Chapter 2

General materials and methods

2.1. Study sites, animals and sampling

Three study sites were selected within the summer-rainfall area of South Africa: one near Rust de Winter, Gauteng Province, one in Impendle, KwaZulu-Natal Province, and one in Kraaipan, North-West Province (Fig. 1.2). A summary of the trial periods and frequencies of visits, breeds of animals, sample sizes, and anthelmintics used is given in Table 2.1.1 The grazing practices, vegetation types, winter supplements and long-term average rainfall for each study site are given in Table 2.1.2. During the day, the goats at Rust de Winter browsed on the natural vegetation while the farmer at Site 2, Impendle, allowed the animals to graze communal pasture surrounding his homestead. At Site 1, Impendle, and at Kraaipan the animals were grazed on communal pasture tended by a shepherd. All the animals were penned in kraals at night. The sheep at Rust de Winter initially grazed with the goats, but from May 1999 they were grazing separately in an enclosed paddock of fallow land and were also penned separately at night.

Climatic data were supplied by the South African Weather Bureau from the weather station nearest to the study sites (Table A1.1) and are given in Figs. A1.1-A1.3.

During visits in November 1999 to January 2000, all the animals present were aged by examining their incisors and sexed. The profiles for the herds on those days are given in Table 2.2.

At Rust de Winter, all the weaner and adult goats and sheep present at each visit were sampled/scored. For the two farmers (Sites 1 and 2) at Impendle, a representative sample of the weaner and adult goats was selected based on the first animals brought into the crush at the first visit, and when available the same goats were sampled/scored throughout the trial period. Unfortunately, the initial sample sets for each of the two sites at Impendle had diminished in

Table 2.1.1

Study sites : summary of trial periods and frequencies of visits; breeds of animals; sample sizes; and anthelmintics used

Study site	Trial period	Frequency of visits	Breed	Approximate numbers of animals present at each visit	Mean number of animals sampled/ scored (range)	Anthelmintic used (dosage)[b]
Rust de Winter (25°16'52"S; 28°38'51"E)	Sep 1998 - Apr 2000	Fortnightly	Boer goat crossbreed	18-47	31 (18-47)	Levamisole ($7.5\ mg\ kg^{-1}$)[c]
			Dorper sheep crossbreed	3-7	5 (3-7)	Ditto
Site 1, Impendle (29°34'39"S; 29°47'7"E)	Nov 1998 - Apr 2000	Monthly	Indigenous Zulu goat crossbreed	±60	18 (9-22)	Ivermectin ($0.2\ mg\ kg^{-1}$)[d]
Site 2, Impendle (29°35'4"S; 29°47'15"E)	Ditto	Ditto	Ditto	±35	19 (14-24)	Ditto
Kraaipan (26°19'16"S; 25°16'44"E)	Oct 1998 - Apr 2000	Monthly[a]	Indigenous goat crossbreed	±60	16 (10-23)	Levamisole ($7.5\ mg\ kg^{-1}$)[c]
			Indigenous fat-tailed sheep crossbreed	±60	14 (5-22)	Ditto

[a]Excepting for two visits over three months at start of trial.
[b]All anthelmintics were administered *per os*.
[c]Predominantly Tramisol™ liquid (Hoechst Roussel Vet, now Intervet); on a few occasions initially, Levisol™ liquid (Bayer).
[d]Ivomec™ tablets for sheep (Logos Agvet); used extra-labelly in goats: goats less than 25 kg in weight were given ½ tablet (5 mg) each and goats between 25 and 50 kg in weight were given 1 tablet (10 mg) each.

