4. Materials and methods

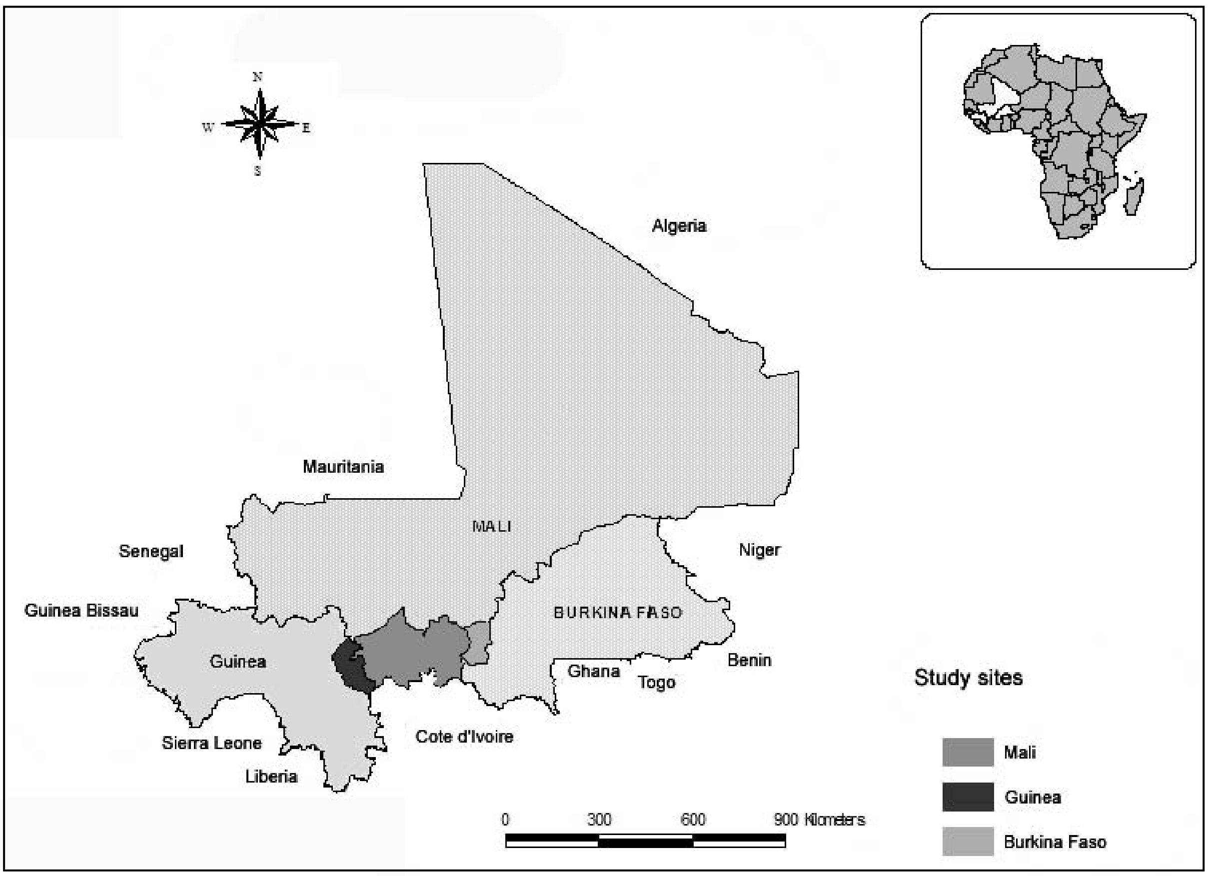
The study had three objectives: describing the epidemiology and management of trypanosomosis in villages under risk of drug resistance; evaluating 'best-bet' strategies for AAT control in villages under risk of resistance; and developing a mathematical model for trypanosomosis incorporating resistance to trypanocides.

4.1 Study setting

The descriptive studies were carried out in Kénédougou province in Burkina Faso, the Circles of Sikasso, Bougouni and Yanfolila in the Sikasso region of Mali, and Mandiana prefecture in the department of Upper Guinea. Assessment of best-bet strategies for the control of trypanosomosis under presence or risk of drug resistance was carried out in the following settings:

- Participatory vector control was evaluated in villages in Kénédougou, Burkina Faso
- Trypanotolerant cattle-keeping was evaluated in Kénédougou, south Mali and Guinea
- Promoting rational trypanocide use was evaluated in three target (user) groups:
  - Paravets in Kénédougou, Burkina Faso and Mandiana, Guinea
  - Farmers in Sikasso, Bougouni and Yanfolila, (south Mali)
  - Animal health service providers in Mandiana, Guinea

Figure 4.1 Map of the study area



Source: Project document

Study area in Burkina Faso

The first study area was in Kénédougou province, Burkina Faso. One of 45 provinces of Burkina Faso, Kénédougou occupies 3% of its land area and is on the border of Mali and not far from Cote d'Ivoire. Benefiting from a sub-humid climate and fertile soil, Kénédougou is considered a region of high agriculture potential. In the drier north, cotton and cereals are the main cash crops; the more humid south is renowned for orchards and root-crop production and farming is more diverse. Other crops cultivated for subsistence and sale include sorghum, rice, maize, millet, groundnuts and legumes. Uncultivated land occupies 20% of the area; mainly exploited by women, this provides firewood, herbs, shea nut and honey. There are two main systems of cattle-keeping; pastoral and agro-pastoral. For pastoralists, cattle-keeping is their central livelihood strategy, milk is a major dietary component and sale of cattle the main source of income. Herds are large; the system is extensive and low external input. Many pastoralists practice transhumance, grazing cattle in the north during the rainy season and moving south after the harvest, but there is an increasing trend towards permanent settlement. Relations with agro-pastoralists are generally good; a complementary role has developed with pastoralists providing expertise, livestock products (milk but more importantly manure) and sale cattle, while benefiting from crop residues and grazing during the months when there are no standing crops. In the agro-pastoralist system, cattle are kept mainly for their contribution to crop production. Animal traction is used in land preparation, weeding and transport; manure is a valuable fertiliser. Zebu cattle are kept as well as trypanotolerant Baoulé and Métis, the latter a stable cross between Zebu and Baoulé. Fewer cattle are kept than in pastoralist systems but inputs per animal in terms of nutrition, housing and veterinary treatments are higher. Kénédougou is divided into four animal health (zoosanitary) districts. Our study took place in two of these: Orodara a zone of technical support (veterinary agents have withdrawn from the provision of clinical services and their role is extension) and Koloko, a veterinary post where, among other roles, the veterinary agent is authorised to give preventative and curative treatments

