Chapter 4 - Approaches to the measurement of nematode infection

This chapter considers methods appropriate for the study of parasitism in wildlife, and uses them to devise ways of addressing the research aims of this project. The rationale and execution of the fieldwork undertaken in Kazakhstan are described, along with field, laboratory and statistical methods. The details of methods used in the identification of nematodes, and the statistical analysis of parasite counts, are discussed in chapters 5 and 6 respectively.

4.1 Approaches to data collection

The particular problems of measuring parasitism in wildlife are discussed below, along with the methods available for gastrointestinal nematodes of ruminants.

4.1.1 Direct measures of parasitism

Methods for measuring parasitism in free-living wildlife have on the whole been adapted directly from techniques used in domestic animals. Most directly, they involve the recovery and enumeration of parasites from the organs of the dead host. The majority of gastrointestinal nematodes are easily recovered by opening and washing the digestive tract (Reinecke, 1984). The procedure is straightforward and requires only the most basic equipment, but requires the death of the host. Leaving aside ethical problems, this may be undesirable if the viability of the population being studied is of concern. Removing animals will also potentially affect the dynamics of both host and parasite populations, so provoking artificial changes in the system under study. These problems are circumvented if only those animals found dead are analysed, but this will produce bias (see chapter 2). It may also be difficult to find freshly dead animals, especially in species that range widely at low overall density, such as the Saiga.

The direct measurement of parasitism in free-living wildlife can also be complicated by logistical problems, which are ignored in most techniques developed for the *post mortem* examination of domestic animals. Much sampling of wildlife occurs in the

field in remote locations: material must then either be transported back to a laboratory for study, or examined in the field. Sampling methods that require unrealistic facilities, resources or precision are unlikely to be useful in these circumstances. Time may also be limiting, while sample size is especially important when sampling for parasites (see chapter 6). Little attention has been paid in the literature to the development of rapid, simple methods for parasite recovery that require only basic equipment and minimise the amount of material that is to be transported back from the field. Many past studies of parasitism in wildlife, meanwhile, have relied on opportunistic access to dead animals, and often suffer from biased sampling, inefficient recovery and low sample size (Grenfell and Gulland, 1995; Borgsteede, 1996; Tompkins *et al*, 2002*a*). The present study presents an opportunity to sample a relatively large number of healthy animals that are culled at the same time.

4.1.2 Indirect measures of parasitism

Indirect methods for detecting gastrointestinal nematodes have been developed for humans and domestic animals. Chief among these is the faecal egg count (FEC). Methods differ in detail, but in most, nematode eggs are separated from the faecal material by flotation, and quantified by counting eggs at a given dilution.

Since Stoll (1930) pioneered the use of faecal egg counts (FEC) for measuring gastrointestinal nematode parasitism in sheep, their reliability at both the individual and population levels has been questioned, due to their notoriously high variability (Rossanigo *et al*, 1991). While part of this is due to variation in underlying parasite distribution (Roberts and Swan, 1982), further inaccuracies are introduced by fluctuations in faecal output, both between hosts and with time (France *et al*, 1988; Brundson, 1970), variation in egg output by worms on a diurnal basis or with age (Brundson, 1970), and variable faecal consistency (Peters and Leiper, 1940). Similar sources of inaccuracy have been identified in FEC of intestinal helminths in man (Hall, 1982). Even at the level of the individual host, the repeatability of faecal egg counts may be poor (De Vlas *et al*, 1992; Gasbarre *et al*, 1996). At the population level, the usefulness of FEC as a measure of parasite abundance as well as prevalence relies on the correlation between them and adult worm burden. Such a correlation has been demonstrated for several nematode genera in growing cattle (Bryan and Kerr,

1989*a*; Murrell *et al*, 1989), and less convincingly in lambs (Thomas and Boag, 1973), but other studies have found no relationship between worm burden and FEC for nematodes in young cattle (Rubin, 1967; Brundson, 1971; Smeal *et al*, 1977), nor for *Oesophagostomum* in sows at pasture (Rose and Small, 1980). This may be due to density-dependent parasite fecundity resulting from acquired immunity. Egg output was found to fall faster than worm burden in calves experimentally infected with *Ostertagia ostertagi*, suggesting that fecundity is affected by the host response (Michel, 1969*a*, 1969*b*). Reduction in FEC with experience of infection was also demonstrated for nematodes in goats by Vlassoff *et al* (1999), and for *Ostertagia ostertagi* in calves by Gasbarre *et al* (1990), who further related the pattern to an increase in circulating anti-*Ostertagia* antibodies.

Kingsbury (1965), McKenna (1981) and Tarazona (1986) all found that FEC broadly reflected worm burden in sheep if both measures were categorised as low, moderate or heavy. However, low counts of *Nematodirus* eggs, in particular, did not rule out high worm burdens, and many very high egg counts (>3,000epg) were due primarily to infection with the very fecund genus *Haemonchus*. Pre-patent infections are not detected by FEC. This may be important in the diagnosis of clinical nematodosis in individual cases, particularly when pathology is largely caused by the immature forms, as in nematodirosis (McKenna, 1981).

Whether or not faecal egg output is correlated with intensity of infection across a wide range of worm burdens, very low numbers of fecund adult worms will produce low numbers of eggs (providing mating takes place). The use of FEC for detecting infection, and the reliability of measures of prevalence based on FEC, will then depend on test sensitivity. Faecal egg density is typically low in cattle due to the diluting effect of the large volume of faeces (Michel, 1968; Burrows *et al*, 1980), and adaptations to standard flotation methods for this species involve examining larger amounts of faecal material. This can be achieved by reducing the dilution of faeces (MAFF, 1986), or, alternatively, by examining supplementary material using the unmodified standard test. In the former case, faecal debris is concentrated and may make reading the test difficult, while carrying out serial tests is time consuming. The increased accuracy achieved by examining ever larger quantities of lightly infected faecal material may not be worth the extra time and effort needed, and the method

selected should reflect expected levels of infection and the sensitivity and accuracy required. This seems to have been a matter of qualitative judgement in most published studies, with standard dilutions prevailing. Methods devised for the detection of relatively intense infections in domestic animals may therefore not be suitable for lighter infections, for example in wildlife or extensively farmed livestock (Arneberg and Folstad, 1999). Development of a rationale for selecting a method that optimises sensitivity, accuracy, sampling effort and time in the laboratory, would enhance the usefulness of FEC as a tool for the investigation of parasitic infection in wildlife.

The usefulness of FEC as a measure of parasitism is further limited by our inability to reliably distinguish between the eggs of different species. Efforts to resolve this through detection of parasite antigen in faeces, either directly through ELISA (Ellis *et al*, 1993), or by amplifying ribosomal DNA using PCR (Zarlenga *et al*, 2001), have been partially successful, but are not yet in widespread use. Differentiation of trichostrongylid eggs for all but a few easily recognisable species relies on faecal culture. The ratios of larvae so recovered may bear little relationship to the species composition of the worm population of origin (McKenna, 1990).