Table 2.1.2

Study sites : summary of grazing practices, vegetation types, winter supplementation and rainfall

Study site	Grazing	Vegetation[a]	Winter supplementation (1999)	Rainfall[b]
Rust de Winter	Private farm of 620 ha.	Mixed bushveld	Bone meal and salt lick	610 (Rust de Winter, 10)
Site 1, Impendle	Communal	Highland sourveld and Döhne sourveld	Poor quality *Eragrostis* spp. hay	993 (Donnybrook, 35)
Site 2, Impendle	Ditto	Ditto	Whole maize kernels - frequency and quantity not obtained	Ditto
Kraaipan	Communal	Sourish mixed bushveld	Bone meal and salt lick	539 (Mmabatho, 60)

[a]Acocks, 1975.
[b]Long-term average annual rainfall in mm (weather station, approximate kilometres in a direct line from study site). Source: South African Weather Bureau.

number by the end of the trial, owing to sales or slaughtering of animals and at least one death. At Kraaipan, a representative sample of the goat and sheep flocks was chosen in a similar manner as for Impendle, but the animal numbers also started to dwindle and for this reason every 10th animal brought into the crush in May 1999 was added to the sample group. This resulted in four goats and four sheep being added to the representative sample groups.

Animals were utilised for faecal egg count reduction (FECR) tests (see 2.2 and 2.4 below) towards the end of the trial. These animals had not been included in the sampling groups mentioned above, except for one of the goats and one of the sheep at Kraaipan. All the goats had no permanent incisors, except for three of the goats at Site 1, Impendle, two at Site 2, Impendle, and 10 at Kraaipan, which goats had two to four permanent incisors. Four of the sheep at Kraaipan had two to six permanent incisors while the rest of the sheep had deciduous incisors only. None of the animals had been treated with an anthelmintic effective against nematodes within 12 weeks of the start of the FECR tests at Rust de Winter and Kraaipan, and within 16 weeks at Impendle.

Table 2.2

Profile of total flocks by age and sex

Age in years[a] (no. of permanent incisors)	Up to ± 1.0 (0)	1.0 and < 1.5 (2)	± 2.0 (4)	± 2.5 (6)	2.5 (8)	3.0 (worn mouth) [b]	Totals
Rust de Winter - Goats - 15 Dec 99							
Female	17	3	2	2	15	0	39
Male	15	1	0	0	0	0	16
Wether	2	1	0	0	0	0	3
Totals	34	5	2	2	15	0	58
Rust de Winter - Sheep - 15 Dec 99							
Female	1	0	0	1	2	0	4
Male	0	0	1	0	0	0	1
Totals	1	0	1	1	2	0	5
Site 1, Impendle - Goats - 23 Nov 99							
Female	10	7	5	3	15	0	40
Male	8	0	0	1	1	0	10
Totals [c]	18	7	5	4	16	0	50
Site 2, Impendle - Goats - 23 Nov 99							
Female	10	2	3	2	13	1	31
Male	12	0	0	0	0	0	12
Totals [c]	22	2	3	2	13	1	43
Kraaipan - Goats - 4 Jan 00							
Female	21	7	1	7	20	4	60
Male	2	1	0	0	1	1	5
Weather	13	6	0	0	0	0	19
Totals	36	14	1	7	21	5	84
Kraaipan - Sheep - 4 Jan 00							
Female	20	4	1	5	25	0	55
Male	5	0	0	0	0	0	5
Weather	10	0	0	0	0	0	10
Totals	35	4	1	5	25	0	70

[a]Adapted from Kwantes, 1994.
[b]Probably ≥ 8.0 years old.
[c]One animal missed per site.

2.2. Parasitological diagnostic techniques

Faecal samples were collected at each visit from the animals at Rust de Winter and the representative sample sets at Impendle and Kraaipan (the "trial" animals). Additional samples were

collected from April 1999 at Kraaipan and from May 1999 at Impendle to ensure that there would be sufficient faeces for a good yield of third-stage nematode larvae (L_3) when cultures were made (see below). The faecal samples were processed for nematode faecal egg count (FEC), using a modified McMaster technique (Van Schalkwyk et al., 1995). This method is described in Appendix 2. Minor modifications to the method as well as the trade names of the equipment used are recorded in square brackets in this appendix. In brief, two grams of faeces were weighed off per animal. To this were added 58ml of sugar solution and the mixture homogenized using an electric mixer (IKA®-Labortechnik, Janke and Kunkel, N.T. Laboratory Supplies, Johannesburg). Two chambers of a McMaster slide (Eggs-Acto™ McMasters, E. Krecek, South Africa) were filled with the solution, and after allowing the slide to stand for at least two minutes, the nematode eggs in the chambers were counted under a compound microscope. The number of eggs counted per sample was multiplied by 100 to give the final result in eggs per gram of faeces (epg), according to the following formula :

