

Chapter 2 Literature review

2.1 Common helminth parasites of dogs in South Africa: The distribution and prevalence of helminth parasites of dogs in South Africa are not well known. To date, only two publications (Ortlepp, 1934; Verster, 1979) have addressed the distribution of worm parasites of dogs in parts of South Africa. Ortlepp's study was restricted to the Pretoria area; Verster's report included other areas in South Africa.

Helminth parasites of dogs are important because they threaten the health and well-being of one of man's favourite pets, the dog, and can also infect humans. Zoonotic dog helminths possibly have more deleterious effects in humans than is commonly appreciated (Woodruff, 1975). It is difficult to diagnose zoonotic helminth infection in humans, as the worms rarely reach maturity (Woodruff, 1975) and therefore do not produce eggs that assist with the diagnosis.

The pathogenicity of a zoonotic worm varies (e.g., from the dermatitis caused by *Ancylostoma* spp. to the lethal consequences of *Echinococcus* spp. (Verster, 1986)). The latter is further complicated because the eggs of *Echinococcus* and *Taenia* spp. (e.g., *Taenia multiceps*) are indistinguishable and both can infect humans (Fripp, 1983).

Nematode parasites:

Hookworm disease is one of the major zoonotic diseases of the human population of warm, moist, tropical and subtropical countries (Fripp, 1983). In Pretoria 69% of

dogs necropsied (Verster, 1979), and in Zimbabwe 38% of faecal samples examined (Mukaratirwa and Busayi, 1995) showed *Ancylostoma* spp. Obwolo et al. (1991) found that 100% of dogs in their study had eggs of *A. caninum* in their faeces. *A. caninum* (Fig. 2.1) has been known, on rare occasions, to reach the intestine of humans and develop there to maturity (Dove, 1932; Hunter and Worth, 1945). This worm, and *A. braziliense* (Fig. 2.2), are more commonly associated with the migration tracts they cause in the skin of humans following percutaneous infection.



Fig. 2.1 *Ancylostoma caninum*
showing two pairs of three-
pronged teeth (arrows)

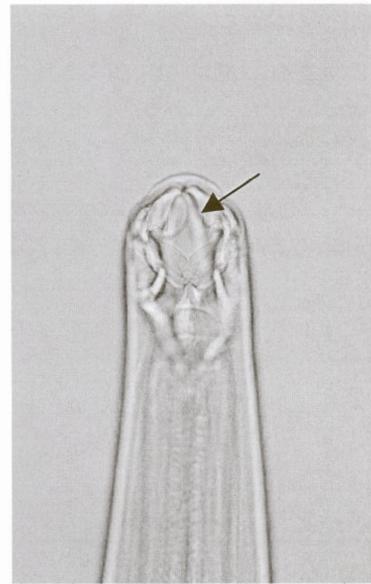


Fig. 2.2 *Ancylostoma braziliense*
showing ventral cutting plates
(arrow)

Biocca (1951) considered *A. braziliense* and *Ancylostoma ceylanicum* as two distinct species from descriptions of *A. ceylanicum* by Looss from Ceylon (modern day Sri Lanka) in 1911 and *A. braziliense* by De Faria from Brazil in 1914 and 1916. Later, Rep (1966) regarded them as synonymous. Indeed, today *A. ceylanicum* is not included in most veterinary helminthological textbooks, which suggests that this parasite is still commonly accepted as being identical to *A. braziliense*. The author

agrees with Biocca's statement for the reason that he (Biocca) was able to demonstrate anatomical differences between the two nematodes. He presented a valid argument that *A. braziliense* causes cutaneous larva migrans (CLM) in humans, whereas *A. ceylanicum* infects the small intestine in humans. For the purpose of this study, however, all hookworms identified in dogs that did not belong to the species *A. caninum*, and that resembled *A. braziliense* morphologically, were regarded as *A. braziliense*.

T. canis does not generally cause clinical disease in adult dogs, but does in pups. Infections of this parasite are relatively common; eggs are frequently found in the faeces of bitches and pups younger than one year old (Holland et al., 1991; Woodruff, 1975). In a study from Dublin, Ireland, all dogs were negative for helminth eggs on faecal flotation tests. However, 6.2% of the stray dogs and 5.3% of the canine faecal samples picked up from the streets were positive for *T. canis* eggs, and 51.2% of humans surveyed were seropositive for *T. canis* (Holland et al., 1991). In the same study, 38% of the soil samples from family gardens and 6% from public parks and open lots were also positive for *T. canis* eggs. This study suggests that the transmission rate of *T. canis* from the environment to humans is much higher than the transmission rate of this nematode between dogs or the levels of contamination of *T. canis* eggs in the environment. In a Zimbabwe survey, faecal samples from 7% of all the dogs tested contained eggs of *T. canis* (Mukaratirwa and Busayi, 1995). Toxocarosis in humans is probably more important than is recognised, as the infection rate is higher than previously realised; infections often remain undetected (Woodruff, 1975). Three human syndromes are recognised (Bass et al., 1983; Holland et al., 1991; Kinceková et al., 1996), viz. visceral larva migrans (VLM), ocular larva

