal being classified into the “healthy” FAMACHA<sup>®</sup> 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<sup>®</sup> evaluation, decreasing the effect of confounding by anthelmintic treatment (Chapter 3).

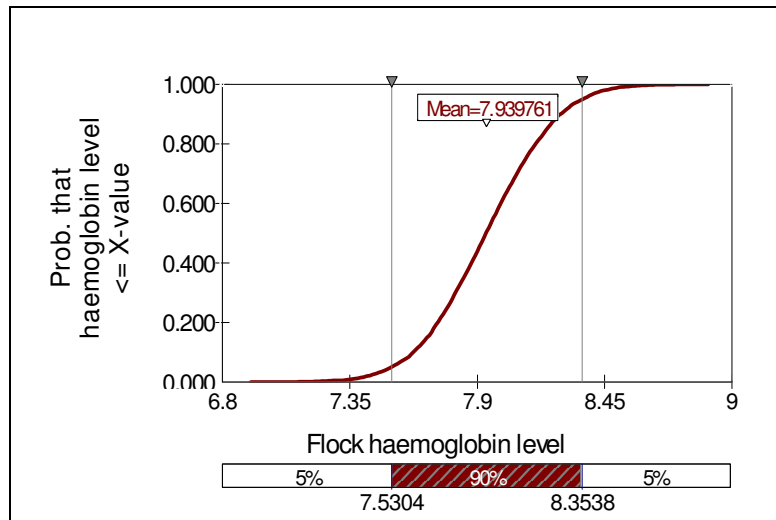


FIG. 6.2. Cumulative distribution function for flock haemoglobin level (g/dl) for a maximum calculated rainfall entropy value ( $H$ ) of 2.

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.

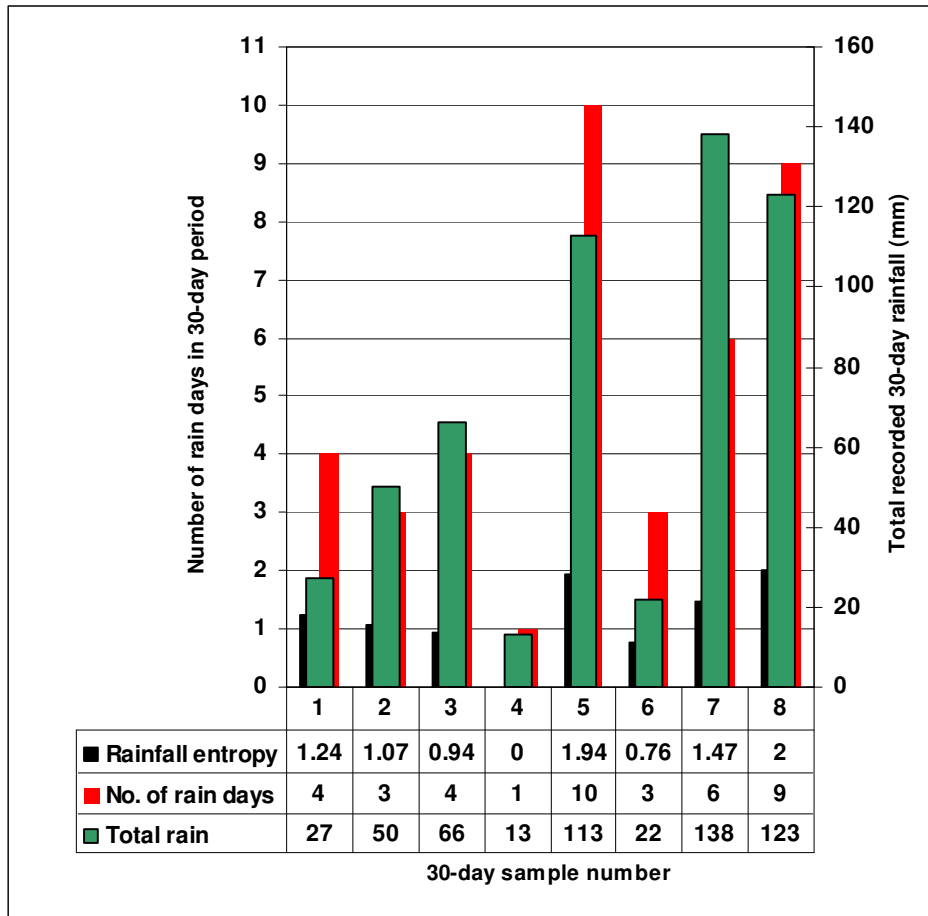


FIG. 6.3. Farm 1. The relationship between rainfall entropy, number of rain days and total recorded rainfall for eight selected 30-day periods.

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<sup>®</sup> system on the two farms. The results of the application of these techniques not only supported the continued use of the FAMACHA<sup>®</sup> 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<sup>®</sup> category 2 in addition to all sheep in FAMACHA<sup>®</sup> categories 3–5, as he had been doing. This is not ideal for the application of the FAMACHA<sup>®</sup> method, as it would be preferable to re-train the evaluator, but due to the flexibility of FAMACHA<sup>®</sup> (i.e. it has a resolution of five categories), the problem could be immediately corrected by temporarily dosing one category “up” to include FAMACHA<sup>®</sup> category 2. The inaccuracy of FAMACHA<sup>®</sup> 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<sup>®</sup> system, use was made of Receiver Operating Characteristic curve analysis, with haematocrit as the reference variable. Although valid results were obtained for the FAMACHA<sup>®</sup> method on the two farms in Chapter 3, it is important that an equally valid method is used to empirically select relevant FAMACHA<sup>®</sup> thresholds for treatment, according to the required sensitivity of the test, and the accuracy of FAMACHA<sup>®</sup> 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  $\leq 22\%$  and  $\leq 19\%$ , 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  $\leq 22\%$  and  $\leq 19\%$ , respectively. As a further step in the evaluation of the FAMACHA<sup>®</sup> results for the two farms, two-graph Receiver Operating Characteristic analysis was used to select cumulative FAMACHA<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> treatment thresholds. FAMACHA<sup>®</sup> cut points that achieved a desired sensitivity of at least 0.9 for FAMACHA<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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  $\leq 1\ 000$  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<sup>®</sup> 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<sup>®</sup> 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. circumcincta* 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<sub>3</sub> 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<sub>3</sub> enter the mucous membrane or glands in the wall of the abomasum, where they moult into fourth stage larvae (L<sub>4</sub>) 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 14<sup>th</sup> 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<sup>®</sup> 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<sup>®</sup> categories 3, 4 and 5, misclassification bias meant that on average, only sheep in FAMACHA<sup>®</sup> categories 4 and 5 were being treated. This can clearly be seen in Table 3.5, where the assigned median haematocrit value for FAMACHA<sup>®</sup> category 3 was 20 %, while the observed median haematocrit value was 15 %, which is the assigned median haematocrit value of FAMACHA<sup>®</sup> category 4. The significance of this finding is that of the 1 957 individual FAMACHA<sup>®</sup> evaluations for the 2001/2002 season for both classes of sheep, only 160 evaluations (8 %) were represented by sheep in FAMACHA<sup>®</sup> 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<sup>®</sup> 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<sub>3</sub> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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.