$$\text{FEC (in epg)} = \frac{\text{number of eggs counted x volume of sample}}{\text{volume of McMaster chamber x number of chambers counted x mass of faeces in sample}}$$

$$\text{FEC (in epg)} = \frac{\text{number of eggs counted x 60}}{0{,}15 \text{ x } 2 \text{ x } 2}$$

$$\text{FEC (in epg)} = \text{number of eggs counted x } 100$$

Strongyloides, *Nematodirus* and *Trichuris* eggs were counted separately from the other nematode eggs, which are herein referred to as "strongyle" eggs (Order Strongylida - Molin, 1861).

Samples were screened for trematode eggs by means of the sedimentation method (Van Wyk et al., 1987, Appendix 2) which was modified for pooled samples as follows. Half a gram of faeces (1g for the sheep at Rust de Winter) was weighed off from each of 10 faecal samples (five faecal samples for the sheep at Rust de Winter) randomly selected from those collected at each visit to a site. The faeces were pooled and softened and/or homogenized with an electric mixer (IKA®-Labortechnik, Janke and Kunkel, N.T. Laboratory Supplies, Johannesburg) in water. The faeces

were then sieved through a 150µm sieve (United wire test sieve, Nigel, South Africa or equivalent) into a 38µm sieve (Labotec test sieve, Johannesburg or equivalent), using water sprayed from a nozzle at high pressure. The remaining sediment was washed into a two or three litre glass jar. This was filled with water and allowed to stand for at least 15 minutes. The supernatant was then decanted and the sediment washed by filling up the jar again. This process was repeated approximately three times until the resulting supernatant was clear. Thereafter the sediment was poured into a measuring cylinder, made up to 200ml with water and mixed well by blowing air through the suspension with a pipette. Twenty millilitres of this suspension were examined in a gridded perspex container (70mm x 70mm, E. Krecek, South Africa) under a stereomicroscope for trematode eggs.

The number of eggs per gram of faeces was calculated as follows :

$$\text{FEC (in epg)} = \frac{\text{Number of eggs present}}{\text{Mass of faeces}}$$

$$= \frac{\text{Number of eggs counted x 10}}{10 \text{ x } ½}$$

$$= \text{Number of eggs counted x 2}$$

Faeces remaining after the FECs had been processed were cultured for third-stage nematode larvae (L_3). The method described in Van Wyk et al. (1987) for the collection of large numbers of larvae was used (Appendix 2). In brief, the faeces were mixed with vermiculite and a small amount of water, and lightly compacted in large fruit jars (approximately one litre). The faeces were placed in an incubation room at a temperature of approximately 25°C until November 1999 when a new room was used and the temperature then adopted was approximately 26°C. L_3 were harvested into small jars (approximately 100ml) by flushing the sides of the jar and the surface of the faeces with water. After allowing the larvae to settle to the bottom of the jar for at least 15 minutes (but often considerably longer) a sample(s) of larvae was (were) drawn up from the bottom of the sample with a Pasteur pipette (Liquipettes™, Elkay, Ireland or equivalent), placed on a slide(s), stained with a dilute iodine solution and where possible at least 50 L_3 were identified under a compound

microscope. The keys of Van Wyk et al. (1997a, Appendix 2) and Dunn (1978) were used. Where few larvae were recovered from a culture, the larvae from a sample were allowed to settle in a test tube. No attempt was made to differentiate *Teladorsagia* spp. from *Trichostrongylus* spp. The proportions of the strongyle L_3 were used to estimate the proportional FECs of the various strongyle worm genera.

The animals were bled from the jugular vein into evacuated ethylene diamine tetra-acetic acid (EDTA) tubes (Vacutainer Systems^{TM}, Becton Dickinson, France). Two heparinised microhaematocrit tubes (Marienfeld, Germany, or equivalent) were filled with blood per sample and centrifuged (Kubota 3100, N.T. Laboratory Supplies, Johannesburg or Hermle Z230 HA, Germany) for seven minutes at 12000 revolutions per minute. The haematocrits were read for each capillary tube and the mean of the two readings used in the analyses.

