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.

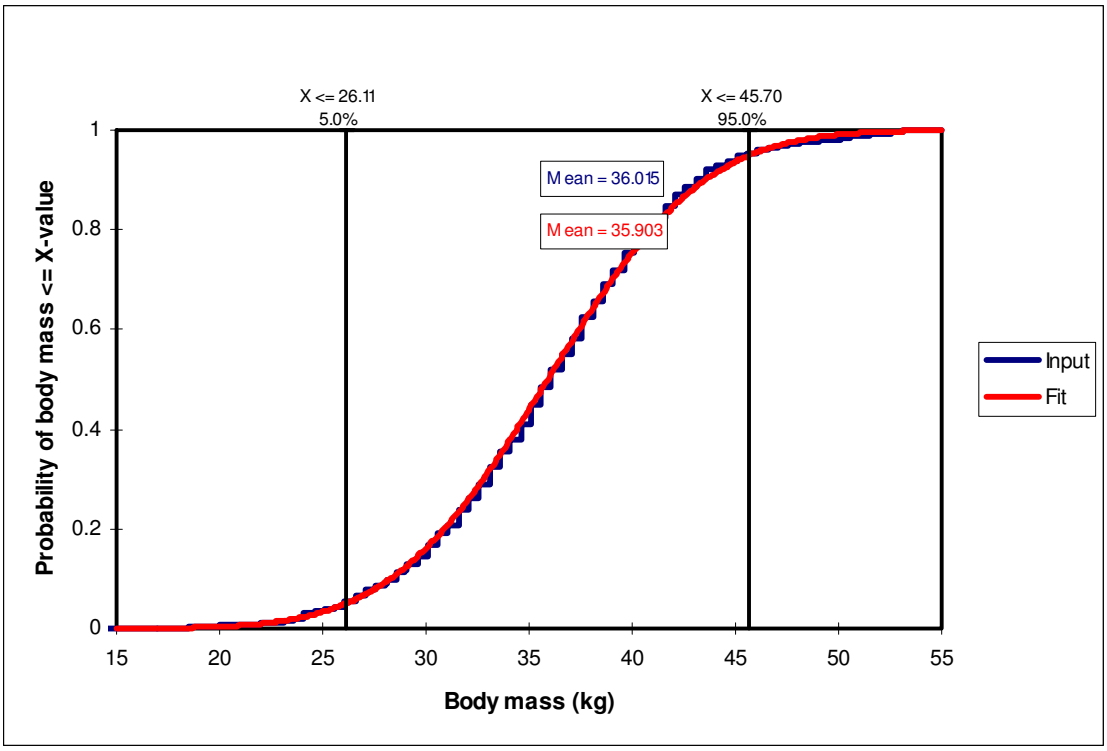

FIG. 5.1 Cumulative distribution function for the body mass of a sample of 179 RAMREP sheep on Farm 1 for the 2000/2001 *Haemonchus* season. The stepped blue line represents the observed body mass values in the sample and the red line represents the @Risk-fitted Normal (35.90,3.65) distribution.

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.

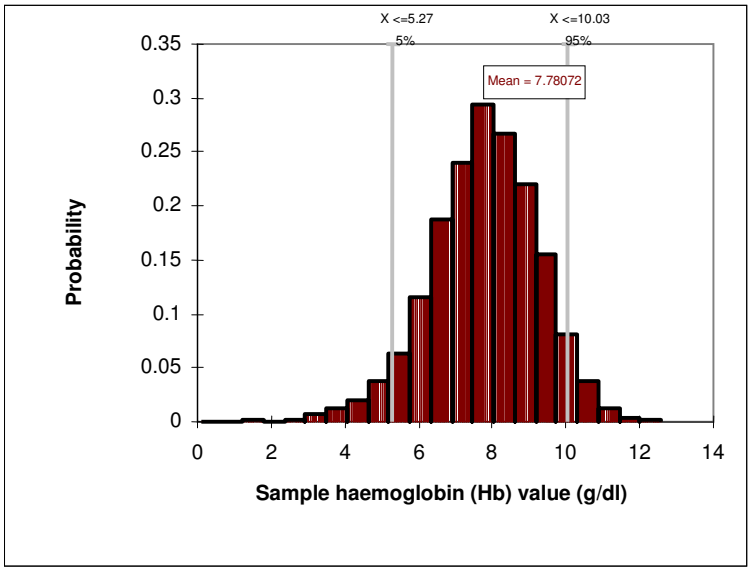

FIG. 5.2 The Discrete distribution for the FAMACHA© variable for a EWEREP sample (n = 133). The mean haemoglobin value was 7.78 g/dl, and 90 % of the simulated haemoglobin values were between 5.27 and 10.03 g/dl.