Despite their problems, FEC have several important advantages over *post mortem* parasite recovery. Faeces can be collected quickly from a large number of hosts, without killing or harming them, and only a small amount of material is necessary, alleviating transport problems. A large number of faecal samples can provide a more useful reflection of parasite abundance in sheep at the flock level than a small number of *post mortem* counts (Stampa and Linde, 1972), in spite of their higher variability. Samples can also be processed quickly, and when time is taken into account, FEC may be the method of choice for parasite surveys at the population level, provided they are known to be correlated with adult worm burdens. Where parasite transmission is the focus of study, the extent to which animals contaminate the environment with free-living parasite stages is of direct interest, and is most appropriately measured directly using FEC. Wild animals, however, must be captured if faeces are to be taken directly from them. If the animals are killed for sampling, the advantages of FEC over direct collection of adult nematodes are largely lost. However, if faeces from known individuals can be collected non-invasively (for example, by sampling middens, or following grazing individuals), and there is

evidence for a relationship between FEC and adult worm burden, faecal samples could be very useful in wildlife studies. Even where individual sources of the faeces cannot be identified, an adequate number of samples can give an approximate measure of parasite abundance in the population as a whole. FEC were used in parasitological monitoring of individual tagged feral Soay sheep on St. Kilda (Gulland and Fox, 1992; Gulland *et al*, 1993). Elsewhere, FEC provided a reasonably good reflection of the burdens of adult *Marshallagia marshalli* and *Ostertagia gruehneri* in Svalbard reindeer (Irvine *et al*, 2001), but were less satisfactory in prediciting individual nematode burdens in blue wildebeest in South Africa (Horak *et al*, 1983). Unvalidated FEC have also been used to compare parasitism in adult and sub-adult African buffalo (Penzhorn, 2000).

Other indirect measures of parasite infection include serum antibodies to *Ostertagia ostertagi* (Gasbarre *et al*, 1990), peripheral eosinophil counts for *Teladorsagia circumcincta* (Stear *et al*, 1995*a*), serum pepsinogen or gastrin for abomasal nematodes (Brundson, 1971; Fox, 1997), and packed cell volume for haemonchosis (Le Jambre, 1995). However, these methods are either non-specific (i.e. do not reflect any parasite species in particular), poorly correlated with parasite abundance (Murrell *et al*, 1989), expensive or impractical for use in the field. The use of serological tests in species for which they have not been validated is also liable at best to introduce additional uncertainty, and at worst to not work at all. Stear *et al* (1995*a*) found that FEC provided a better reflection of adult *Teladorsagia circumcincta* burden in sheep than either peripheral eosinophil counts or plasma pepsinogen concentrations. Serum pepsinogen concentration, on the other hand, better predicted food intake and weight gain in captive wild reindeer than FEC, even though the correlation with abomasal nematode burden was poor in both cases (Arneberg and Folstad, 1999).

4.1.3 Overdispersion and sample size

Both direct and indirect measures of parasitism must take account of overdispersion in parasite distributions, which has many implications for study design. Methods for comparison of sample means that assume Normal distribution about the mean are flawed (Rózsa *et al*, 2000), as are many methods for determining the sample size necessary to identify significant differences between means (Thrusfield, 1995). Even

non-parametric tests for sample means may not be applicable, if distributions of samples differ (Sokal and Rohlf, 1995). The increased chance of finding heavily infected hosts in large samples also poses the risk of Type I error when comparing samples of unequal size.

The implications of overdispersion for the statistical analysis of parasite data are discussed further in chapter 6. For study design, perhaps the most important point is that larger sample sizes will be required to make reliable comparisons between means. It is, however, difficult to quantify the required sample size, particularly if there is no previous information on the expected form of the distribution. The chance of finding parasites in a given host, and hence measured prevalence, is also affected by parasite distribution. Many methods for predicting the sample size needed to detect parasitic infection at a given prevalence, however, remain valid, since they make no distributional assumptions (Thrusfield, 1995).

Regardless of data form, there is a relationship between the sample size and the number of comparisons that can be made, while resources will always set an upper limit to the number of animals that can be sampled. In surveys of gastrointestinal nematodes, FEC can generally produce a larger sample size than gastrointestinal washes given similar resources, but will usually give only a vague indication of the species composition of adult worm populations. For these reasons, this study uses faecal egg counts complimented by gut washes in a sub-set of animals, so maximising the information gathered. Sampling considerations are discussed in section 4.3.

4.1.4 Sampling bias

Bias in the selection of animals for sampling is a common feature of wildlife parasite surveys, as already discussed in chapter 2. Even when hunting healthy animals specifically for research, bias is difficult to avoid, since not all members of the population are likely to be equally available or make equal targets. Purposive sampling, which aims to sample ‘representative’ individuals, can be a useful way of ensuring that important strata of host types are sampled, but is likely to artificially depress apparent variation within strata, relative to random sampling (Thrusfield, 1995). Random sampling of wildlife populations is likely to be an unrealistic

aspiration in many cases, due to licensing restrictions, and unavoidable bias in the animals that present themselves for sampling.

Greater control is often possible over livestock, but even here random sampling is often an illusion. Abattoir surveys, for example, can only ever be representative of those animals presented for slaughter. Surveys at the farm level, on the other hand, may be non-random due to variable farmer co-operation, and unevenness in the farm types sampled. Thus, random selection of farms will lead to disproportionate representation of animals from small farms, and results should not be extrapolated to the population as a whole. There are many ways of trying to avoid bias in farm surveys (Thrusfield, 1995). In this study, however, practical considerations limited their applicability, as will be seen in section 4.3.

4.1.5 Free-living parasite stages

In addition to measures of parasitism in the host population, it may be useful to estimate the size and distribution of the free-living part of the parasite population. In the trichostrongyloids, the density of infective larvae on the herbage can be used as an index of the relative risk and likely intensity of new infection on a particular pasture at a particular time of year. In veterinary research, this approach has formed a part of both general epidemiological studies aimed at building an understanding of typical patterns of infection in a region, and specific investigations aimed at managing disease at the farm level through optimal grazing strategy (see chapter 8). Less use has been made of estimates of pasture larval contamination in studies of free-living wildlife, possibly due to practical considerations, though they were used by Gulland and Fox (1992) in studies of Soay sheep.

In estimating larval density on vegetation, herbage is most simply collected directly from the pasture, and larvae extracted by washing and sedimentation (Taylor, 1939), or by sieving (Lancaster, 1970). Many more complex techniques have been developed, and are reviewed by Couvillion (1993). However, all suffer from very large variation both within and between studies, and from a questionable relationship to the number of larvae likely to be actually ingested by grazing animals. Most significantly, larvae are unlikely to be distributed evenly over pasture, since neither

ruminant grazing patterns (and hence pasture contamination), nor conditions for larval development and availability, are uniform in space (Levine, 1963; Boag *et al*, 1989; Krecek *et al*, 1990). Most techniques aim to compensate for this unevenness by sampling from a suitably large number of points on the pasture. However, there have been few attempts to standardise for size of pasture or density of herbage, and studies conducted at different times may be confounded by variation in larval availability with time of day, climatic conditions, and forage type (Couvillion, 1993). Comparison between studies is often meaningless due to differences in laboratory techniques or operator variation.