The efficacies of the anthelmintics used in the trial were assessed by means of the FECR test (Coles et al., 1992; Presidente, 1985; Van Schalkwyk et al., 1995), which uses the reduction in FECs following anthelmintic treatment as an indication of anthelmintic efficacy. In each FECR test, the faeces remaining after the FECs had been done were cultured as follows for L_3 recovery: for the initial date per test all the faeces were pooled together (pre-treatment culture), while the faeces for the second date were pooled separately per group (post-treatment cultures). The proportions of L_3 were applied to the strongyle egg counts to estimate the relative contribution of each genus (Coles et al, 1992; Presidente, 1985).

Table 2.3 gives the mean FECs of the animals at the visit dates immediately prior to the dates on which the anthelmintic treatments for the FECR tests were carried out. The animals were ranked according to these FECs from lowest to highest. The animals were then divided into groups of two or three, depending on whether one or two anthelmintics were being tested. Each individual within each group was then randomly assigned to a treatment or control group (Table 2.3) with the help of a table of random numbers. The initial and post-treatment dates of the FECR tests and the sizes of the groups included in the tests are also recorded in Table 2.3.

Table 2.3

Faecal egg count reduction tests : details of groups

Location	Mean FEC (interval)[a]	Treatment date of FECR test (interval)[b]	Anthelmintic (dosage)[c]
Goats			
Rust de Winter	2171 (14)	24 Feb 2000 (13)	Levamisole (7.5 mg kg^{-1})
Site 1, Impendle	3775 (28)	14 Mar 2000 (15)	Ivermectin (0.2 mg kg^{-1})
Site 2, Impendle	3147 (28)	14 Mar 2000 (15)	Ivermectin (0.2 mg kg^{-1})
Kraaipan	778 (28)	29 Feb 2000 (10)	Levamisole (7.5 mg kg^{-1}) Rafoxanide (7.5 mg kg^{-1})
Sheep			
Kraaipan	8230 (28)	29 Feb 2000 (10)	Levamisole (7.5 mg kg^{-1}) Rafoxanide (7.5 mg kg^{-1})