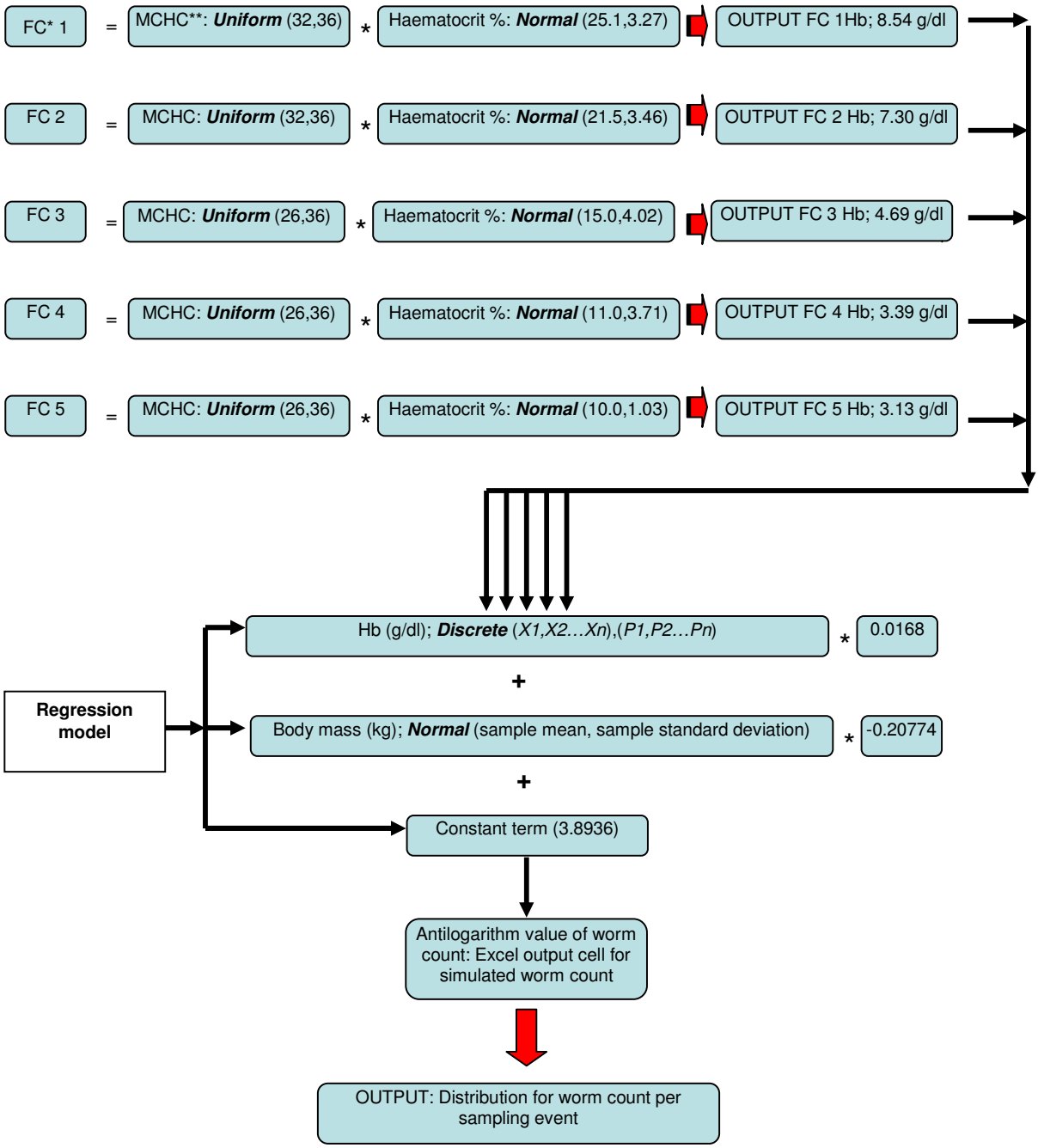

FIG. 5.3 Schematic diagram of the model used to simulate mean worm count of sampled sheep. Fitted statistical distributions are given in bold italicized letters. Bold red arrows indicate Monte Carlo simulated outputs of the model. (*FC = FAMACHA©, ** MCHC = mean corpuscular haemoglobin concentration)

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).