migrans (OLM), and covert toxocarosis (subclinical with or without eosinophilia). OLM is clinically indistinguishable from retinoblastomas (Woodruff, 1975).

The study by Verster et al. (1991) reported that heartworm, *Dirofilaria immitis*, does not occur naturally in South Africa, because it has not been detected in dogs (except for a few cases reported in imported dogs), though its vectors are present. Van Heerden et al. (1980) reported that it is common in Kenya and it has been reported in Mozambique (E V Schwan and R C Krecek, 1997, personal communication). *D. immitis* can also infect humans, and although infections are self-limiting, they may cause changes which are radiographically visible, called "coin lesions" which may be misinterpreted as neoplasia and consequently result in unnecessary thoracic surgery (Bowman, 1995).

In its natural host, the dog, *S. lupi* causes the development of granulomas in its predilection and aberrant sites. In the oesophageal walls these may cause difficulty in swallowing, chronic coughing and vomiting, as well as ossifying spondylitis and hypertrophic osteopathy if situated in the aorta or thoracic oesophagus. This helminth is closely associated with oesophageal tumours, which originate in the granulomas, and may cause aneurysms if the wall of the aorta is involved (Fitzsimmons, 1966). The importance of this parasite of dogs is underestimated in Southern Africa (Mukaratirwa and Busayi, 1995; Reinecke, 1983), as it is more common than is realised. Obwolo et al. (1991) found that 47% of the faecal examinations from dogs in Zimbabwe had eggs of *S. lupi*. This nematode may also infect man (Woodruff, 1975).

T. vulpis in South Africa has been reported in Durban (Reinecke, 1983), where warm and wet conditions exist. Reinecke (1983) suggested that clinical signs develop only during severe infections. It is regarded as a zoonosis (Woodruff, 1975), although it has not been reported in humans in South Africa.

Cestode parasites:

In his study, Schoning (1994) determined that only half the dogs actually infected with cestodes gave positive results on faecal flotation. He found that tapeworms were best seen during necropsy. Schoning also considered that the use of the adhesive tape swab technique (Deplazes and Eckert, 1988) is the most efficient method for detection of taeniid infection in live dogs.

2.2 Study areas: Several criteria were used in selection of the five study areas in resource-limited communities. The first criterion was level of helminth control and intervention (i.e. deworming). Preference was given to minimal intervention. Secondly, existing linkages with the community were preferable. This was by current co-operation or animal welfare organisations that were already active in the area. Lastly, other factors considered were climate, accessibility, political stability and safety of co-workers. A summary of the study areas and the samples collected is given in Table 2.1.

Table 2.1 Village, province and categories of samples collected from dogs as well as questionnaires in five resource-limited study areas in South Africa

Village and province	Boksburg, Gauteng	Bloemfontein, Free State	Jericho, North-West	Zuurbekom, Gauteng	Mamelodi, Gauteng
Blood samples	✓	✓	✓	✓	✓
Faecal samples	✓	✓	✓	✓	✓
Adhesive tape swabs	✓	✓	✓	✓	✓
Organ samples	✓	✓			
Questionnaires	✓		✓	✓	✓

Three of the five study areas were visited as part of a Veterinary Needs Appraisal (VNA) (Mettrick, 1993) commissioned by the national government, and the other two were used for long-term cross-sectional studies. In order to plan proactive strategies (such as vaccination and correct management) for animal disease control it is essential that the veterinary needs of target communities be well understood. It is also vital that the animal owners in resource-limited communities be actively involved in the assessment of their own needs and implementation of strategies. The VNA method offers a holistic approach to this problem (McCrindle, 1998). The three short-term study areas (Jericho, Zuurbekom and Mamelodi) were each visited for a one-week period during which a VNA was carried out. The dog-owners were also interviewed using a questionnaire (Chapter 6). The dogs were examined, and adhesive tape swab, faecal and blood samples were collected for further processing and examination for helminth parasites and haemoprotozoa in the laboratory.