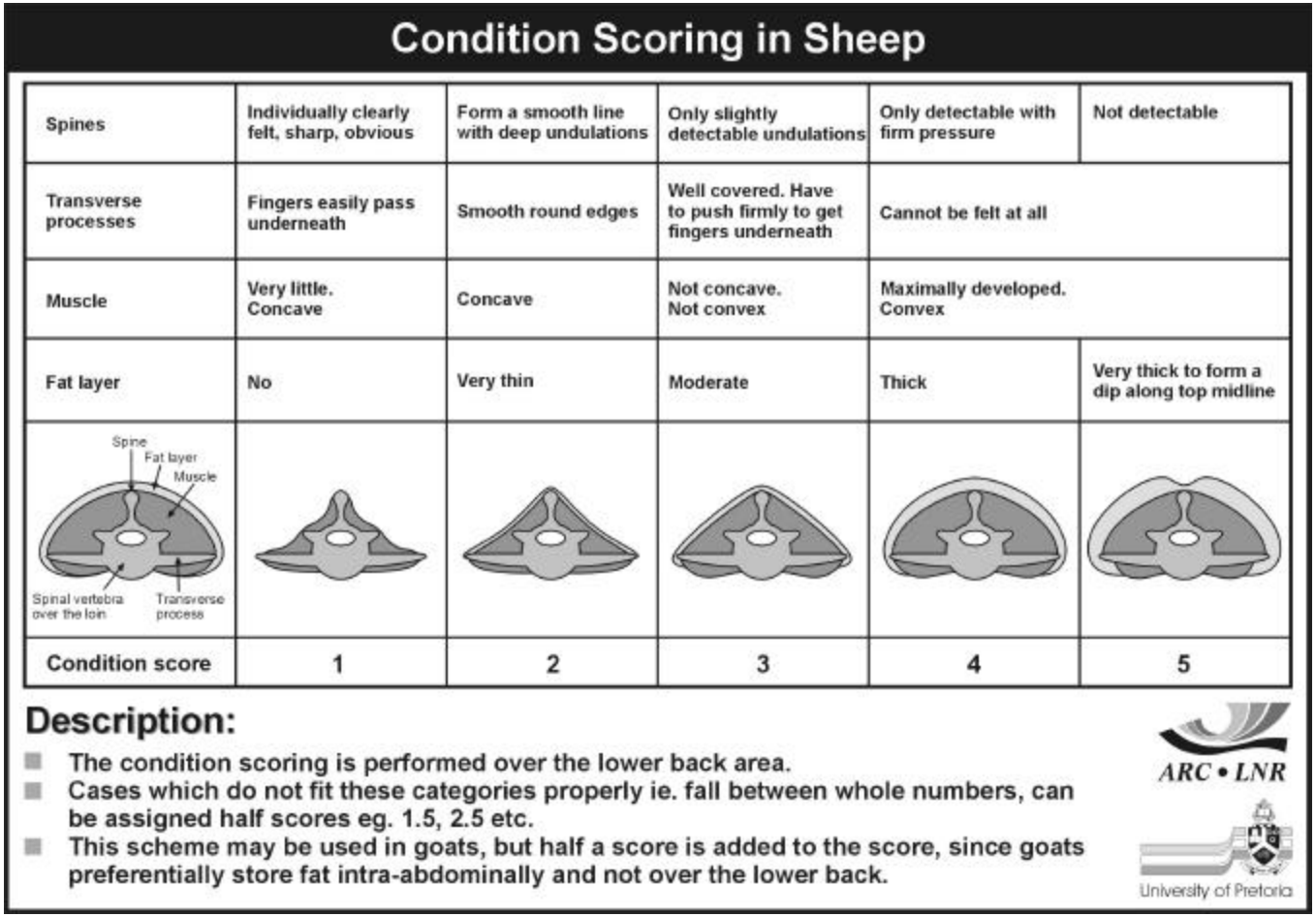
[a]Mean strongyle faecal egg counts in eggs per gram of faeces at last visit before FECR test (interval in days between last visit and FECR test).
[b]Treatment date of FECR test (interval in days between pre- and post-treatment collection of faecal samples).
[c]All anthelmintics were administered *per os*.

The animals were body condition scored according to the chart depicted in Fig. 2.1 which method agrees with that of Williams (1990).

2.3. Scoring for level of anaemia

At the scheduled visits, the author or one of the assistants on the project scored each animal for level of anaemia using the FAMACHA© colour card (Fig. 2.2). The author ensured that each assistant for whom scores were recorded had been adequately trained in the method. Excepting for the few visits that the author could not undertake, the scoring was always performed under his direct supervision. Occasionally, monitoring was done in-between scheduled visits by the farmer at Site 1, Impendle, and by the animal health technicians (AHTs) assisting with the project at Kraaipan. However, these scores were not included in any of the analyses discussed in this dissertation. Only the animals that were considered to be pale, i.e. colour chart categories four and

Fig. 2.1 : Condition scoring in sheep



five, were treated with an anthelmintic. At times, animals scored as category three were erroneously treated by the AHTs at Kraaipan and the farmer at Site 2, Impendle, initially misunderstood the aim of the trial and treated all his goats sometime between 24 November and 22 December 1998. Between 23 November and 21 December 1999, 10 goats were apparently treated by the shepherd of the farmer at Site 1, Impendle, but the animal identifications were not recorded.

To promote farmer co-operation, animals that showed signs indicative of *Oestrus ovis* infection (profuse mucous nasal discharge and difficulty in breathing through the nose) or infection with cestodes were at times also treated with an appropriate remedy. With respect to the trial animals, only one goat (at Kraaipan) was treated with rafoxanide [Nasalcur™, Hoechst Roussel Vet (now Intervet), 7.5 mg kg^{-1}] for *Oestrus ovis* infection during the trial. One to two sheep were treated on four occasions at Rust de Winter, and one to eight were treated on twelve occasions at Kraaipan. Twenty-two of the goats at Site 1, Impendle, and 25 of the goats at Site 2 were treated with niclosamide [Ex-a-lint™, Hoechst Roussel Vet (now Intervet), 50 mg kg^{-1}] during January
1999 for cestodes.