Study area in Mali

The second study area was in the Sikasso region of Mali; specifically the Circles of Sikasso, Bougouni and Yanfolila. With annual rainfall between 800-1200 mm, Sikasso is the most agriculturally productive of the seven regions of Mali. Agriculture is the main source of employment and food, and the basis of the local economy. The main subsistence crops grown are maize, sorghum, millet and rice; in most years a surplus is produced which is sold outside the area. Cotton and groundnut are important cash crops. Most farmers now use cattle to cultivate these crops. Of secondary importance are root crops, legumes and fruit, and small-scale market gardening, for which draft cattle are not used. Fish resources are abundant especially in Yanfolila. Industry is little developed, just two cotton factories, a factory for processing shea nuts, gold-mines and a dam *cum* hydro-electric plant. In Yanfolila there is extensive unsettled and uncultivated land; here hunting, bee keeping and gold-washing are important economic activities. Until comparatively recently (60s), the livestock system was traditional, with trypanotolerant cattle owned by the extended family and kept mainly for savings and social obligations (bride price and sacrifices) and only secondarily for milk and meat production; animal traction was not used. Even here, traditional cattle-keeping is being replaced by a commercially oriented, draft-based system. Both long and short distance transhumance is practised and the number of cattle entering Yanfolila is estimated at one tenth of the local population. There are eight recognised Zebu breeds in Mali (Maure, Touareg, Azawak, Peul Soudanais, Peul du Macina, Peul Toronke, Peul Sambourou and Peul Bororo), while two taurine races are commmon (N'Dama and Baoulé) as is the Méré or Métis, a stable cross between Zebu and taurine. Government animal health services are delivered by a pluri-potential rural development agency and regulated by a supervisory agency. This model has not been successful and Mali is in the process of returning to a line ministry system (Affognon, 2005).

Study area in Guinea

The third study area was in Upper Guinea. The largest and poorest of the four regions of Guinea, it lies in the north west of the country, bordered by Mali and Cote d'Ivoire. Mandiana, where the study was carried out, is a large prefecture to the east of Upper Guinea, with 12 sub-prefectures, one large town (Mandiana) and 73 villages. Consisting of wide flood-plains separated by extensive plateaus, the climate is typically Sudano-Guinean with a dry season of six to seven months followed by abundant rains (1200-1800 mm/year). The population is predominantly Malinké, with a significant minority of settled Fulani (Peul Wossoulou) and smaller numbers of Sousou and Forest Region ethnic groups. The principal crops are maize, sorghum, groundnut plus fonio (a type of millet) in the north and manioc in the south. In low-lying river areas, rice is often the most important crop. Production of cotton, the main cash crop, has declined in recent years due to the poor functioning of the cotton parastatal. Animal traction is used in the production of these crops, and a minority of farmers keeps large herds for milk, meat and sale. Livestock keeping is traditional and mainly N'Dama are kept; Guinea is the birthplace of this breed. Government animal health services consist of four veterinary officers located in the provincial head quarters of Mandiana and eight veterinary posts. Associated with the veterinary post is a network of paravets (46 in total), trained in the 80s and 90s by a World Bank funded project.

4.2 Study design

4.2.1 Situational analysis

The situational analysis drew upon cross-sectional descriptive epidemiological and socio-economic surveys (farmers and animal health service providers), carried out at different times and in different countries. Surveys were complex and multi-stage, (the sampling strategy is described in the next section). Formal transversal surveys were supplemented by Participatory Rural Appraisals.

4.2.2 Evaluation of AAT control strategies

Study designs for evaluating the interventions for improved AAT control, outcome measures and *ex ante* sample size estimations (or where sample size was fixed by logistic constraints, power calculations) are described in the next paragraphs.

For the evaluation of participatory vector control (VC), the study design was longitudinal, controlled, non-randomised, clustered, quasi-experimental with double pre-test. The main outcome measures were AAT prevalence and cattle survival. Randomisation was not possible; of the seven villages were trypanocide resistance had been assessed only four were willing to carry out participatory vector control and these were *ipso facto* chosen as intervention villages. Although, randomised control trials are the gold standard for evaluating effectiveness (Table 4.1), under field conditions they are not always feasible and well designed quasi-experimental studies such as that described here are considered adequate for policy decisions (Varkevisser *et al.,* 1991).

The sample size of eight villages (four test and four control) and 100 cattle per village was large enough to detect a decrease in prevalence from 10% to 1% (one-sided test), with a power of 80%, alpha 5% and assuming an intra-class correlation coefficient of 0.02 (Uilenberg, (1998).

Table 4.1 Hierarchy for assessing quality of evidence (NHS, 1996)

Rank	Method used for evaluating effectiveness	Category
1	Systematic review of all randomized control trials, including assessment of methodologies	Experimental
2a	One or more well-designed randomized control trial	Experimental
3a	Well designed before and after studies with control, or time series	Quasi experimental
3b	Well-designed cohort studies	Quasi experimental
3c	Well-designed case control studies	Non experimental
4a	Dramatic effects from uncontrolled experiments	Quasi experimental
4b	Descriptive studies, *ex ante* and *post hoc* intervention evaluation	Non experimental
5	Expert opinion, theoretical derivations, analogies	Non experimental
6	General opinion	Non experimental

The evaluation of keeping trypanotolerant cattle was essentially descriptive. A cross-sectional design was used to compare the prevalence in different breeds. To assess benefits of trypanotolerant cattle, longitudinal data from the participatory vector control study and the Rational Drug Use study were used. For impact of breed on mortality, herds with only trypanotolerant cattle (n=328 herds) were compared to those with only trypanosusceptible cattle (n=175 herds). Given the number of herds, and assuming a mortality of 10% in trypanosusceptible herds and reduction of at least 50% in mortality in trypanotolerant herds then the power was 61% (test of comparison of proportions, one-sided, alpha =5%).

Three experimental trials were carried out, targeting farmers, paravets and service providers.

1. A cluster randomised, controlled trial (CRCT) was used to evaluate the provision of drug use information to farmers. Restricted allocation was used as the number of clusters was relatively small and preliminary work showed marked variation between zones. Accordingly clusters were stratified on zone. The design was partially double blind. Farmers did not know to which group they were assigned (the control group received placebo information). The technicians assessing animal health parameters were blinded, but staff assessing farmer knowledge were not. CRCTs are appropriate where it is difficult to apply an intervention to individual subjects.

This is always the case for community level interventions such as participatory vector control, but may also be indicated when interventions are likely to be shared with family and neighbours (contamination). CRCTs require special analytical techniques and methodological shortcomings in analysis and reporting are common. A recent meta-analysis of CRCTs in sub-Saharan Africa found that only 20% of the studies considered clustering in sample size calculations or discussions of power; and less than 40% took clustering into account in calculating confidence intervals or p values (Isaakidis and Ioannidis, 2003). Previously reported studies on AAT have not accounted for clustering.