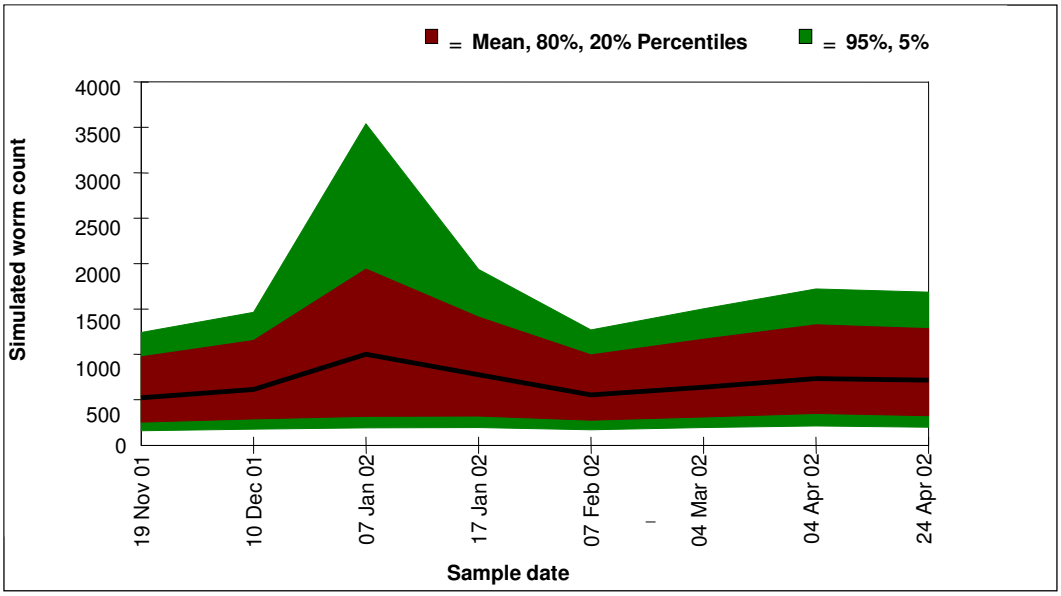

FIG. 5.4a EWEREP (n = 130). Model output for simulated worm count, 2001/2002 season. The black line represents the simulated mean worm count.

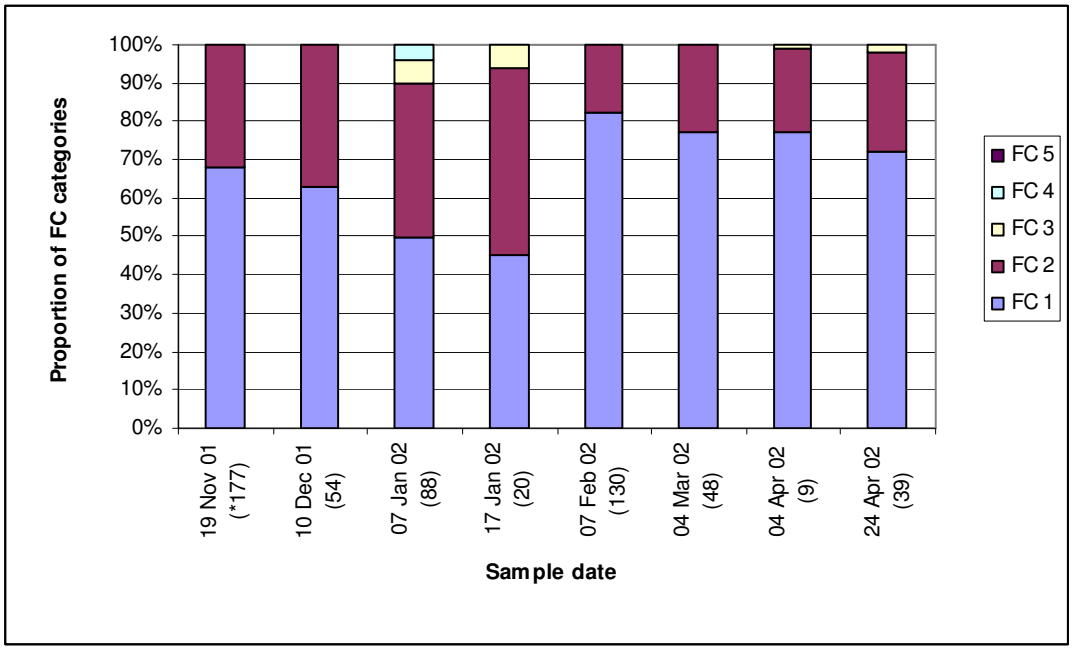

FIG. 5.4b EWEREP: Proportional representation of the FAMACHA© categories per sample. Rainfall between sampling events is given in parentheses, in mm. (*Rainfall for preceding 4 weeks). FC = FAMACHA©.