Since estimation of larval density usually aims to quantify infection risks to grazers, refinements to techniques have sought to emulate ever more closely the grazing behaviour of hosts (Bryan and Kerr, 1988). It is unlikely, however, that human samplers will be able to accurately simulate ruminant grazing behaviour. Larvae are likely to be concentrated near the faecal mass, for instance (Williams and Bilkovich, 1973), and the extent to which animals graze these areas may vary with their nutritional status or stocking density (Hutchings *et al*, 1998). Grazers can be used as sampling tools, the researcher collecting the ingested herbage through surgical fistulae (Gettinby *et al*, 1985), or by killing tracer animals after 2-4 weeks and counting their worm burdens (Waller *et al*, 1981). However, the inaccuracies inherent in human sampling techniques are then replaced by vagaries in the grazing patterns of individual animals, and the fate of larvae once ingested. Gettinby *et al* (1985) found that fistulated sheep provided no less variable an indication of pasture contamination than did direct sampling of herbage, while Waller *et al* (1981) found that variation in estimates of pasture contamination were much higher using tracer animals than direct sampling, particularly for *Nematodirus*. The use of tracer animals in wildlife studies is, in any case, likely to be impractical in almost all cases.

Most workers concede that absolute numbers of larvae recovered from herbage by direct sampling are all but meaningless. However, relative numbers obtained in different areas and at different times of year can give an approximate idea of the relative risk and quantity of larval ingestion, provided methods are standardised and take into account the non-random distribution of larvae by ensuring that adequate

samples are taken. In this study, standard methods were adapted to take account of expected low larval density (see section 4.3).

4.1.6 Objectives of fieldwork

Objectives were formulated by devising hypotheses based on the key research questions outlined in earlier chapters, deciding how these were to be tested, and itemising the data required to do so. This process provided a guide to the fieldwork: the chief objectives are listed in Table 4.1.

Table 4.1. Objectives of fieldwork. The chapter which is most relevant to the objective is noted in the right hand column. Table continued overleaf.

	Objective	**Test**	**Data**	**Fieldwork**	**Chapter**
1	List parasite species known to infect saigas.	Survey Russian literature.	Species lists in saigas.	Find and translate previous work.	3
2	List parasite species currently infecting saigas.	Parasite identification.	Presence/absence of species.	Sample a representative number of saigas for parasites.	5
3	Quantify current levels of parasitism in saigas, especially by gastrointestinal nematodes.	Collect sample statistics that reflect parasite abundance.	Prevalence and mean intensity of infection by species.	Count parasites recovered from an adequate number of hosts.	6, 7
4	Determine whether parasitic infection is likely to affect saiga fitness.	Evidence of association between parasite infection and measures of reduced fitness.	Evidence of pathology or reduced body condition in heavily infected saigas.	*Post mortem* examination of saigas, including pathological examination and assessment of body condition.	7
5	Determine whether there is effective immunity to parasites in saigas at their present levels.	Compare parasite abundance in saigas of different ages, to assess whether exposure confers resistance.	Parasite abundance in young and adult saigas.	Worm counts from animals of different age, and a reliable index of the age of individual saigas.	7
6	List parasites of domestic ruminants in Kazakhstan, and current understanding of their local epidemiology.	Survey Russian literature, and unpublished work.	Species lists and abundance data, past experimental work, expert opinion.	Find and translate previous research, evaluate ongoing work in the field.	3
7	Determine the main factors affecting the abundance and distribution of parasites of domestic ruminants now.	Analyse associations between parasite burden and factors such as location, husbandry and grazing management.	Measures of parasite burden in domestic ruminants from a variety of locations and husbandry / grazing systems.	A large number of faecal samples. Also, a smaller number of gut washes to confirm species present, and the existence of a relationship between parasite burden and faecal egg count.	7
8	Determine whether saigas and sheep genuinely share parasite species.	Compare parasites from saigas and sheep, and decide whether there is overlap.	Parasite specimens from saigas and sheep.	*Post mortem* recovery of parasites from both hosts.	5

Table 4.1, continued.

	Objective	**Test**	**Data**	**Fieldwork**	**Chapter**
9	Identify likely places and times of parasite transmission between saigas and sheep.	Devise a rational model that incorporates existing data on saiga and sheep densities and movements, and parasite development and survival parameters.	Saiga population density and movement, stock population density and movement, parasite development and survival parameters.	Published and unpublished data, and expert opinion.	8
10	Assess likely effects of climate, and changes in host density and movement, on parasite abundance and transmission within and between host species.	Compare model predictions with past data, and extend predictions to hypothetical situations.	Climatic data, and parasite abundance in saigas and livestock in the past.	Information gathering.	8, 9
11	Devise and evaluate useful methods for the detection and measurement of parasitism in wildlife in difficult field conditions.	Compare the performance of different methods with each other, and with more established methods in the literature.	Test performance, and data on parasite distributions within and between hosts that will affect test reliability.	Direct and indirect parasite counts from saigas and livestock.	6
12	Make sensible recommendations for the control of parasite transmission in and between saigas and domestic livestock, and for prioritising and advancing further work.	A combination of model output, and a critical assessment of available data and key shortfalls in it.	All the above.	All the above.	10

4.2 Fieldwork design and execution

4.2.1 Time spent in Kazakhstan

Visits to Kazakhstan took place between 1997 and 2002, as summarised in Table 4.2. Saigas and livestock in several different areas were sampled for helminth infection (Fig. 4.1). Information on previous parasitological studies, as well as population and climatic data, were also obtained in Kazakhstan from archives and published sources.

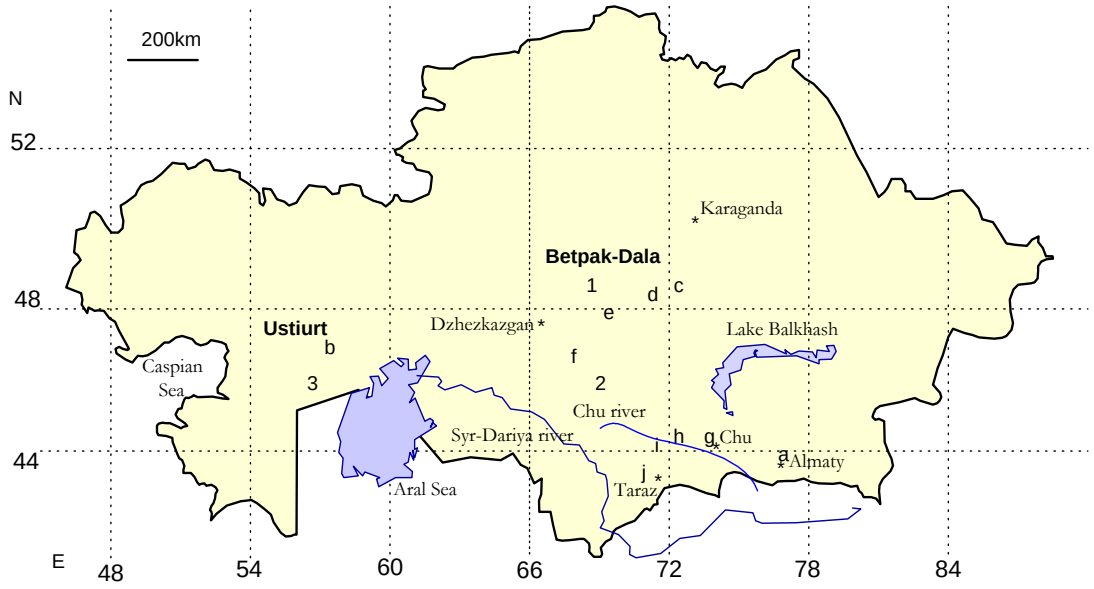

Figure 4.1. Locations of sampling sites of saigas and livestock in Kazakhstan, in chronological order. 1 and 2 represent saiga hunts in autumn 1997, which progressed southwards to follow the migrating herds, 3 is the saiga calving ground visited in Ustiurt in spring 1998. Livestock samples: a = Almaty (autumn 1997), b = Ustiurt (spring 1998), c-f = northern transect (Betpak-Dala; summer 1998), g-i = southern transect (Chu; autumn 1999), j = Taraz (Dzhambul; autumn 1999). Locations a and j in southern Kazakhstan (SK) are outside the saiga range. *= town

Table 4.2. Details of fieldwork in Kazakhstan. Durations of expeditions include practical preparations and processing of samples.