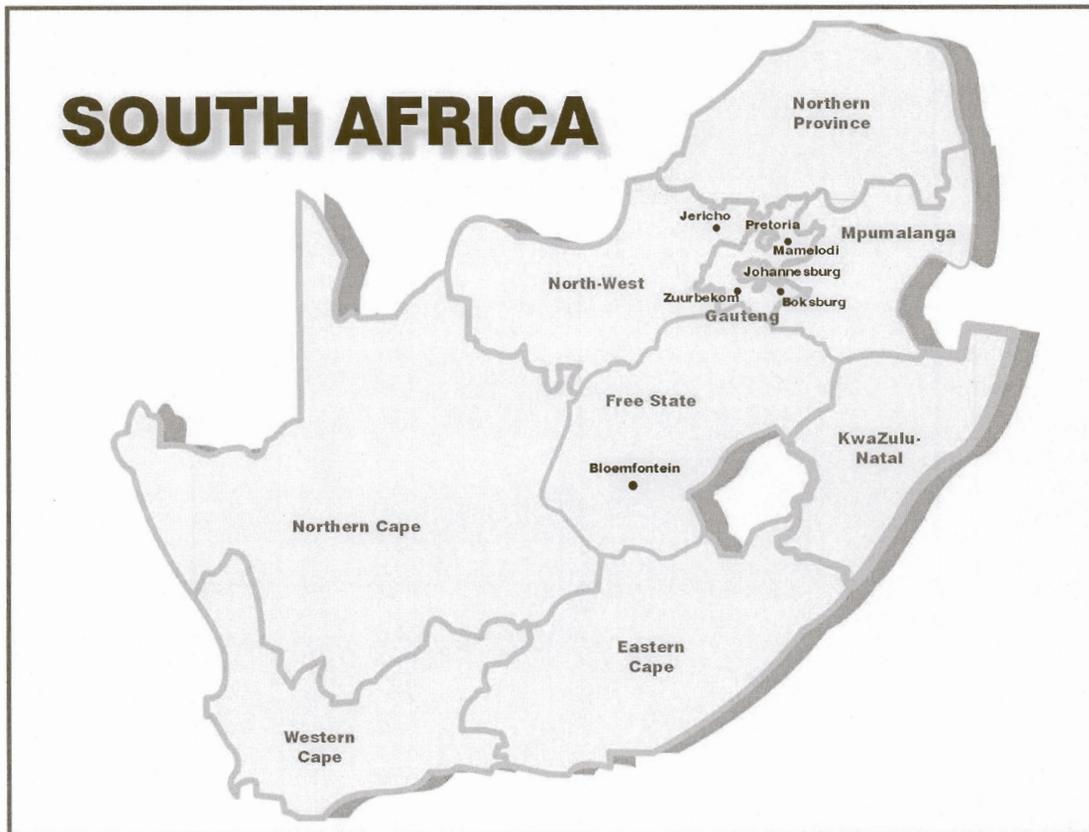


Fig. 2.3 The geographic locality of the five study areas in South Africa. These included Jericho, Mamelodi, Zuurbekom, Boksburg and Bloemfontein

The fourth study (Boksburg), one of the long-term cross-sectional studies, included questionnaires and collection of samples from live dogs (adhesive tape swabs, faecal and blood). Organ samples were also collected at necropsy from dogs, which had been impounded, and euthanized by the Boksburg Society for the Prevention of Cruelty to Animals (SPCA). The fifth study, undertaken in Bloemfontein, was also cross-sectional; blood, faecal and adhesive tape swab samples were collected from euthanized dogs at the Bloemfontein SPCA, but it was not possible to complete questionnaires, as there was no contact with the owners of the dogs.

2.3 **Biological samples from live animals and at necropsy:**

Sample collection from the live animal:

Permission was obtained from the owners before samples were collected from live dogs. In study areas where samples were collected from live dogs and where there was contact with the dog-owners, questionnaires were completed with information provided by the owners. The questionnaires (Appendix A) were completed by asking the owner the necessary questions in a semi-structured interview. The method for interviewing owners and the completion of questionnaires is discussed in Chapter 6. The aim was to determine the economic position of the owner and how this affected the dog's health and the care that it received, and the dog's social importance in the household. It also provided information on the dog's health, nutrition, movements and the environment in which it spent most of its life. For obvious reasons, aggressive dogs were not sampled. Sick or anaemic dogs were not sampled to avoid causing excessive stress.

Necropsy sampling:

These dogs, originating from resource-limited areas, had been impounded by the Boksburg and Bloemfontein SPCAs and were sampled after having been euthanized through intravenous administration of a barbiturate overdose.