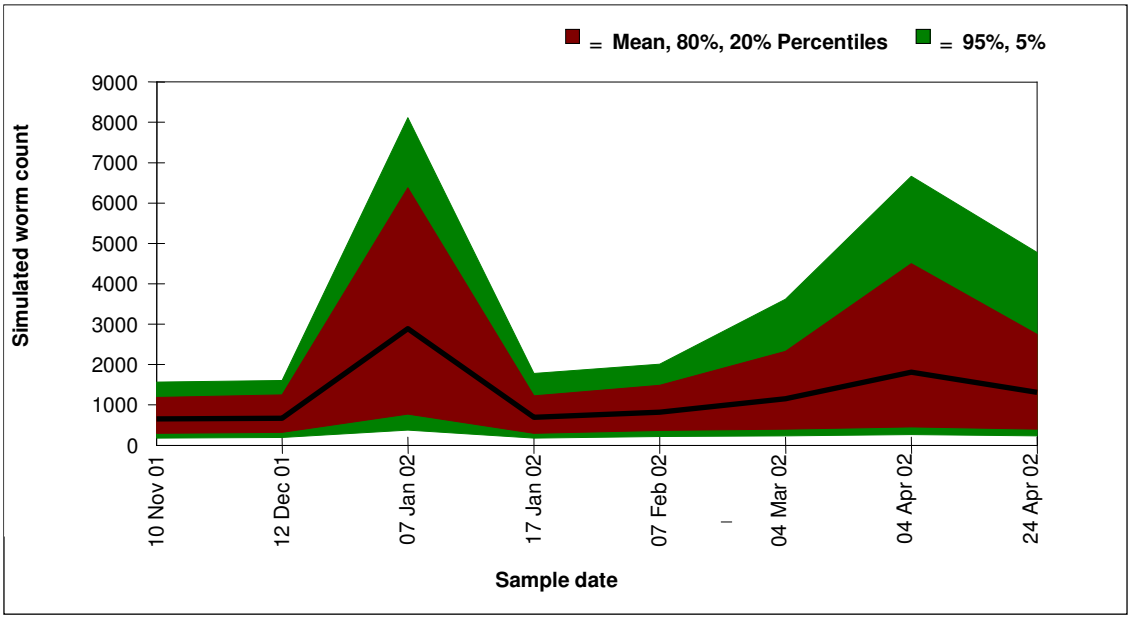

FIG. 5.5a RAMREP (n = 120). Model output for simulated worm count, 2001/2002 season. The black line represents the simulated mean worm count.