The sample size of 20 farmers in 46 clusters was large enough to detect an improvement of 10% (from 65% to 75%) in knowledge scores at alpha of 5% and power of 90%. (Based on a previous knowledge test on farmers in Burkina Faso showing an ICC of 0-0.29 and SD of 8.8%). For assessing the medium-term effects of RDU the sample size of 20 animals in 46 clusters was large enough to detect an increase in PCV of 3% (from 25 to 28%) at alpha 0.05 and power of 80%. The ICC (0.098) and SD (5.71) were derived from data from the first epidemiological survey in Burkina (baseline for participatory vector control assessment.

2. A before-and-after, controlled, trial was used to evaluate establishing village primary animal health workers (paravets). The main outcome was improvement in knowledge/skill. Randomisation was not possible because a principle of CAH is that paravets are chosen by their community (GTZ, 1984). In before-and-after studies, secular trends or sudden changes make it difficult to attribute observed changes to the intervention and often overestimate intervention effects (Lipsey and Wilson, 1993), but identification of a suitable control group is often problematic (Eccles *et al.,* 2003). In this case, because training was given sequentially, paravets in the second round could act as controls for those in the first. Both first round and second round candidates were chosen by the community using the same criteria, so the control group is likely to be similar to the test group. The importance of paravets in the provision of AAT-related health services was assessed by an observational cross-sectional survey of service providers and clients using a health behaviour model, and by the data from the KAP. The power of the experimental evaluation (testing knowledge and skills using the t-test) was 87% given an estimated number of 15 paravets in each round, a predicted score of 50% in the trained group and at most 33% in the untrained group and a standard deviation of 15%.
3. Training existing service providers was evaluated by a randomised, controlled, before and after experimental trial. The main outcome for evaluating the training of service providers was improvement in diagnosis and dosage of trypanocides.

4.3 Participant selection

For the situational analysis, cross-sectional AAT prevalence studies were carried out in eight villages in Burkina Faso, 81 in Mali and 30 in Guinea (results in Guinea are documented elsewhere). A Knowledge Attitude and Practice (KAP) survey was carried out in eight villages in Burkina Faso, 16 in Mali and 11 in Guinea. Additional information on farmer diagnosis and herd structure was available for 46 villages in Mali. Participatory Rural Appraisals took place in four villages in Burkina Faso and three in Guinea.

The participatory vector control trials were carried out in the same eight villages in Burkina. The evaluation of trypanotolerant cattle used data from the KAP and additional information from the vector control study, cross-sectional study and RDU study. Provision of RDU to farmers was trialled in 46 villages in Mali (including 12 of the 16 in which the KAP had taken place); establishing paravets trained in integrated trypanosomosis control took place in the eight villages in Burkina Faso; the third experiment of providing continued professional development (CPD) covered all the existing formal sector animal health service providers in Mandiana, operating in 235 villages and hamlets.

The sampling strategy for village selection was multi-stage, cluster-randomised sampling, stratified to ensure representation of villages at high risk of resistance (summarised in Table 4.2).

Table 4.2 Criteria for selecting study villages and number selected in each country

Country	Burkina Faso	Mali	Guinea
Village with cows & accessible in rains	166 villages	462 villages	90 villages
Village randomly selected for CSS	45 (previous phase)	44	30
Village selected for CSS by judgement	1 (as low risk)	25 (as high risk)	15 (high risk)
Randomly selected with high prevalence	9	5	3
Judgement selected and ISMM test	2	13	15
Village in which ISMM resistance tested	11	18	18
Village in which resistance confirmed	3	7	0
KAP study carried out	8	46 (16 full KAP)	10
Strategy testing carried out	8	46	90

- In Burkina Faso, a previous study (including a cross-sectional study of AAT prevalence in 45 villages selected randomly from the 166 villages of Kénédougou) identified nine villages at high-risk of resistance and suggested resistance was present in six (Woitag, 2003). Seven of the nine villages went on to participate in the second phase of the project described here. One village was excluded because of low disease prevalence, and one because of insufficient cattle. An additional low-prevalence village (Wolonkoto) was selected on the basis of similarity to the other villages participating in the study to complete the number of study villages to eight. Subsequent longitudinal studies were carried out in two villages and resistance confirmed in one village.
- In Mali, 46 villages were selected in three Circles (Sikasso, Bougouni and Yanfolila). In east Sikasso, 25 villages from a sampling frame of 100, were selected for prevalence studies. Seven of these had AAT prevalences over 10% and five were selected for longitudinal studies. An additional ten villages were selected based on their proximity to these, and an abbreviated resistance test carried out. In west Sikasso and in Bougouni and Yanfolila 34 villages were selected. Nineteen were randomly selected from a sampling frame of 362 villages meeting inclusion criteria (accessible during the rains and less than 70 km from the circle capital) and 15 on the basis of high risk of resistance, as assessed by local experts, (using criteria of high

prevalence, high use of drugs and treatment failures). In three of these villages longitudinal studies were carried out.

- In Mandiana, 30 out of 90 villages in the sampling frame of suitable villages were randomly chosen for prevalence studies. In three villages incidence was more than 10% and longitudinal tests were carried out. For the KAP study three sub-prefectures were selected, located in north, central and south Mandiana. Two were chosen because epidemiological surveys had revealed a high prevalence of trypanosomosis and suggestion of resistance to trypanocides on the basis of treatment failures (potential 'hot-spots' for resistance development). In these areas AAT prevalence was 13% and 10% respectively. A comparable sub-prefecture of low prevalence was selected on the basis of judgement by local animal health professionals as a 'low disease' control group. Here the level of infection, or prevalence, was 2%.

Within villages, all cattle-keepers were surveyed making this a comprehensive survey. The response rate for prevalence and KAP studies was high >90% in Burkina Faso and Guinea (>90%) and moderate (>60%) in Mali.

4.4 Data collection tools

The study had three overall objectives, namely: describing trypanosomosis epidemiology and control (situational analysis); developing and testing best-bet strategies for trypanosomosis control; and modelling trypanocide resistance. Methods used for data collection were:

4.4.1 Situational analysis

Four different instruments were used for obtaining information from farmers on the farming system, the problems with trypanosomosis and resistance.

1. A Knowledge Attitude and Practice (KAP) questionnaire was administered in Guinea (10 villages), Burkina Faso (eight villages) and Mali (16 villages). This is a formal data collection method which uses quantitative survey techniques to determine knowledge, belief, attitude and practice. It is the most widely used method in heath-seeking behaviour research (Hausmann-Muela *et al.,* 2003), and is increasingly applied to other sectors, such as education and natural resource management (Lok-Dessallien, 2005). Based on initial meetings with the communities and literature review, standardised questionnaires were developed in French. The questionnaire was as short as possible to reduce the burden on respondents and the risk of biased responses through fatigue. Sensitive questions on purchase and administration of medicines were placed at the end of the questionnaire. The questionnaire was administered in local language (the administrators translating the questions and responses). Picture cards were used to improve understanding and ensure farmers recognised the entity described and not just the name. Open questions minimised affirmation bias. The questionnaire was pre-tested in each country to ensure that questions were comprehensible, unambiguous and acceptable. Modifications were made according to the different conditions in each country. The final version of the questionnaire was administered by trained interviewers: six in Burkina Faso, eight in Mali and three in Guinea. (All were male, lived in the region, were educated to at least secondary level and were familiar

with livestock.) Questionnaires were checked soon after completion and any inconsistencies or gaps corrected by a follow-up interview with the appropriate respondents.