2.3.1 Blood samples

Blood was collected from the live animal in bleeding tubes that contained Ethylenediamine Tetraacetic Acid (EDTA) anticoagulant using the superficial antebrachial vein while the animal was restrained, preferably by its owner. When dogs

were euthanized, blood samples were collected directly from the heart immediately after death (Fig. 2.4).



Fig. 2.4 Collection of a blood sample from a dog after euthanasia

Each blood tube was identified and placed in a cooler for transportation to the laboratory (Fig. 2.5). The purpose of collecting blood samples was twofold: to examine blood smears for the presence of haemoprotozoan parasites and microfilariae in the blood, and to identify the latter to species level, if found present.

The blood samples were stored at *ca.* 4°C and were processed within two months of collection. Coagulation occurred if the samples were left longer than this before being processed, which posed problems with the application of the filter tests.



Fig. 2.5 Equipment used for the collection and processing of blood samples

Two blood smears were made in the laboratory, one thin and stained with Cam's Quick Stain (Diff Quick) (Pratt, 1985) and one thick, stained with Giemsa stain. All the samples were also screened using the modified filter technique (Sloss et al., 1994), in which transparent 3 μm aperture polycarbonate filters were employed. The blood was not haemolysed with formalin because this would have interfered with the subsequent staining of the filters. After filtration of a 0.5 ml volume of blood from each sample the filters were mounted on microscope slides, left to dry, and then stained with Giemsa stain. Thin blood smears were examined for the presence of haemoprotozoa and thick blood smears and filters were examined for microfilariae. When the presence of microfilariae was detected on a filter by light microscopy, another slide was prepared in a similar fashion. The filter was mounted and stained using the acid phosphatase staining technique (Balbo and Abate, 1972) to identify the microfilariae.

Collection of faecal samples at necropsy generally occurred during the evisceration procedure (Section 2.3.4) when the rectum was bisected. A faecal sample of about 1 - 2 g was collected from the rectum of each dog and placed in a Faecalizer® well, which was then closed with a lid, marked, and placed in a cooled container.

Faecal samples for flotation tests (Pratt, 1985; Reinecke, 1983) give the best results if processed fresh or kept cool. Therefore, throughout these studies, faecal flotations were carried out within 24 hours. Under field conditions faecal samples may be kept for up to three days without deterioration of nematode eggs taking place, if stored (never frozen) at 5 - 7°C. No chemicals were used for preserving the faecal samples during this project.

The standard flotation technique was used for the examination of the faeces for the presence of nematode eggs. Although faecal flotation remains the best procedure for identifying intestinal nematodes (e.g., *Ancylostoma* spp., *T. canis*, *T. leonina* and *T. vulpis*) in live dogs, false negatives may occasionally occur, as the technique is dependent on the presence of eggs (Schoning, 1994). Occasionally some cestode eggs may be detected when this method is used. Each faecal sample was mixed with flotation fluid, which, due to its specific gravity being higher than that of the eggs, causes the latter to rise to the surface. The eggs were then picked up from the surface with a cover slip, which was mounted on a microscope slide and then examined with a light microscope using low magnification. The identification of helminth eggs was made according to Thienpont et al. (1979).

2.3.3 Adhesive tape swabs

Adhesive tape swabs (Deplazes and Eckert, 1988) were prepared to demonstrate cestode eggs, and/or segments, if present, on the peri-anal skin or hair from most of the dogs in the survey, including those euthanazed (Fig. 2.7). Most live dogs allowed the procedure to be performed without objecting to it. After restraining the animal, its peri-anal area was dabbed with the adhesive side of the tape (Fig. 2.8), which was then flattened with the adhesive down on a clean microscope slide.



Fig. 2.7 Equipment used for collecting adhesive tape swabs

Taeniid eggs are thick-shelled, and are able to withstand dry conditions and high and low environmental temperatures for up to a year. Swab samples do not therefore require any special treatment, even if left in a slide folder on the laboratory shelf for months, provided the place where it is stored is kept free of insects and rodents. In the laboratory, samples were examined at low magnification under a light microscope.



Fig. 2.8 Collecting an adhesive tape swab from a dog in Mamelodi

2.3.4 Organ samples

Organ samples (Jacobs et al., 1994) were collected from euthanized dogs. The heart and lungs with trachea attached were removed after the aorta and cranial and caudal vena cava had been tied off about 10 cm from the heart, depending on the size of the dog. The cranial end of the oesophagus and rectum were also tied off and the entire gastro-intestinal tract was removed. These organs were placed in a cooler box with ice packs for transport to the laboratory. The Boksburg samples were processed over the next 3 days following their collection, and the Bloemfontein samples were frozen until processed.