Trip	Date	Duration (weeks)	Location	Task	Data collected
1	Sep-Dec 1997	6	Almaty	Background information, language familiarisation, expedition preparations.	
		6	Betpak-Dala	Saiga hunting expedition.	Samples of helminths from 133 saigas (gut washes).
2	Apr-May 1998	6	Ustiurt	Saiga calving expedition.	Samples (gut washes) from 11 saigas; also faecal and herbage samples from saiga pasture, and 50 faecal samples from livestock.
3	June-Aug 1998	3	Almaty, Karaganda, Dzhezkazgan	Visits to government offices, laboratories and farm administrations.	Information on farming, stock numbers, veterinary infrastructure and parasitological monitoring.
		4	Betpak-Dala	Farm survey.	Faecal samples from 300 domestic ruminants.
		2	Almaty	Sample processing.	
4	Apr 1999	2	Almaty	Present preliminary findings to conference on biological resources of Kazakhstan.	Review of literature in Russian.
5	Oct-Dec 1999	4	Almaty and Taraz (Dzhambul)	Licences and administration. Search of literature and archives.	Published and unpublished past surveys of saiga and livestock parasites.
		4	Dzhambul *oblast*	Survey of sheep parasites.	Gut washes from 30 sheep; faecal samples from 200.
6	Dec 2000	2	Almaty	Zoology conference. Literature review.	Published and unpublished data on parasite development; update literature review.
7	Mar 2002	2	Almaty	Presentation of results, and feedback from experts. Address gaps in literature.	
Total		40 weeks	Kazakhstan		

4.2.2 Expeditions

Expeditions were planned to collect helminth samples from saigas and livestock in the field, and test hypotheses drawn from Table 4.1. Priority was attached to the collection of different samples according to their importance to the chief objectives of the study, and to the availability of existing data (Table 4.3).

Expeditions and sample collection were subject to severe practical constraints. Much of the saiga range is remote from centres of human habitation: as an example, the saiga calving grounds in Ustiurt are seven days' journey from Almaty, and two days from the nearest town. Logistical and budgetary considerations meant that most parasitological work was undertaken alongside other scientific objectives, on joint expeditions. Additional constraints were imposed by regulations governing the hunting of saigas: most *post mortem* examination of saigas was restricted to the official hunting season in October and November. Hunting was predominantly undertaken by state-controlled hunting co-operatives, and carcases sold for food. In November 1997, only female and sub-adult saigas were taken. Ten male saigas were culled in Ustiurt in May 1998 under special licence: the single female sampled was found injured, and euthanased. In 1998, following increases in illegal hunting and worrying population decline, the government of Kazakhstan imposed a total moratorium on saiga hunting (Milner-Gulland *et al*, 2001). This forced a restructuring of sampling plans, which originally aimed to compare parasite burdens in saigas in different regions and in different years and seasons.

Sampling of livestock was also subject to state regulation and logistical limitations. By 1998, most abattoirs and meat plants in or close to the saiga range were no longer operational, and slaughter was undertaken by stock owners or meat retailers. This, along with manpower limitations and lack of facilities, ruled out central *post mortem* sampling of large numbers of animals. Given good prior knowledge of parasite diversity in livestock in Kazakhstan, and the ease with which the three key genera of gastrointestinal nematode can be distinguished coprologically, the livestock survey used faecal egg counts, with a small number of sheep sampled *post mortem* to verify the dominant nematode species and establish a correlation between faecal egg density

and adult worm burden. Sampling focused on sheep, with some goats, cattle and camels included for comparison.

The samples taken from saigas and livestock are summarised in Table 4.4, and their locations in Fig. 4.1. More detailed information on the organisation and bureaucratic requirements of the 1997 and 1998 expeditions can be found in Lundervold (2001).

4.2.3 Other data sources

Population and range size estimates for saigas were obtained from Bekenov *et al* (1998), and more recent figures from Milner-Gulland *et al* (2001), and from the Institute of Zoology in Almaty. Official data on livestock numbers and production were obtained from Kazakhstan government sources, and corroborated with figures from administrative authorities at the *oblast*, *raion* and farm levels. Most of this work to 1998 was undertaken by Sarah Robinson, and is discussed in Robinson (2000). Climatic data were also those collected and used by Robinson (2000).

Published work on parasites of saigas and livestock in Kazakhstan was obtained principally from the Institute of Zoology, and the Veterinary Scientific Research Institute, both in Almaty, and the K.I.Skrjabin Veterinary Scientific Research Station in Taraz (Dzhambul), and also from the Russian Academy of Agricultural Sciences, Central Scientific Agricultural Library, and the K.I.Skrjabin All-Russian Scientific Research Institute of Helminthology library, both in Moscow. Unpublished records of parasite surveys were obtained from the above-mentioned sources in Kazakhstan, as well as from the Dzhezkazgan *oblast* veterinary parasitology diagnostic laboratory (now subsumed into Karaganda *oblast*). With the exception of Bekenov *et al* (1998), findings were reported in Russian, and translated as part of this project. Russian terms and place names used in this thesis are transliterated according to the Library of Congress system (Brown, 1996). Where place names have changed since independence, the new name is used, followed by the old name in brackets.

Table 4.3. The sampling plan in order of priority. Objectives cited relate to Table 4.1. Sample sizes are discussed in the text, section 4.3.

Host species	Sample		Purpose	Objectives
	1	2		
Saiga	Counts of abomasal nematodes.		List parasite species, prevalence, abundance and distribution.	2, 3, 8, 11
Saiga	Counts in young saigas.	Counts in adult saigas.	Age-intensity as an indicator of density dependence.	5
Saiga	Counts in thin animals.	Counts in fat animals.	Association between parasitism and body condition.	4
Sheep	Faecal egg counts in extensively grazed sheep.	Faecal egg counts in village sheep.	Effect of grazing strategy on parasite burdens.	7
Sheep	Faecal egg counts on different farms.		Effect of location on burdens of different species.	7, 9
Sheep	Gut washes.	Faecal egg counts from same animals.	Parasite species composition and egg output.	7, 8, 11
Saiga	Check for other parasite species.		List species present.	2
Livestock	Faecal egg counts in different host species.		Relative contribution to pasture contamination.	9

4.3 Field methods

4.3.1 Saiga hunting and post mortem examination

Saigas in Betpak-Dala were killed by hunters of the Karaganda hunting inspectorate as part of the annual cull, and those in Ustiurt under licence by employees of the Institute of Zoology. Groups of migrating saigas were identified at night using a vehicle-mounted searchlight, and pursued. When close to the group, the vehicle stopped and as many saigas as possible were shot. Carcases were assembled and loaded onto a truck, then taken back to a central camp, where viscera were removed. On long hunts, evisceration was carried out before loading, and the abomasa separated and placed into labelled plastic bags for transport to camp.