2. A survey on AAT management by farmers was carried out in 46 villages in Mali. A shorter version of the KAP questionnaire, this focused only on cattle breed and experience of AAT (morbidity, mortality, duration of illness signs used for diagnosis, and treatments).
3. Animal health service providers were surveyed in Kénédougou and Mandiana (n=73), to provide information on quantity and quality of animal health services for AAT management.
4. Participatory Rural Appraisals (PRA) were held in Burkina Faso and Guinea. PRA is "*a family of approaches and methods to enable rural people to share, enhance, and analyse their knowledge of life and conditions, to plan and to act*" (Chambers, 1994). An essentially qualitative approach, it emphasises group-work, consensus, appropriate imprecision, visual analysis, and relationship-building between outsiders and communities. PRA facilitates a reversal of power from outsiders to farmers. Though applied widely in agricultural and livestock development, PRA has been little used in AAT control (Pretty and Voduhe, 1994).

Epidemiological studies to understand the health and disease situation comprised entomological studies, assessment of AAT prevalence and other relevant diseases, and drug resistance studies.

1. Fly trapping was carried out by placing (on average) eight biconical and two Nzi geo-referenced traps for six hours in fly predilection sites (un-shaded areas beside rivers, identified by farmers). Odour baiting was not used. Insects were identified and counted by species and sex. Numbers of tsetse flies and other biting flies implicated in mechanical transmission of AAT were noted. Age was determined by the wing fray method for males and ovarian dissection for females. Live and recently fed flies were dissected to determine trypanosome infection rates using the method described by Lloyd and Johnson (1924). In brief, flies were pinned to a dissection board and the mid-gut, salivary glands and mouthparts removed and examined using a microscope at x25 objective and x10 eyepiece.
2. AAT prevalence surveys were carried out in 56 villages. In Burkina Faso and Guinea cattle were selected by judgement; selectors chose animals they believed to be representative of the village herd. In Burkina Faso 100 cattle were chosen per village; except for three villages with less than 100 cattle where all cattle were selected. In Guinea 40 to 80 cattle were chosen per village. In Mali a sampling frame of all the cattle in the villages was constructed and 80 animals were randomly selected. At sampling cattle were ear-tagged and the breed, sex and age noted (age was confirmed by inspection of teeth). Blood sampling was carried out by jugular or caudal venepuncture using EDTA or heparin coated vacutainers; samples were placed on ice, and analysed within six hours. To determine packed cell volume (PCV), blood in microhaematocrit capillary tubes was centrifuged at 8000g for five minutes. PCV was read using a Hawkesley micro-haematocrit reader (Hawkesley, Lancing UK). Trypanosome infection was detected using the Buffy Coat Technique (BCT) (Murray *et al.,* 1977). In brief, the microhaematocrit capillary tube was sectioned 2mm below the buffy coat, which was expressed on a clean glass slide,

covered with a half-coverslip and examined for the presence of trypanosomes. Thin blood smears were made by placing a drop of blood on a clean glass slide, air drying and fixing with methanol for ten minutes. Staining with Giemsa (10% Giemsa in Weise buffer, pH 7.2 for 35 minutes) was carried out in the laboratory and slides were examined for haemoparasites (using a x100 objective and x10 eyepiece and oil emulsion).

3. Cattle girth was measured in centimetres using a tailors' measuring tape. The conversion tables of Bosma (1992), developed for south Mali, were used to convert cm girth to kg body weight. A trial was carried out in the study area to compare the weight obtained using girth measures with that using an electronic balance, as recommended by FAO.
4. Faecal samples were obtained *per rectum* using plastic bags. Samples were labelled and placed in cool-box containers with ice. Analysis was carried out within 24 hours using the Ovassay Diagnostic (Synbiotics, 1995), a test in which faeces are added to a saturated salt solution, and nematode eggs float to the top of the liquid where they adhere to a cover slip. Standardised amounts of faeces and solution were used for the examination to give semi-quantitative results (Table 4.3).

Table 4.3 Semi-quantitative assessment of faecal egg counts

Per field	Per cover slip	Code	Interpretation
< 1	1 to 10	+	Little infection
1 to 2	11 to 40	++	Some infection
3 to 4	41 to 200	+++	Moderate infection
>4	>200	++++	Heavy infection

Sedimentation was used to detect trematode eggs (Hansen and Perry, 1994). Faeces were mixed with water, left to sediment, the supernatant decanted, the sediment re-suspended, re-sedimented and finally decanted to a petri dish for examination after adding a drop of iodine. Pooled samples were used for the detection of lungworm larvae, using the Baermann apparatus. Hydrophilic larvae migrate from the faeces placed in a strainer and collect in the base of a water column, from which a drop is taken to be examined. All sick animals in the RDU study in Mali and one tenth of the animals in the vector control study in Burkina Faso were sampled.

Trypanocide resistance tests are not reported here (Diall *et al.,* 2005), but as results are used in analyses, the test is summarised: cattle were randomly allocated into control and test groups; the test group was given ISMM and both checked fortnightly for 2 to 3 months for AAT infections.

4.4.2 Evaluation of AAT control strategies

Four different interventions for improving trypanosomosis management in the presence or risk of trypanocide resistance were assessed.

Data collection for participatory vector assessment

In Burkina Faso, participatory (high-level) vector control (VC) was carried out. The eight villages participating were divided into four intervention villages and four control villages, on the basis of willingness to participate. Insecticide-treated screens and cattle were used for VC. Screens were made from polyester (electric blue, produced by Comitex, Mali), by local tailors. Screens were one metre square with a 4cm hem at top and bottom and three 40cm hemmed slits at right angles to the base. Screens were treated with Deltamethrin 12.5% emulsifiable concentrate (Decis®, SOFACO, Abidjan). One litre of Decis® was added to four litres of water in a container made by sawing a barrel in half. After agitation, 20 screens were added, sufficient to absorb the solution. Screens were drained on sticks placed over the barrel and then spread flat on the ground to dry. The dried screens were rolled up and taken to the watercourse. Straight pieces of wood were cut from nearby trees and the bark stripped. A piece of wood was placed in the fold at the top of the screen and fixed with two strands of wire at opposite ends. A second heavier piece of wood was placed through the bottom fold and held in place with two metal tacks. Screens were suspended from tree branches, approximately every 100m, at right angles to the river, on both banks, in un-shaded areas, about 1m from the ground. If necessary, vegetation was cut back around the screen. Sixty to 70 screens were placed per village in areas of cattle-tsetse contact. Screens were put in place in April in the first year and removed in June. In the second year they were placed in January and removed in June. After removal screens were washed, repaired if necessary and stored in clean, dry surroundings. While screens were in place, they were checked every two weeks by the communities, who cut down encroaching vegetation, untangled screens caught in branches and dealt with any attempted thefts or vandalism.