For preserving organs for nematode and trematode recovery at a later stage, freezing (Jacobs et al., 1994) is the recommended method. However, in the current study, it was found that ice crystals damaged the soft tissue of the cestodes, which made their identification to species level by examination of mature proglottids almost impossible. The integrity of the internal structures became so disrupted that they could not be recognised, and the hooks become dislodged from the scolex. The ideal method for

preserving cestodes for species identification is to "relax" them by placing them in lukewarm saline solution when fresh and thereafter in the preservative (e.g., 10% formalin or 70% alcohol). During this project collection of live material was not always possible. The organ samples were preserved by freezing and, although the taeniids could not be identified to species level, the number of scoleces could still be counted. Their presence was recorded as "taeniids" or *Taenia* spp.

The heart and its associated major blood vessels were opened up in the laboratory to determine whether any mature *D. immitis* were present. The trachea and bronchi were opened and examined for the presence of *Filaroides osleri*. Similarly, the entire gastro-intestinal tract was opened and its content and mucosal scrapings flushed over a 150 µm aperture sieve in two stages. The contents of the stomach were sieved first, followed by that of the small intestine and caecum. The material retained by the sieve was examined with the aid of a magnifying diamond sorting lamp. Any helminths present were recovered and preserved in 70% alcohol, a 70% alcohol and 5% glycerine mixture, or 10% formalin to be later identified, sexed and counted using a light- or stereo microscope under low magnification. The identification of helminths was according to Reinecke (1983).

Differentiation of dog ascarids

The identification of nematodes of dogs was done after the worms were mounted on a microscope slide under a cover slip with lactophenol as the clearing agent (Sloss et al., 1994). This "clears" the nematode, which enables the examination of its internal organs and other structures in order to identify it to species level.

The fourth-stage larvae (L₄) and some of the immature adults were small enough to mount as described above. However, technical difficulties arose with the mounting and examination of the mature ascarids of dogs. Firstly, the size of the microscope slides routinely used is too small for mounting these whole worms. Secondly, as the worms are quite robust and may be up to 2,5 mm thick, the surface tension of lactophenol is insufficient to prevent the lactophenol from running out of the space between the slide and the cover slip.

An alternative method for clearing and examining the ascarids was developed during this study. The mature adults were immersed in lactophenol in 20 ml screw-cap containers for two to three days until they had cleared sufficiently. They were then placed in the lid of a 12 cm petri dish, and its base was placed inside the lid and on top of the worms in order to flatten them for examination. Sufficient lactophenol was poured between the lid and the base of the petri dish to immerse the worms and to enable most of the air bubbles to escape, which allowed improved visualisation.

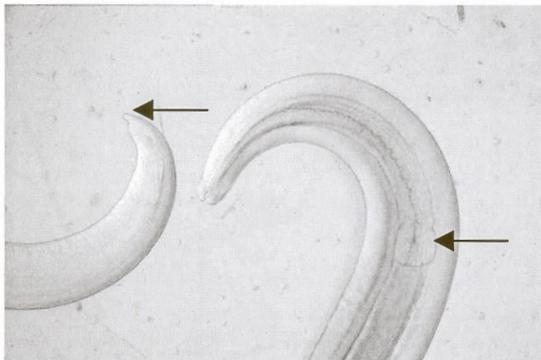


Fig. 2.9 *Toxocara canis* head and tail, terminal appendage on the male tail (left arrow) and oesophageal bulb (right arrow)



Fig. 2.10 *Toxascaris leonina* head and tail. Note there is no oesophageal bulb, nor is there a terminal appendage on the male tail

Four to six worms could be accommodated in one petri dish and they were examined under a stereo microscope to distinguish *Toxocara* from *Toxascaris* and to differentiate between the sexes without damaging the specimen by searching for eggs. The two species are differentiated from each other on the basis of their oesophagus morphology: *Toxocara* has an oesophageal bulb, whereas *Toxascaris* does not (Figs. 2.9 - 2.10).

2.4 Estimation of body condition: Estimation of a dog's body condition may serve as a tool to assess whether there are problems with its health or nutrition. Because the size and breed of a dog has an influence on its weight and may vary even in healthy dogs (Laflamme, 1993), it is more practical to advise owners on the condition of their dogs with the use of a body condition scoring (BCS) system such as the nine-point system of Laflamme (Laflamme et al., 1994).