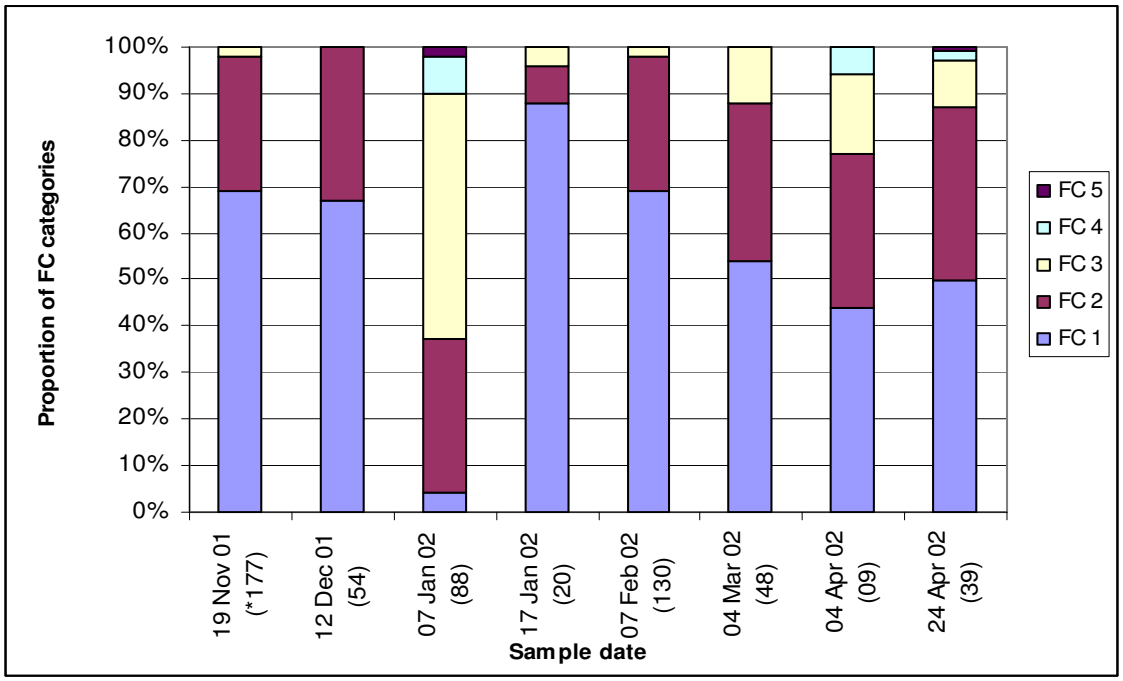

FIG. 5.5b RAMREP: Proportional representation of the FAMACHA© categories per sample. Rainfall between sampling events is given in parentheses, in mm. (*Rainfall for preceding 4 weeks). FC = FAMACHA©.

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.

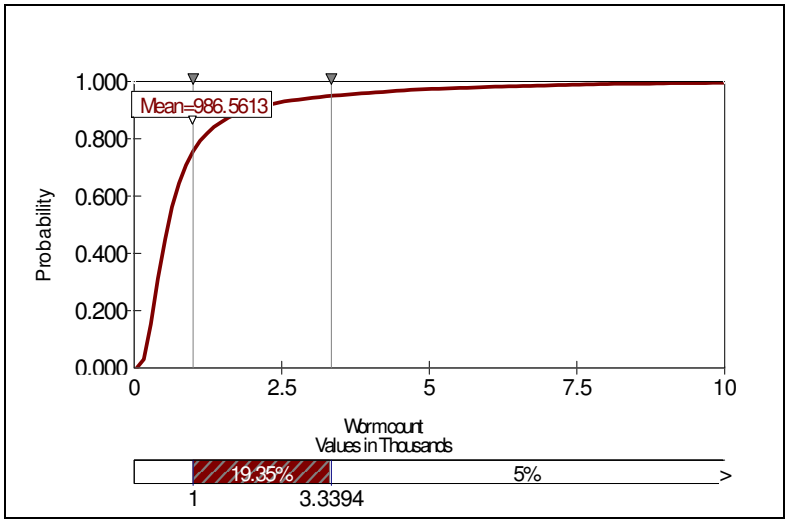

FIG. 5.6a Ascending cumulative output distribution for worm count for the EWEREP class, 7 January 2002. Refer to text.

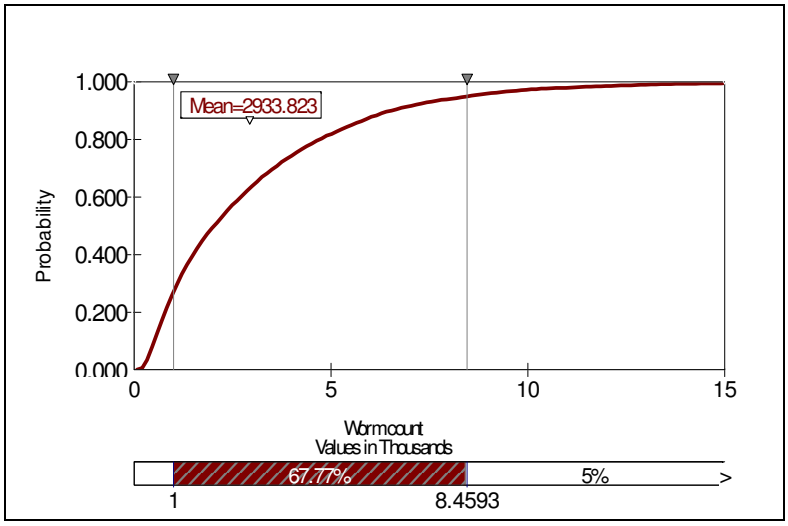

FIG. 5.6b Ascending cumulative output distribution for worm count for the RAMREP class, 7 January 2002. Refer to text.

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.