Cattle were sprayed monthly with deltamethrin during two rainy seasons. Based on local advice, a 0.01% solution rather than the 0.005% recommended by FAO (1994) was used. One to three litres of spray were applied per animal using a back-pack sprayer (not supplied by the project). Particular attention was given to tick attachment sites (axillae, groin and escutcheon) and the front legs (tsetse preference sites). Spraying was on dry days. In one village, Bayticol® pour-on (Flumethrin 1%) was used for initial treatments of cattle at a dosage of 1ml per 100kg applied in a stripe along the back; after one month this village choose to switch to spraying.

Data were collected on: AAT prevalence and tsetse apparent density (AD); cattle health and production; and acceptability of control to users. AAT prevalence and tsetse AD were measured by the epidemiological surveys three times a year (rains, cold dry season and hot dry season). The impact on cattle production and health was measured by periodic epidemiological checks on a cohort of 100 animals per village, by a questionnaire carried out at baseline and after two years (KAP) and by PRAs. Mortality was the main outcome measured and days worked, calving rate, abortion rate and expenditure on drugs the secondary outcomes. The acceptability of control was evaluated by participatory stakeholder assessment and by revealed behaviour (participation in spraying and screen operations).

Data collection for the evaluation of trypanotolerant cattle

The strategy of keeping trypanotolerant cattle was evaluated firstly by epidemiological surveys comparing the incidence of trypanosomosis and of treatment failures in different breeds both at individual and herd level. Secondly, data on the production of different breeds collected during the participatory vector control and evaluation of RDU information studies were analysed. Thirdly, a questionnaire survey on breeds kept by farmers, herd mortality, motivation for keeping different breeds, and use of trypanotolerant cattle as a deliberate strategy for managing trypanosomosis was carried out in three countries (Burkina Faso, Mali and Guinea).

Data collection for the evaluation of RDU

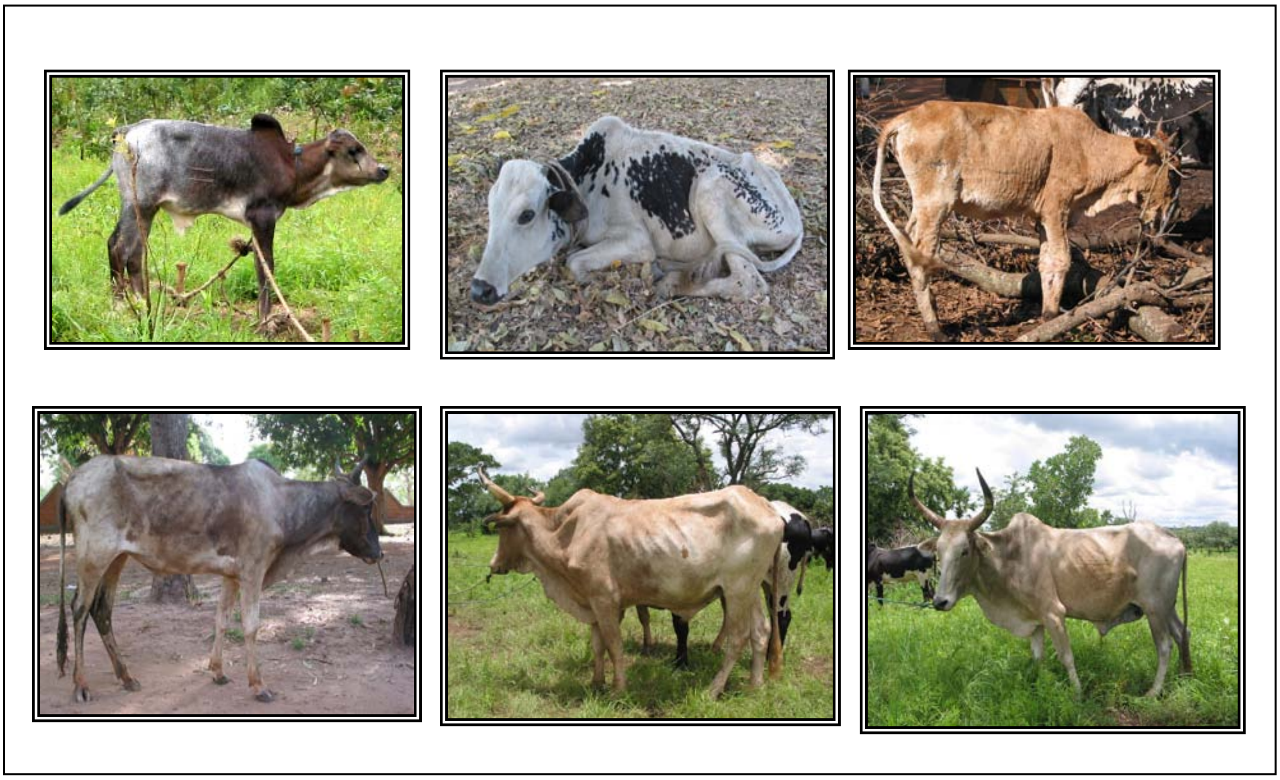
Three interventions were tested: providing information to farmers, training a new cadre of service providers (paravets), and continuing professional development for existing service providers.

For the first trial, KAP surveys and PRAs were used to identify problems with drug use by farmers. Messages were then constructed to address information gaps. Next, studies were carried out with farmers to determine which their preferred channel and format of information. Pamphlets were then developed and pre-tested, with improvements after each test until they were comprehensible by all literate and 70% of illiterate farmers (the sixth version).

The 46 study villages were divided into three regions and half the villages in each region randomly selected to receive pamphlets. Pamphlets on RDU were distributed to farmers in test villages, without explanation of the contents. Control villages received a placebo pamphlet on vector control, and farmers did not know to which group they were allocated (blinded). The outcome measures were pre-chosen and related to change in knowledge, farmer satisfaction, change in practice and change in clinical outcomes. Primary outcomes were decrease in under-dosages, decrease in injection complications and improvement in haematocrit.