Body condition was graded by daylight according to the amount of abdominal and retro-peritoneal fat, and each carcass allocated a score of 1 (poor, almost no fat), 2 (average: fair amount of fat present, but kidneys clearly visible) or 3 (good: plentiful fat, completely obscuring kidneys). A similar index was used in deer by Waid *et al* (1985), and in peccaries by Corn *et al* (1985). Age was determined in the first instance by an experienced observer from the Institute of Zoology in Almaty, on the basis of body size and head shape: animals were categorised as juveniles in their first year of age (i.e. around six months old), yearlings (18 months), or adults. In Ustiurt, ages were multiples of one year. The central incisor teeth were taken from each animal, and the complete mandibles from some, in order to age animals more accurately. In the tooth sectioning technique (TST), age is estimated from annuli in the cementum of a transverse section of the tooth root (Gruzdev and Pronyaev, 1994; Pronyaev *et al*, 1998). In the tooth eruption and wear technique (TEWT), measurements of the mandible, and assessment of tooth eruption and wear, provide a guide to age (Pronyaev *et al*, 1998). Both were carried out at the Norwegian Institute for Nature Research in Trondheim, Norway, and detailed test methods and reliability are discussed in Lundervold (2001) and Lundervold *et al* (2003).

The first 50 animals killed in Betpak-Dala, and all those in Ustiurt, were subjected to a general parasitological examination, which consisted of visual inspection and digital palpation of the integument, liver, trachea, lungs, diaphragm, mesentery and, in 22

animals, the nasal chambers and heart. The liver and lungs were inspected for metacestodes, and incised for detailed examination. In 20 animals, the liver was sectioned into small (0.5cm square) cubes, which were washed in water and examined with the naked eye against a pale background for trematodes. Thick and thin blood smears were taken from 10 animals, air dried, and fixed in ethanol. The gastrointestinal tract of all saigas killed was examined in more detail (section 4.3.3).

The number of saigas shot, as well as the age and sex composition of the sample, was determined by licence requirements and hunting success. The number examined was limited by time, manpower and working conditions. Priority was given to obtaining a large number of abomasal samples. The sample size, *n*, required to detect a parasite species with a probability of 95%, given that it is present in 5% of a saiga population of 250,000, was calculated using the formula given in Thrusfield (1995, p.187), and found to be 60. Equivalently, the sample size needed to detect a prevalence of 5% with an absolute precision of 5% was calculated using a second formula (Thrusfield, 1995, p.183), below:

$$n = \frac{1.96^2 . P_{\text{exp}} . (1 - P_{\text{exp}})}{d^2} \qquad (4.1)$$

where P_{exp} represents the expected prevalence, and d the absolute precision required.

Using this formula, 73 samples were deemed necessary. The same formula gave 96 as the sample size required to detect a prevalence of 50% with an absolute precision of 10%. This was set as the minimum sample size aspired to for abomasal nematodes. Sampling continued beyond this, both to find rarer species, and to provide a sounder basis for a comparison of means between subgroups. The sample size necessary for this could not be calculated before analysing samples and extracting information on parasite abundance and distribution. The maximum possible number of animals was therefore sampled (Table 4.4): their age and sex distributions are given in Table 4.5.

Within the constraints of the licences, there was no systematic bias in hunting effort. However, individuals slower in fleeing may have been more likely to be shot. There was no way to test for this source of bias in the field. However, the hunting methods used are usual for saiga culls, and are comparable to those of previous studies.

Table 4.4. Samples collected from saigas and livestock in Kazakhstan for examination of gastrointestinal nematodes. Includes material collected on previous expeditions in 1996 and spring 1997, but not previously analysed: abomasal material from these did not permit enumeration of worm burdens, and is not included in total sample size. All locations except Almaty and Taraz (i.e. southern Kazakhstan, SK) are within the saiga range.

Species	Date	Location	Gastrointestinal washes			Faeces
			Abomasum	Small intestine	Large intestine	
Saiga	Nov 96	Betpak-Dala	(11)	-	-	20
	May 97	Betpak-Dala	-	-	-	4
	Nov 97	Betpak-Dala	133	21	3	49
	May 98	Ustiurt	11	11	9	56
(Subtotal)			**144**	**33**	**12**	**129**
Cattle	May 98	Ustiurt	-	-	-	9
	Summer 98	Betpak-Dala	-	-	-	78
Camels	May 98	Ustiurt	-	-	-	3
	Summer 98	Betpak-Dala	-	-	-	6
Goats	May 98	Ustiurt	-	-	-	2
	Summer 98	Betpak-Dala	1	1	1	48
	Nov 99	Chu	3	2	1	5
Sheep	Oct 97	Almaty (SK)	2	-	-	
	May 98	Ustiurt	-	-	-	48
	Summer 98	Betpak-Dala	-	-	-	208
	Nov 99	Chu	23	16	5	105
	Nov 99	Taraz (SK)	5	5	5	5
(Subtotal)			**34**	**24**	**12**	**517**
(Total)			**178**	**57**	**24**	**646**

Table 4.5. Saigas examined for parasites in Kazakhstan. Includes 10 adult males and one adult female killed in Ustiurt in May 1998.

Age category	Male	Female	Total
Juvenile (6 months)	43	44	87
Yearling (12-18 months)	10	17	27
Adult	0	30	30
Total	53	91	144

4.3.2 Livestock sampling

In both Chu and Taraz (Dzhambul), slaughter of sheep was carried out in private houses, and proper *post mortem* examination was not possible. Instead, meat vendors in the market were requested to bring gastrointestinal tracts from sheep they killed, along with information on the age, breed and origin of the animal. The market value attached to intestines, and limited enthusiasm among vendors, meant that abomasa were forthcoming but intestines and faecal samples less so.

Faecal sampling was conducted on farms along two transects, running from east to west (Fig. 4.1). In the north, the transect was along the Karaganda to Dzhezkazgan road, along which most farms are located, while the southern transect roughly followed the river Chu, where most of the livestock which come into contact with saigas in the south of their range are found. As for gut washes, faecal sampling relied to a large extent on owner co-operation. Since the privatisation of state and collective farms, livestock rearing has continued to be organised around the villages that formed the centres of population on these farms, and local administration and veterinary authorities are also organised on this basis. Sampling on the territories of former collective farms therefore relied on the co-operation of local authorities: while this was often given freely, administrators would have some influence on which owners were selected for sampling. Likewise, owners would sometimes select stock to round up on the basis of their convenience, and sample size was often limited by owner patience as much as statistical requirements. Pooled faecal samples from several animals can have advantages in saving time while still giving a reasonable estimate of herd or flock mean FEC (Nicholls and Obendorf, 1994), and pooled samples were collected in some locations. However, individual samples were generally preferred since they can be used to estimate parasite distribution as well as total abundance.

Given the difficulties of obtaining truly random samples of livestock, an attempt was made to ensure that the greatest possible variety of herd and flock sizes, locations and husbandry techniques was included. Animals grazing close to the villages and those further out on the steppe and desert pastures were sampled; within flocks and herds efforts were made to include animals of different age and body condition, countering a tendency by owners and stockmen to present their best or most docile animals. At least forty animals were sampled from each group where possible, but many groups were smaller than this. In the villages, lone animals were common.