For the present study, there was a need for a BCS system to assess the overall condition of the dog. It is desirable to be able to objectively measure body condition and correlate it with factors such as nutritional status or levels of helminth parasitism that may attribute to lower BCS.

The principle of BCS was originally developed as a visual estimation of body fat proportion for assessing the adequacy of nutrition, and it is at present commonly used in production systems such as cattle, sheep, goats, horses and donkeys. Veterinarians often use live mass estimation based on BCS for calculating drug doses for treatment of animals. Although this method can be variable as a means of estimating the live

mass of animals (Jones et al., 1989), it was used here as an overall measure of condition in large numbers of dogs in communities. The intent was to make a comparison between BCS and animal health practices, nutritional practices, levels of parasitism and other environmental factors.

BCS is significantly correlated with body weight for large dog breed females only, and highly correlated with percent overweight for large and small breed dogs of both sexes (Laflamme, 1993). The practical application of BCS in dogs may be more valid in order to determine the nutritional status, and in the presence of optimal nutritional conditions to diagnose chronic conditions such as parasitism and other debilitating diseases.

The BCS system used in this study is given in Table 2.2. This system is modified from the original nine-point system (Laflamme et al., 1994). Figs. 2.11 - 2.15 illustrate the characteristics used in this study. This five-point system was developed for the current study.

Table 2.2: Body condition scoring (BCS) system for dogs (modified from Laflamme et al., 1994)

Body condition scoring (BCS)	Description
1	Very thin. Ribs, lumbar vertebrae, pelvic bones and all bony prominences easily visible. No palpable fat. Some loss of muscle mass (Fig. 2.11).
2	Thin, underweight. Ribs easily palpated with minimal fat cover, tops of lumbar vertebrae and pelvic bones may be visible. Waist easily noted, viewed from above. Waist and abdominal tuck evident (Fig. 2.12).
3	Ideal. Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked up when viewed from the side (Fig. 2.13).
4	Heavy, overweight. Ribs palpable with fat cover. Fat deposits become evident over lumbar area and base of tail. Waist may become barely visible. Abdominal tuck may be absent (Fig. 2.14).
5	Obese. Ribs not palpable under very heavy fat cover. Heavy fat cover over thorax, spine, lumbar area and base of tail. Fat deposits may be present on neck and limbs. Waist and abdominal tuck absent. Obvious abdominal distension (Fig. 2.15).