Farmer knowledge was measured before giving information, and at two weeks and five months after giving information. Knowledge in the following five areas was measured: diagnosis, choice of medicine, dilution, dosage and injection, as follows:

1. Knowledge of AAT signs was assessed by four sets of pictures comprising one bovine with a sign of AAT and two without (Figure 4.2). Farmers were asked to identify the animal with AAT.
2. Knowledge of AAT treatments was assessed by a card with photographs of different drugs: six incorrect treatments and two correct treatments (Figure 4.2). One mark was given for a correct response and one subtracted for an incorrect response.
3. Knowledge of trypanocide dilution and reconstitution was assessed by asking farmers to demonstrate adding water to a large sachet of Veriben® using an empty 20ml syringe supplied by the questionnaire administrator who then converted the amount shown into millilitres.
4. Farmer knowledge of dosage was assessed by farmer demonstration (with an empty syringe) of how much of the reconstituted Veriben® (previous question) they would give to a male adult Zebu. The administrator wrote down the response in millilitres.
5. Farmer knowledge of injection sites was measured by a picture of a cow with a grid superimposed (Figure 4.2). Farmers indicated where injections are made and the administrator recorded the grid reference. One mark was awarded for every correct injection site identified and one mark deducted for every incorrect site. This was converted to standardised score.



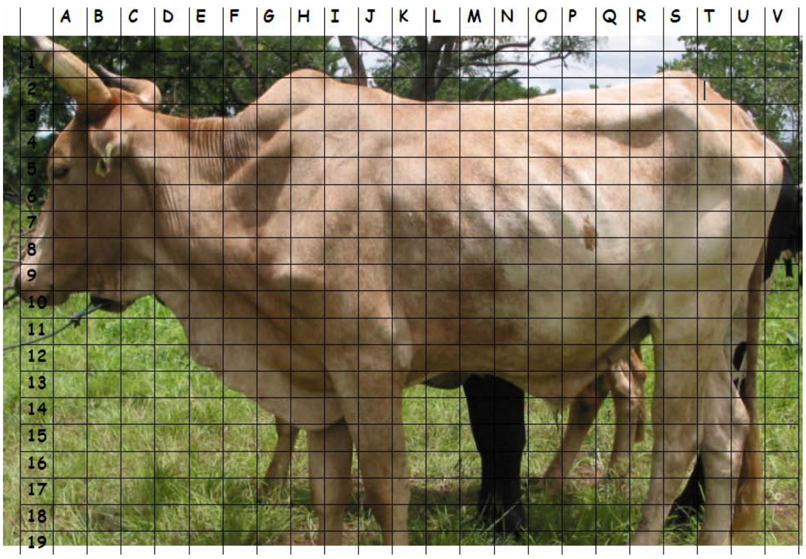


Figure 4.2 Tools used to assess farmer knowledge and skill (not to scale).
The first box shows two sets of three animals; one of the three has signs characteristic of AAT and farmers identify which. The second box shows veterinary drugs; farmers show which are used to treat AAT. The third has a picture of a cow; farmers indicate injection sites and questionnaire administrators note the grid references.

Farmer treatment of sick cattle was assessed in animals identified as sick with AAT by farmers (20 cattle per village). They treated the animals themselves, and showed how much trypanocide each animal treated actually received using an empty syringe.

Clinical outcomes were assessed by an initial evaluation of the 20 farmer-selected sick animals (clinical examination, girth measure, temperature, PCV and BCT) repeated after two weeks. Cattle were then assessed for: injection side effects (i.e. swelling, pain, lameness or abscess at injection sites) and treatment failure (by presence of trypanosomes on BCT examination on day14).

Change in herd health was assessed by measuring PCV, AAT prevalence and weight at day zero and at five months. Technicians assessing this did not know to which group farmers belonged.

Owner satisfaction with the treatment was assessed by asking farmers to evaluate the improvement of the animal. This ranged from minus 3 (great deterioration or death) through 0 (no change) to plus 3 (great improvement).

An evaluation of establishing primary animal health services took place in Kénédougou, Burkina Faso, in the villages where vector control was evaluated plus an additional three villages without control. Training was given to 24 farmers from eight villages; first a residential training of one week and then four to six training sessions in the village. Participatory techniques suitable for adult learners were used. The subjects and teaching method were based on those used in the training of Community Animal Health Workers, see Table 4.4.

Table 4.4 Course content for farmer training

Differential diagnosis	**Practicals**	**Group discussion**
• Weight loss, staring coat, weakness • Diarrhoea • Respiratory problems • Abortion • Sudden death • Skin problems • Eye problems • Nervous problems	• Clinical examination • Weight estimation, re-hydration solutions • Calculating dosages and dilutions • Post-mortem examination • Vector control with screens • Cattle insecticides for tsetse and tick control • Buying medicines, • Making simple medicines • Price analysis	• Traditional medicines • Trypanocide resistance • Role of state, private sector and farmers • Nutrition

The main outcome variables were changes in farmer knowledge and skill. Impact of training paravets on trypanosomosis-related animal health services was also assessed.

1. Farmer knowledge and skill was evaluated before training, at the end of the first residential training and ten months later before the refresher training. At the time of the second training, knowledge and skill were also compared to other untrained farmers who had also been selected by their community for training. The test was mainly skill-based; farmers were asked to carry out specific tasks such as make up an injection or read a thermometer and scored on their ability to do so. Diagnostic ability was tested using photographs of disease signs.

2. A survey on knowledge and information systems (KIS) for trypanosomosis control was carried out in Burkina Faso. This used RAAKS (Rapid Appraisal of Agricultural Knowledge Systems) tools

(Saloman and Engal, 1997). RAAKS is a methodology for understanding the Agricultural Knowledge and Information System (AKIS), that is the social organisation of innovation (Roling and Engel, 1992). The RAAKS survey (n=100 farmers) on information needs and sources was used to assess the importance of paravets as information and advice providers.

3. The survey of formal and informal service providers (section 4.4.1) included 22 of the 24 paravets trained in Burkina Faso. This provided information on the quantity and quality of animal health services offered and allowed estimation of paravet availability, accessibility, affordability and acceptability relative to other service providers.

4. Impact of paravets on cattle mortality was estimated in Guinea by farmer reports.

An evaluation of giving training on Rational Drug Use to existing service providers took place in Guinea. All formal sector service providers were included in the study (10 government vets, eight private sector vets/technicians and 30 paravets). For three months before training, service providers recorded all veterinary transactions in special notebooks. Service providers were then randomly assigned to two groups. One group received training comprising a one day workshop and also a set of tools designed to improve AAT case management (weigh band, anaemia chart, thermometer, *aides mémoires* for trypanosomosis signs and trypanocide dosages). Each group continued to record all veterinary transactions over the next three months. The main outcomes were correct diagnosis, correct choice of drugs and correct dosage.

4.5 Data analysis

Questionnaires were entered into a Microsoft Excel® or Microsoft Access® database. Statistical analysis was carried out with Stata® version 8 software.

Statistical models - general

The statistical models summarised in Table 4.5 were used to investigate the relationship between outcome and explanatory variables.