2.4. Statistical analyses

The data were entered in SAS (SAS Institute Inc., Cary, NC, USA) and this software was used throughout the analysis of the data. Sensitivity, specificity, predictive value of a negative and predictive value of a positive were calculated for the FAMACHA© clinical assay in goats. Smith (1995) defines sensitivity as the proportion of infected or diseased individuals with a positive test, or in the case of the FAMACHA© clinical assay, the proportion of anaemic animals correctly identified as anaemic. Test specificity is defined as the proportion of disease-free individuals that test negative, or the proportion of non-anaemic animals that are categorised as such. In the case of the FAMACHA© method, predictive value of a negative is the probability that an animal is not anaemic when the test result is negative for anaemia and vice versa for the predictive value of a

Fig. 2.2 : FAMACHA© anaemia guide (colours are not a completely accurate reflection of the original card)

positive. The sensitivity and specificity were tested statistically by means of Fisher's Exact Test for a two-by-two contingency table.

Two data sets for 1998/1999 and 1999/2000, respectively, were created from the FAMACHA© scores and haematocrit values obtained from the trial goats. Two-way frequency tables of haematocrit by FAMACHA© were drawn up, with FAMACHA© values four and five (or three, four and five) considered positive for anaemic animals and FAMACHA© values one, two and three (or one and two) considered negative test results, respectively (Table 2.4). Haematocrit was used as the gold standard by which anaemia was measured and two cut-off values for anaemia were assigned (less than 18% and less than 19%, respectively). In establishing the properties of a test, cut-off values are assigned to define the level of a test result that is needed to make or reject a diagnosis, in this case a diagnosis of anaemia (Smith, 1995).

The author chose to maximise the sensitivity and specificity of the FAMACHA© method in goats when the average of the sensitivity and specificity attains its highest value. This was calculated by the following equation for the present data:

$$(\text{sensitivity} + \text{specificity}) / 2 \quad \ldots (1)$$

In order to determine the percentage of animals treated in each case (FAMACHA© cut-off of four as opposed to FAMACHA© cut-off of three), the following calculation was also applied to the data:

$$(\text{true positive} + \text{false positive}) / \text{total number of animals} \times 100 \quad \ldots (2)$$

With respect to the FECR tests, anthelmintic efficacy was calculated by two methods: that of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al., 1992) and that of Presidente (1985). In the WAAVP method, the arithmetic mean of the treatment and control groups at 10 to 14 days after treatment are utilised to calculate the percentage reduction of FECs and the upper and lower 95% confidence intervals. Resistance is determined to be present

Table 2.4

Two-way frequency table of haematocrit by FAMACHA© with haematocrit cut-off of 18% (or 19%) and FAMACHA© scores 4 and 5 (or 3, 4 and 5) considered positive test results

		Anaemia	
		Present Ht[a] <18% (Ht <19%)	Absent Ht ≥18% (Ht ≥19%)
FAMACHA© score	Positive 4,5 (3,4,5)	True positive (TP)	False positive (FP)
	Negative 1,2,3 (1,2)	False negative (FN)	True negative (TN)

[a]Haematocrit.

Sensitivity = TP/(TP+FN) x 100
Specificity = TN/(FP+TN) x 100
Predictive value of a negative = TN/(FN+TN) x 100
Predictive value of a positive = TP/(TP+FP) x 100

if the percentage reduction is less than 95% and the lower confidence interval is less than 90%. If only one of the conditions is met, resistance is only suspected. In the method of Presidente (1985), the geometric or arithmetic means of FECs both on the day of treatment and 10 to 14 days thereafter are used in the calculation of the percentage reduction. Geometric means were used in the current calculations. Resistance has been said to occur when the percentage reduction is less than 80% in goats (Kettle et al., 1983). Reduced efficacy of anthelmintics in goats may be the result of a faster metabolism of the drugs in this species. Hence the lower value is used for goats when no difference between the dose for sheep and goats is indicated. The WAAVP method is considered to be a more conservative measure of anthelmintic efficacy (Coles et al., 1992). Focus has been placed on this method to allow for comparison between data of different authors, but the method of Presidente was included for completeness' sake.