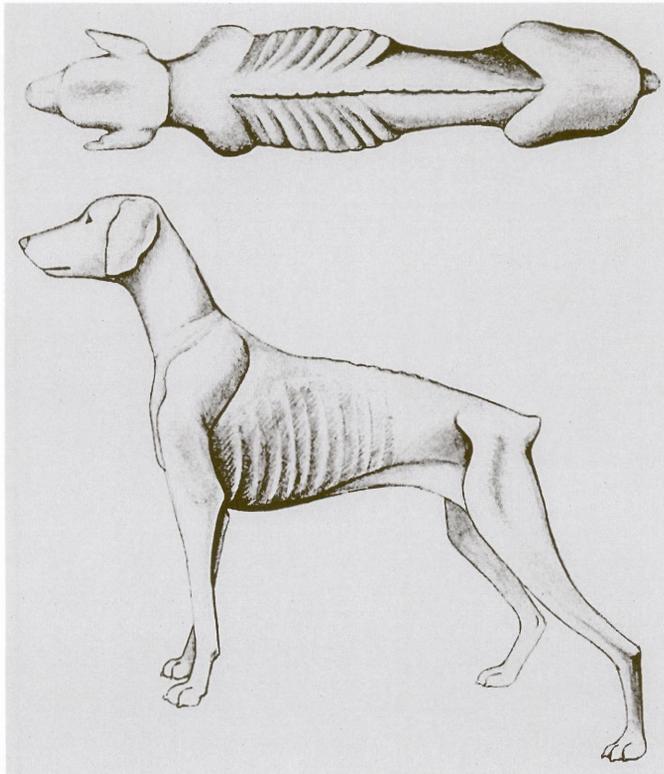


Fig. 2.11 Dog condition score 1

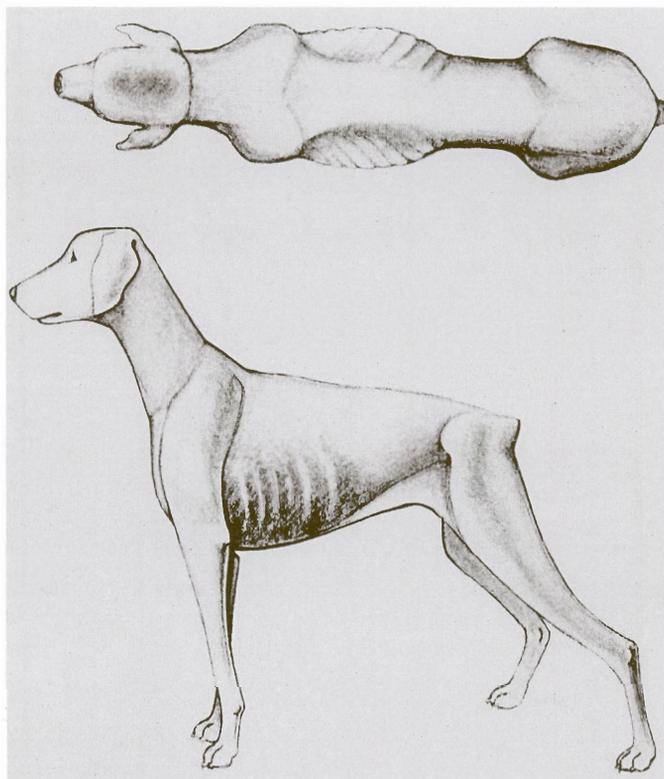


Fig. 2.12 Dog condition score 2

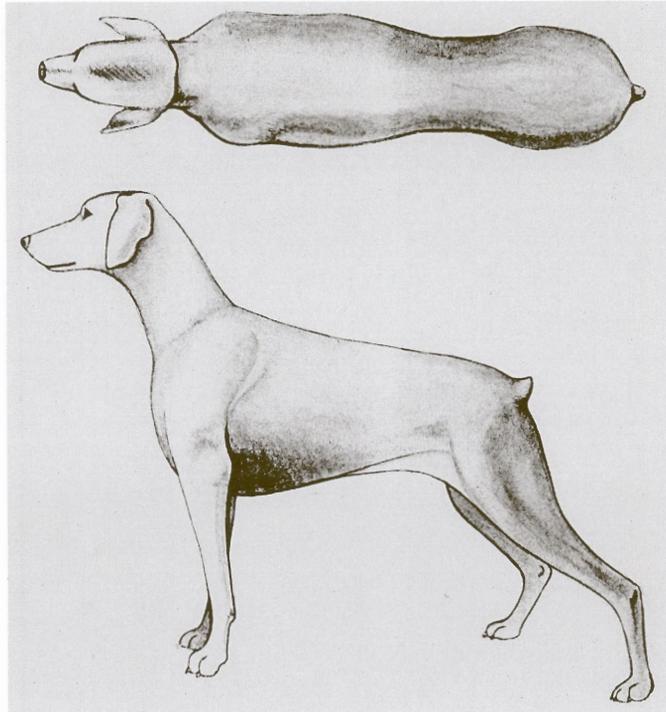


Fig. 2.13 Dog condition score 3

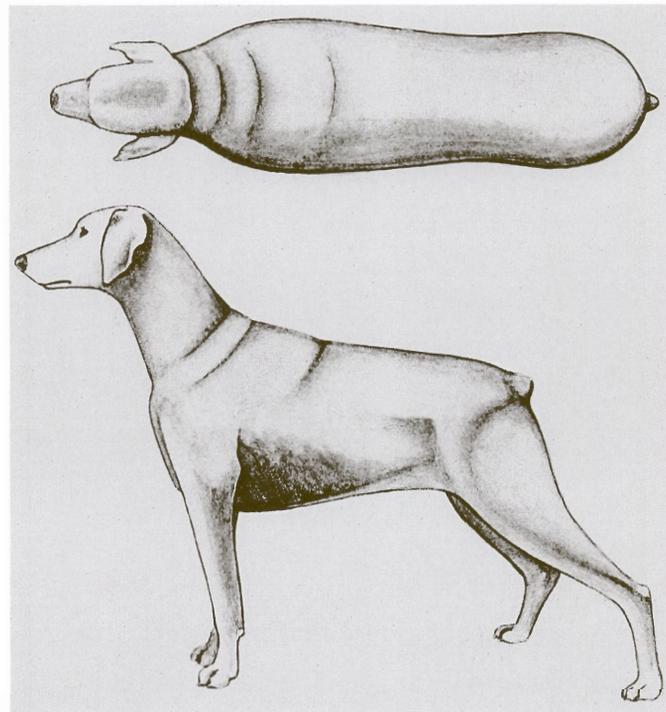


Fig. 2.14 Dog condition score 4

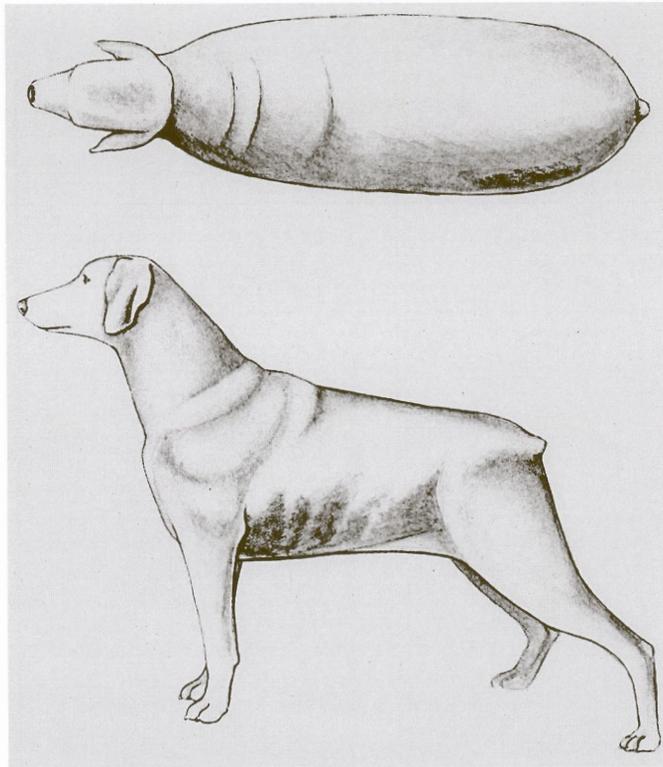


Fig. 2.15 Dog condition score 5

This method was particularly useful, as the scoring of body condition is quite consistent throughout and allows for the full spectrum of dog breeds of all shapes and sizes. It is objective and provides the means for easy, accurate assessment in all respects.

2.5 Criteria for estimation of dog age: Estimation of dog age is important because it is information required to determine the status of health levels in dogs. For example, younger dogs are more susceptible to helminth disease as evidenced by the clinical picture. The age categories were pups, sub-adults, adults and old dogs. The cut-off ages for these four groups were chosen for specific reasons.

Young dogs were regarded as pups if they were suckling from the bitch, and were still dependent on her milk for most of their nutritional needs. Pups may also be infected with *A. caninum* through the milk. Young pups of domestic dogs have maternal immunity only, as their own immune systems are developing and only gain full function at about the age of ten to twelve weeks (Tizard, 1996). The limits set for this age category were birth to three months (complete maternal independence).

Sub-adults are fully independent as far as their nutritional needs are concerned. They have a well-developed digestive capacity and a high metabolic rate, as they are still growing. They are typically very active, playful and inquisitive. The immune system is not yet mature and competent, and the animal is developing into sexual maturity. Milk teeth are being replaced with permanent teeth, and the dog's hair coat and body conformation are maturing. This age category is defined as three months to one year.