Table 4.5 Statistical models used in data analysis

General Linear Univariate Model	• The t-test was used for comparing two groups (normal continuous data) • ANOVA was used for multiple groups and confounding factors • Multiple regression was used when there were multiple predictors
General Linear Multivariate Model	• Factor analysis was used for detection of underlying structure in data
Generalised Linear Model	• Logistic regression was used for dichotomous variables • Exploded logistic regression was used for ranked data • Rare event logistic regression was used for uncommon outcomes • Robust regression was used for clustered and heteroskedastic data • Poisson regression was used for count data • Generalised estimating equations were used for clustered longitudinal data • Cross sectional time series was used for wide and short longitudinal data • Generalised linear latent and mixed modeling was used for multilevel data

All models followed a general approach to model building, fitting and diagnostics, as follows: to avoid spurious significance and over-fitting, we distinguished between Exploratory Data Analysis

(EDA) used to investigate patterns and generate hypotheses, and Confirmatory Data Analysis (CDA) used to test already formulated hypotheses. For EDA, variables with theoretical or empirical basis for inclusion, either as predictors, confounders or precision variables, were assembled. Next biologically or sociologically plausible two-way interactions or non-linear terms were pre-defined (e.g. $herd^2$, age^2, breed*PCV). Exploratory univariate analysis was carried to better understand the data. Where two variables measured the same factor, that with least measurement error was chosen. Variables with many missing values were avoided, and similar variables were combined into an index. Next, a model was constructed using all the variables identified as relevant in the preliminary analysis. Variables were removed one at a time based on high p value and small standardized regression co-efficient. If inclusion of an otherwise non-significant variable caused a hypothesis variable to have a clinically/practically important change in its coefficient, this variable was considered to be a confounder and included in the model. Interactions were then added and models with and without interaction terms compared for goodness of fit using Akaike's information criterion and the Bayesian information criterion. Interactions significant at the 10% level of alpha, and improving fit were incorporated into the model. Where CDA was used to test hypotheses, only the variable related to the hypothesis being tested and known confounders (e.g. dummy variables for clusters) or precision variables (variables associated with the response which can decrease uncertainty and improve precision) were included, to avoid spurious significance.

Model fit was assessed by the R^2 statistic in the case of linear regression. For logistic regression McFadden's R^2, percentage of correct classifications and the Hosmer-Lemeshow statistic were used. Standard regression diagnostics were carried out (Table 4.6) and are referred to in the results section only when problems were found, and variables dropped or modifications made to the analysis (e.g using robust standard errors).

Table 4.6 Standard regression diagnostic tests used

Test	**Tests for**	**Problem level**
Normality of residuals	Standardized normal probability plot	Visual inspection
White test	Heteroskedasticity	p<0.05
Variance inflation factors	Multicollinearity	>10
Linearity	Component plus residual plots	Visual inspection
Specification	Missing relevant or presence of irrelevant variables	_hat _hat2/
Leverage	Influence-extreme value	>(2k+2)/n
Independence	Index plots, one-way ANOVA	ICC>0
Residuals	Influence-outlier (large residual (atypical value)	> 4/n
abs(DFITS)	General influence test, combines residual and leverage	> 2*sqrt(k/n)
abs(DFBETA)	Specific influence of observation on coefficient	> 2/sqrt(n)

If an observation was problematic on more than two influence measures it was dropped. If there was evidence of heteroskedasticity (on tests and residual plotting), multicollinearity and non-linearity then changes were made by a) transforming data (non-linearity), b) dropping redundant variables (multicollinearity), or c) performing robust regression. If these were not successful then linear regression was not considered suitable and another statistical method was chosen. Specification was less important for CDE, where only one variable was of interest.

Logistic regression has less restrictive assumptions than linear regression. We checked for: outliers (deviance residuals and standardised Pearson residuals); leverage; linear relation between independents and the dependent variable; sampling adequacy (no cell frequency <1 and no more than 20% of cells <5): specification (hat, $hat^{2,}$ and omitted variable test); multilcollinearity and adequate number of observations. If problems were found the analysis was modified.

Another general method used in multiple evaluations was Participatory Rural Appraisals. Tools used, that are described in the literature, included timelines, village map, institutional map, ranking, pair-wise matching, and proportional piling; creative modification were carried out as appropriate in accordance with the philosophy of PRA (Waters-Bayer and Bayer, 1994; Mariner, 2000). The next section describes specific analyses.

4.5.1 Situational analysis

The KAP survey had a complex two-stage design with subjects clustered within villages. Data were analysed using STATA survey commands (Taylor series linearization), with country as stratum and village as primary sampling unit. The assumption that the first stage sample is drawn without replacement or sampling fraction is small was met (no replacement and sampling fraction 3% in Guinea, 8% in Burkina Faso and 16% in Mali). Comparisons were made using lincom commands. Sampling was two stage: first villages were selected from a sampling frame and then villages considered to be at high risk for resistance were added. This over-represented villages at high risk for resistance, so probability weights (pweights) were used. Pweights are the inverse of selection probability and equivalent to the number of subjects in the population of interest (Kénédougou, East Sikasso and Mandiana) from which the sample is drawn represented by each individual in the sample. Sample weights were constructed as shown in Table 4.7. For example, in Mali there were seven high risk villages out of thirty, but the KAP contained five high risk villages in a sample of 16, so each subject in a village selected for high risk represented 0.9 subjects in the population and each subject in a village not selected for high risk represented 1.1 subject in the population.

Table 4.7 Probability weights for villages depending on the method of sample selection.

Selection method	Country	Initial selection	Risk	Proportion	KAP - chosen	Risk	Proportion	Pweight
High risk of resistance	Mali	25	7	0.28	16	5	0.31	0.8960
	Burkina	25	11	0.44	8	5	0.63	0.7040
	Guinea	30	3	0.10	3	2	0.67	0.1500
Other	Mali	25	18	0.72	16	11	0.69	1.0472
	Burkina	25	14	0.56	8	3	0.38	1.4933
	Guinea	30	27	0.90	3	1	0.33	2.7000

- Equity of cattle ownership was described by a Lorenz curve. In this the cumulative percentage of population is plotted along the horizontal axis whilst the cumulative percentage of possession is plotted along the vertical axis. The Gini coefficient corresponds to twice the area between the Lorenz curve and the diagonal and varies from 0 (perfect equality) to 1 (perfect inequality).

- Exploratory Factor Analysis was used to investigate farmer recognition of the more specific signs of AAT. This is a technique used to uncover the latent structure (dimensions) of a set of data producing a small number of factors from a large number of variables which is capable of explaining the observed variance in the larger number of variables. Since factor analysis is based on a correlation or covariance matrix, it assumes the observed indicators are measured continuously and distributed normally. Signs used by farmers in diagnosis were recorded as a dichotomous variable (present, absent) but are indicators of underlying continuous variables, so tetrachoric estimators were used (Drasgow, 1988). To carry out factor analysis, we first generated a correlation matrix for all the variables. Secondly, factors were extracted from the correlation matrix based on the correlation coefficients of the variables and finally factors were rotated in order to maximize the relationship between the variables and some of the factors.
- The importance of diagnostic signs were assessed using Odds Ratios (OR), (the odds of an subject in the group of interest suffering an adverse event relative to a control subject) as this is the only measure appropriate in case-control studies where the prevalence is not known. Probability was calculated as OR/(1+OR). Sensitivity, specificity, negative predictive value and positive predictive value were used to assess farmer diagnosis of trypanosomosis. Sensitivity is the proportion of those with disease who have a positive test; specificity is the proportion of those without disease who have a negative test; Positive Predictive Value (PPV) is the proportion of those with a positive test who have disease; and Negative Predictive Value (NPV) is the proportion of those with a negative test who are free of disease. While sensitivity and specificity are characteristics of the test, the more informative PPV and NPV depend on the prevalence of the disease tested for.