On the southern transect, sampling was hindered by a general dearth of livestock, and difficulties in gaining access to restricted areas such as the Moinkum desert. A number of animals were sampled at a livestock mart in Chu town: most of these animals came either from the town itself, or from the Chu-Iliyski hills some 40km away, a favourite wintering ground for saigas.

Cattle, sheep and goats were assessed for body condition using a scale modified from MLC (1983), and assigned a score of 1 (poor), 2 (average) or 3 (good): Table 4.6 gives details. Animals at market are likely to be biased towards better condition, though not all were destined for immediate slaughter. The age of animals was obtained by asking the owner or stockman, and confirming the plausibility of their estimates by inspecting the teeth.

Table 4.6. The body condition scoring system used for domestic ruminants.

Condition score	Class	Description (sheep)	Description (cattle and goats)
1	Poor	Ribs, and spinous and transverse processes of the lumbar vertebrae, easily palpable. Pelvis prominent when viewed from the rear.	In addition, poor depth of tissue around tail head, such that tail head appears raised and can be easily palpated.
2	Average	Palpable spinous processes, but good muscle cover over the ribs and back, such that transverse processes are not easily felt.	Good muscle cover, intermediate condition.
3	Good	Good muscle and fat cover, rounded rump, and barely palpable spine.	Spine palpable, but good tissue depth around tail head, such that it appears buried.

Farm veterinarians, owners and herdsmen were interviewed on husbandry techniques, measures taken against parasitic infection, and general grazing management. In 1998, this formed part of an overall interview structure described in Robinson (2000) and Lundervold (2001), which included detailed consideration of stock ownership, farm management and animal movement patterns. Parasitological interviews were informal and intended both to provide a background understanding of current and past livestock rearing and grazing techniques in the study area, and to identify possible determinants of parasite load in the animals sampled.

The main objective of the livestock sampling was to detect any substantial differences in parasite abundance between groups of livestock. Given no prior information on parasite distribution or between-group variance, it was impossible to calculate a required sample size (Thrusfield, 1995). Gasbarre *et al* (1996) suggest that 15-20 faecal samples typically give a reasonably accurate measure of mean faecal egg count in a group of calves: smaller samples are of little use given overdispersion in parasite distributions. The effort given to sampling once on site was low relative to that of arranging the expeditions. It was therefore decided to collect the maximum possible

number of samples from the widest variety of animals, prioritising sheep. The total number and locations of livestock sampled have already been given in Table 4.4, and their species, sex and age distributions are in Table 4.7 and Fig. 4.2.

Table 4.7. Livestock sampled for faecal egg counts by location, species, age category and sex. Young indicates less than one year old, adult more than one year. NK = not known.

		Ustiurt, May 98		Betpak-Dala, summer 98		Chu, Nov 99			Total
		Male	Female	Male	Female	Male	Female	NK	
Cattle	young	1	-	7	20	-	-	-	28
	adult	-	8	5	46	-	-	-	59
Camels	young	-	-	-	-	-	-	1	1
	adult	1	2	-	-	-	-	5	8
Goats	young	1	-	9	11	1	1	-	23
	adult	-	1	11	17	1	2	-	32
Sheep	young	4	3	28	19	23	10	7	94
	adult	1	40	38	123	9	34	14	259
	NK	-	-	-	-	-	-	13	13

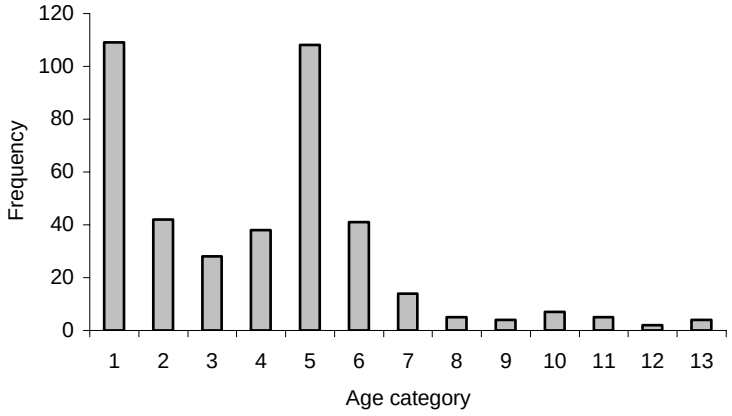

Figure 4.2. Age distribution of sheep sampled in Kazakhstan for faecal egg counts, 1998-99. Age category indicates year of age, such that 1 = first year, etc., and 13 = 12 years or more. Most lambs were spring born. The relatively low numbers of younger sheep, and the peak at 4-5 years of age, broadly reflected the availability of different age groups for sampling (Lundervold, 2001), and may be a result of the recent decreases in livestock numbers described in chapter 3.

The most obvious source of bias is in the year and season of sampling: stock in Ustiurt was sampled in spring 1998, that in Betpak-Dala in summer 1998, and in southern Kazakhstan in autumn 1999. Only limited comparisons between these groups can therefore be made. The timing of sampling was constrained by practical limitations, such as access to remote farms, limitations of time and bureaucracy, and the concurrent aims of joint expeditions. However, one of the aims of sampling was to

assess the likelihood and extent of parasite transmission from livestock to saigas: in all three areas stock was sampled shortly before the arrival of migrating saigas. Faecal egg counts will therefore give an indication of the potential density of contamination of saiga pasture with eggs of different nematode species at the most relevant time.

4.3.3 Collection of helminths from the gastrointestinal tract

Gastrointestinal tracts were processed as soon as possible, and usually the day following culling. On the main saiga expedition in 1997, it was not always possible to do this, either because there were too many samples to process, or because of lack of water or extreme weather. In this case, abomasa were labelled and left outside, where the temperature rarely rose above freezing, and processed later.

Helminths were collected using techniques adapted from MAFF (1986). Abomasal contents were emptied into a bucket, the abomasal mucosa washed separately in clean water, and the mucus layer scrubbed with firm digital pressure and examined for gross pathological change. Washings were combined with abomasal contents, mixed well, and passed through a sieve of 220µm aperture. The volume of the residue was measured, an aliquot of 15ml taken and placed in a sterile universal container, and formalin added to a final concentration of 5-10%.

Small intestines were stripped off the mesentery and processed in a similar manner, except that contents were extracted by manually squeezing out sections around one metre in length, and the mucosa was not normally scrubbed. After sampling in this way, a subset of intestines was opened longitudinally, the mucosa washed and scrubbed, and the residue kept to verify the efficiency of the first method. Large intestines were sampled in the same way as abomasa, and the contents of the caecum and colon were pooled. Any adult cestodes found in the gut were extracted and preserved in formalin, separately from the washings.

After washing, a number of fresh abomasa were placed face down in trays of clean water and left to steep, in an attempt to allow immature stages of nematodes to migrate from the tissue. It was not possible to maintain a constant temperature; however, trays were kept as near to room temperature as possible for 24-48 hours,

before placing the water in a measuring cylinder for several hours, and preserving the sediment in formalin for later examination. In addition, on the 1999 livestock survey, the washed and soaked abomasa were scrubbed further to remove the mucosal epithelium, and any areas of hyperaemia scraped with a scalpel blade to reveal tissue stages of nematodes. These were removed individually and fixed in formalin. Samples of tissue that showed gross pathological change, and larval cestodes found in the viscera, were also preserved in formalin for histological examination.