Adult dogs are more mature, socialised dogs with fully developed, competent immune systems and have reached sexual maturity. They are normally more resistant to diseases and helminth infections. Dogs in this category are one to eight years of age.

Old dogs have specific characteristics that may or may not make them more susceptible to diseases and parasitism. The following signs may be present: tartar on teeth and bad breath, canine teeth worn down, greying, senile cataracts, chronic kidney failure, calluses, aggression due to deterioration of senses, overgrown nails, deafness, arthritis, cancer, obesity or weight loss. The onset of old age in dogs is around eight years (Odendaal, 1998).

2.6 Statistical analyses: The SAS® System* was used for the statistical analyses of the data collected in the five study areas. The data collected were the following: total helminth occurrence; occurrence of *A. caninum*, *S. lupi*, *T. canis*, *D. caninum*, *Joyeuxiella* sp. and *Taenia* spp.; four age groups; five body condition score indices; dog diets (i.e., commercial dog food, leftovers, maize-based and meat); four climatic seasons; faecal flotation results; whether or not dogs were treated with dewormers; five Economic Situation Score (ESS) indices. SAS® is an integrated system of software that provides complete control over data management, analysis and presentation.

Two statistical methods were used for analyses of data collected during this project. The "general linear modelling" procedure (PROC GLM) and the regression procedure (PROC REG) were applied for finding associations between the dependent and independent variables (named above). The relevant data were entered into the SAS programme to test the hypotheses discussed in Chapters 3-5 (i.e., for all five study areas).

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