Field conditions provided a number of challenges, and deviations from standard sampling methods were designed to address these. On the 1997 expedition in particular, extreme cold, lack of a permanent base or even basic facilities, and the arrival of carcases from hunters in large batches with limited time for examination, all meant that detailed or time-consuming techniques were not practical. Equipment, formalin and sample containers had to be minimised in size and amount for transport, and water was generally very limited in both summer and winter. The sieving and taking of aliquots rather than complete gut washes was designed to meet these constraints. Provided material is well mixed, the worms in the aliquot should provide a good reflection of the actual worm burden (Reinecke, 1984), though there is a risk that very rare species will be missed.

In sampling different parts of the gastrointestinal tract, there was a trade-off between thoroughness and sample size. Intestines were particularly time-consuming to process, became friable at very low temperature, and demanded large volumes of water compared with abomasa. Hunters insisted on gutting animals on the move on longer hunts, and there was insufficient space to save both abomasa and the bulkier intestines. At the same time, the main parasite species of interest occur in the abomasum, and abomasal nematodes are generally among the most important for the health of domestic ruminants (Urquhart, 1996). A decision was therefore taken to prioritise the processing of abomasa at the expense of intestines and other organs. Time was not as limiting for sheep samples, and the whole gut was examined whenever it was available. Methods were the same as for saigas.

4.3.4 Collection of faecal and herbage samples

Faeces were taken from the rectum of carcases, or from live animals using digital extraction (Swan, 1970). When there was no time to accurately weigh faeces, at least 3g were taken from each animal. The volume occupied by the uncompressed faeces was marked on the sample tube, and compared later with a standard tube containing exactly 3g to provide an estimate of sample mass. Faeces were broken up before the addition of formalin to 5-10% concentration. In Ustiurt, faeces were collected from the pasture among herds of calving saigas. A long flight distance (300-400m) prevented collection of faeces from known animals. The absence of livestock and the recognisably smaller size of calf faeces, however, mean that most faeces collected were likely to be from adult saigas. Fresh faeces were collected from the centre of the aggregations, while males usually remain on the edge, or in separate groups, so peri-parturient females were more likely to be sampled.

A number of duplicate faecal samples were not formalised, but the faeces were compressed and the tube filled to minimise access to air. Aerobic culture was later attempted under moist conditions in the laboratory, and infective larvae extracted after 14-21 days using a modification of the Baermann technique (MAFF, 1986). Unavoidable variation in conditions during culture meant that both the absolute and relative abundance of larvae recovered were virtually meaningless (see section 4.1.2). The main species of interest can be distinguished on egg morphology alone, and faecal culture was used to confirm infection, and as a source of third stage larvae to aid identification of those recovered from herbage.

Herbage was collected in Ustiurt in May 1998, and in Betpak-Dala in summer 1998, using the protocol originally described by Taylor (1939) and modified by Lancaster (1970). ‘N’ or ‘W’ transects were walked, and herbage collected from approximately 100 locations, at each of four sites: directly in front of the toe, and from one metre ahead and to each side. Past studies have found most larvae close to the base of plants (Silangwa and Todd, 1964; Sykes, 1987), though this may not always be the case (Williams and Bilkovich, 1973). In the absence of firm evidence for preferential grazing by saigas on different parts of the sward, grasses were clipped at ground level, and only green foliage collected from woody plants. Waller *et al* (1981) gives a

method of estimating the required sample size based on observed variance in samples collected in a pilot study, but the one-off nature of our expeditions did not permit this. Instead, both sample number and the amount of herbage collected in each sample were increased relative to standard techniques for intensively grazed livestock pasture. Waller *et al* (1981) estimate that variance in larval counts is no longer decreased significantly after the first three 250g samples. In the present study, eight samples, each of approximately 1kg wet weight were collected from each location, and the distance of each transect was at least 400m.

Samples were processed immediately following collection in order to minimise larval die-off (Fine *et al*, 1993). The herbage was placed in a bucket with 5-10 litres of water and a small amount of surfactant, agitated, and left to soak for at least two hours. After further agitation, plant matter was removed manually and by passing the water through a coarse sieve (aperture 1mm). The filtrate was left to stand for a further three hours, the water decanted off, and the sediment placed in an improvised Baermann apparatus (MAFF, 1986) and left for 12-24 hours. The lower 15ml of water and sediment were then removed, placed in a universal container and fixed in 5-10% formalin for later examination. The plant material was sun-dried over three days, and weighed to obtain an approximate dry mass, so compensating for variation in herbage moisture content between sites.

4.4 Laboratory methods

4.4.1 Worm counts

Abomasal and intestinal washes were analysed in the parasitology laboratory of the Faculty of Veterinary Medicine, University College Dublin. Recovery of helminths followed a method similar to that of Reinecke (1984). Aliquots were placed into a beaker of water and stirred, and the whole passed through a fine sieve to remove background colour. The residue was re-suspended in water, well mixed, and an aliquot of one quarter taken. This was transferred bit by bit into a petri dish and examined closely under the dissecting microscope, under reflected light against a black background. Any adult or immature nematodes were removed and mounted in

lactophenol, which was prepared according to instructions in Mahoney (1968). Coverslips were added, and sealed for long-term preservation.

If fewer than forty adult nematodes were obtained from the first aliquot, further aliquots were examined until this target was exceeded. The total nematode burden for each part of the gastrointestinal tract was calculated by dividing the number of worms found by the proportion of gut contents examined.

4.4.2 Identification of adult and larval helminths

Adult nematodes were left to clear in lactophenol for at least 24 hours before microscopic examination. Females were identified to the level of genus, and males to species, using keys and illustrations in Skrjabin *et al* (1954), Andreeva (1957) and Boev *et al* (1962). The first text has been translated into English (Skrjabin *et al*, 1960), but the illustrations in the original are of superior quality. Where taxonomy in the Russian texts differs from that generally accepted in the current international literature, the latter was adopted, as discussed in chapter 5. Supplementary sources used for identification included Dunn (1978), Levine (1980) and Trach (1986). For male nematodes, the morphology of the spicules and bursa were the main characters used for identification. Representative samples of each species found were taken to Almaty for comparison with catalogued specimens kept at the Institute of Zoology, and difficult specimens to Moscow for confirmatory diagnosis at the Parasitology Laboratory of the A.N. Severtsov Institute of Ecology and Evolution, and the Central Helminthological Museum of the K.I.Skrjabin All-Russian Scientific Research Institute of Helminthology. A number of worms of the genera *Marshallagia* and *Haemonchus* were measured for morphometric analysis: this is described in chapter 5.

Immature nematodes found in the gut were identified to the level of genus and stage (moult) using keys and drawings in MAFF (1986) and the more comprehensive Trach (1983). Pre-imaginal stages were identified on the basis of spicule morphology, and infective larvae on characteristics of the head and sheath tail as described in MAFF (1986). Material from herbage washes was examined under the dissecting microscope after the addition of a drop of iodine, such that unstained ensheathed larvae could be distinguished from the stained background and free-living nematodes.

Adult and larval cestodes were identified under the dissecting microscope to species level, using Dunn (1978) and Boev *et al* (1962). Differential identification was limited to those species previously found in ruminants in Kazakhstan (Boev *et al*, 1962), and special stains and techniques were not used.

4.4.3 Faecal egg counts

Around half of the faecal samples were analysed in the parasitology laboratory of the Institute of Zoology in Almaty, and the remainder using the same methods in Dublin.

Faecal samples were analysed using a combination of four different methods:

(i) Modified McMaster count. This dilution/flotation method was based on the McMaster technique described in MAFF (1986). Approximately 3g of faeces were added to 42ml of tap water. After crushing and suspending faeces, coarse debris was removed using a tea strainer, and 9ml of the well-mixed suspension transferred to a glass test tube. Tubes were centrifuged at 1500rpm for 2 minutes, and the supernatant decanted off and replaced with saturated saline solution. The faecal material was re-suspended and used to fill a standard McMaster slide. The tubes were inverted seven times to ensure that thorough mixing occurred, and separate aliquots taken for each of the two counting chambers. Slides were examined between 10 and 40 minutes after loading, to maximise the proportion of eggs floating (Dunn and Keymer, 1986). Medium power magnification (total 100x) was used. The total amount of faeces examined in two McMaster chambers was 0.02g, and the number of eggs therein, multiplied by a factor of 50, gives the number of eggs per gram (epg).

Examination of the first set of faecal samples revealed that nematode eggs were present at very low densities. To increase the chance of finding eggs in lightly contaminated faeces, three additional techniques were used on a proportion of samples (Table 4.8):

(ii) Low volume McMaster count. In this method, the 3g of faeces were initially suspended in 12ml rather than 42ml of tap water, increasing the total amount of faeces examined on each slide by a factor of three. Each egg observed on one slide (two counting chambers) represents 17epg.

(iii) Coverslip flotation. After withdrawal of the McMaster aliquot, the test tube was refilled to the top with saline, and a microscope coverslip placed on top of the meniscus. The coverslip was then removed using a sharp vertical motion 10-40 minutes later: any remaining eggs should float to the top of the tube, and cling to the coverslip, to be counted under low power (Stoll, 1930; Thienpont *et al*, 1979).

(iv) Loop technique. A subset of the samples was examined using a concentration technique that was widely used in parasite surveys of livestock in the former Soviet Union. This will be called the loop technique, a version of which is described in Boev *et al* (1962). Samples were prepared as for the coverslip technique, but the topmost layer of the meniscus was instead sampled using a fine wire loop some 3mm in internal diameter, and placed onto a microscope slide. This was repeated four times, and the four droplets examined separately without a coverslip. This has the advantage that eggs, which tend to float to the top of the droplets, are clumped together and can be examined free of debris. It was important to compare this technique to the others, so that results could be compared with those in the Russian literature.

A number of supplementary exercises were undertaken to gauge the relative accuracy and repeatability of the above tests. The distribution of eggs between slides, and the chances of finding eggs by the McMaster method in lightly infected hosts, were assessed by examining five slides in series from each of 74 saiga samples. In this case, a separate tube was prepared for coverslip flotation, to compensate for the removal of a large proportion of tube contents for the McMaster slides. Coccidial oocysts, which were generally more abundant than nematode eggs, but equally detectable by flotation (Taylor *et al*, 1995), were counted in 50 samples to provide a supplementary measure of egg distribution between slides. Extra variability within the faecal mass, and that introduced in the preparation of the sample (i.e. dilution and sieving), was considered by examining duplicate samples from 50 animals. In 24 samples, the residue from the sieve was re-suspended in 9ml of saline and processed for coverslip flotation to check for eggs retained at this stage. Coverslip flotation using zinc sulphate, or sedimentation in water, was undertaken on 40 saiga samples to test for trematode eggs (MAFF, 1986).

A separate study on faeces from an Irish foal known to be infected with strongyles was undertaken in Dublin. The objective was to assess the relative sensitivity of each technique in detecting strongyle infection at a known egg density. The faecal material was sieved as described above, and diluted to the low volume McMaster concentration. Subsequent serial dilutions were prepared, four 9ml tubes drawn from each, centrifuged, and topped up with saturated saline solution. Five McMaster slides were prepared from each dilution, and examined in turn between 10 and 50 minutes after filling. The second and third tubes were used for coverslip and loop flotation. After removing the meniscus to a slide as already described, these tubes were topped up and the procedure repeated three times at intervals of 10-20 minutes, to see whether repeated examination of the same material increases the sensitivity and accuracy of the test. The fourth tube was used for a centrifugation-flotation method similar to that described by Lane (1928) and held to be the most sensitive of the commonly used diagnostic techniques for trichostrongylid eggs in ruminant faeces (Stoll, 1930). After re-suspension of the centrifuged pellet in saturated saline, the tube was replaced in the centrifuge at 1500rpm for a further 2 minutes, topped up, and a coverslip placed on the meniscus for approximately 30 minutes. After removal of the coverslip, the process was repeated twice, to recover as many eggs as possible from the sample.

In the samples from Kazakhstan, identification of nematode eggs was limited to the easily recognisable genera *Nematodirus* and *Marshallagia*, which at around 200µm are more than twice the length of those of other trichostrongyloids (Thienpont *et al*, 1979). Most *Nematodirus* eggs have a thick, symmetrical shell and contain 4 or 8 blastomeres when passed, while *Marshallagia* eggs are recognisable by their thinner shell, which is straight on one side and very slightly curved on the other, has rounded poles and contains 16 or more blastomeres (Dunn, 1978; Thienpont *et al* 1979; Sloss *et al*, 1994). Other nematode eggs were noted as trichostrongylid, ascarid, oxyurid or rhabditoid. Coccidial oocysts, which were unsporulated due to preservation in formalin, and cestode eggs were noted, but not counted.

4.4.4 Other samples

Fixed blood smears were stained using Giemsa (MAFF, 1984) and examined under the light microscope at high power to check for blood parasites. Tissue samples were

prepared for histology by the pathology laboratory of the Faculty of Veterinary Medicine, University College Dublin. Samples were mounted in paraffin and sectioned using a microtome, then stained with haematoxylin-eosin (MAFF, 1984).

4.5 Statistical methods

Unless otherwise stated, statistical methods were taken from Sokal and Rohlf (1995), Fowler *et al* (1998), and Siegal and Castellan (1988), and applied using the SPSS software package (SPSS Inc., Chicago). Non-parametric tests were used to compare categorical data, and for initial analysis of parasite counts. Parasite and faecal egg counts in different groups of animals were compared using the Mann-Whitney *U*-test on untransformed data, and prevalence of infection using the chi-square test for association. Correlations between parasite burden and body condition, between FEC calculated using different methods, and between parasite burden and FEC, were assessed using the Spearman rank correlation coefficient. Where a causative link between associated variables could be assumed, as for adult worm burden and faecal egg output, linear regression analysis was conducted using the least-squares method, after inspection for linearity and demonstration of a significant correlation using the Pearson product moment coefficient.

Where data did appear to be approximately Normally distributed, for example measurements of worm length, and about 70% of observations were found to fall within one standard deviation of the mean (Fowler *et al*, 1998), parametric tests were applied. Means were compared using a *z*-test if sample size was higher than 30, or a *t*-test if not. Methods used for multivariate analysis are described in the appropriate sections of chapters 5 and 7.

Methods for comparing sample means are generally subject to assumptions regarding data form, which may not hold for parasite counts (Rózsa *et al*, 2000). A detailed consideration of the distribution of parasites among the saigas and livestock sampled, and its implications for sample statistics and their analysis, can be found in chapter 6. This forms the basis for much of the analysis of parasite counts that follows. The success of the field methods used in addressing the problems at which they were aimed is also discussed in chapter 6.