4.5.2 Evaluation of AAT control strategies

A variety of quantitative and qualitative methods were used to assess the different strategies for trypanosomosis control (participatory vector control, trypanotolerant cattle, provision of RDU information, training farmers in integrated trypanosomosis control).

Participatory vector control assessment

The effectiveness of vector control was assessed using epidemiological surveys. A cohort of 100 animals per village, selected by judgement, were followed over a period of two years, during which time six epidemiological surveys were carried out. Survey data was preliminarily analysed using chi-square test and Response Feature Analysis. In the latter, the responses of each subject are used to construct a single number that characterises a relevant aspect of the subject response profile. This naïve analysis does not take into account clustering or make full use of longitudinal information. Panel series analysis (CSTS) was considered appropriate because of the large number of censoring over the two year follow-up period and the short (4 follow ups) and wide (580 cattle) nature of the data. A random effects model was chosen because of the presence of time-invariant dependent variables, and because and objective was making population generalisations.

Because enumerators were living in the study villages, missing data was not a problem in this study. Drop-out of farmers was very low (<5%) and though there was a relatively high drop-out of cattle (30%), in most cases (87%) the reasons for loss to follow up were recorded.

Multiple outcome measures were assessed, so to avoid problems with chance significance (Ludbrook, 1998) all outcome measures were predetermined and theoretically coherent. Significance levels were not adjusted, but following the recommendations of Zhang *et al.* (1997) a single primary outcome was pre-identified. Haematocrit values, production parameters and survival of the cohort animals were assessed at the same time as the epidemiological surveys. Production and PCV data was assessed using CSTS and mortality data using Survival Analysis.

Mortality was the primary outcome for assessing benefit of VC. We compared survival in control and test groups by plotting Kaplan Meier estimators of the group specific survivor function and applying the log rank test (the survivor function is the probability of surviving to a given time). As this did not allow for confounding variables or clustering, Cox regression was carried out, using robust standard error to allow for clustering on village and stratifying to allow for the confounding intervention. The assumption of proportional hazards was tested using Schoenfeld and scaled Schoenfeld residuals. Missing data causes problems in survival analysis as missing subjects are more likely to have negative outcomes so cannot be assumed to be missing entirely at random. In this study rigorous follow-up kept missing data to a minimum and reasons for missing were recorded to investigate potential bias. Impact was also assessed by the KAP survey; this was administered at baseline and two years later, as data was clustered we used a modified t-test for analysis (Donner and Klar, 2000). Thirdly, impact was assessed using qualitative PRA techniques.

Evaluation of trypanotolerance

Trypanotolerance was evaluated using data collected in the various surveys. Prevalence data was evaluated using Taylor linearisation, and linear regression was performed using EDA linear regression as described earlier. Benefits were assessed using data from the cohort study and CSTS analysis. Acceptability was measured using KAP and PRA .

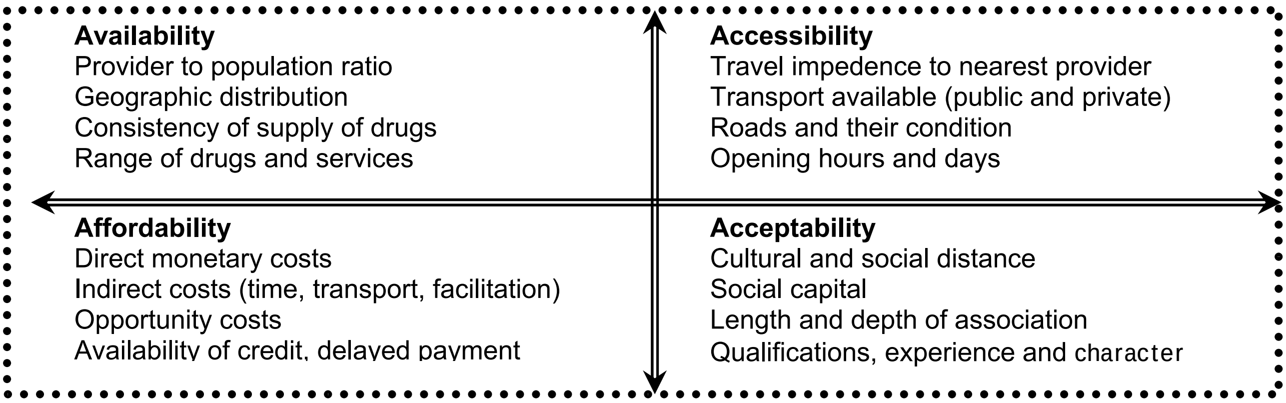
Because most analyses were based on cross-sectional surveys, missing data was not an issue.

Evaluation of RDU information

The intervention of informing farmers on AAT was a CRCT. These have traditionally been analysed by using summary statistics or adjusting the chi square/t statistic by the design effect, this is liable to the ecological fallacy, and individual analysis using multilevel models makes better use of the data. Methods reported in the literature include the generalized linear mixed model, generalized estimating equations (GEE), and hierarchical Bayesian models. All require a relatively large (~25) number of relatively large (~25 member) clusters to achieve their asymptotic performance. In our study there were 46 clusters of approximately 100 cattle, 20 treatments and 10 farmers. GEE models were chosen as more suitable for evaluating differences in population averaged response rates (i.e., treatment vs control group differences at follow-up) (Laird and Ware, 1982; Hui *et al.,* 1998). GEE are less appropriate when there is missing data (because subjects are not assumed to be measured at the same number of time points, thus subjects with missing data on the dependent variable are not excluded from the analysis (Hedeker and Gibbons, 1997; Little, 1995)), but in this relatively short study this was not considered a problem *ex ante*. Because of the relatively short duration of the experiment there were few farmers missing; 96% of farmers were present at the first follow-up and 99% of farmers at the second follow-up. Missing data at farmer level were ignored. However, in the assessment of health herd only 89% of cattle were presented after five months. For missing cattle we adopted the conservative method of carrying forward the last value as this was not likely to over-estimate the benefits of interventions. We also analysed the mortality reported by farmers in the last five months, to check for any unanticipated increase in deaths in the intervention group.

The efficacy of establishing primary animal health services (paravets) was evaluated by assessing improvement of farmer knowledge and skill after training using paired t-tests. The impact of paravets was evaluated using the ‘Four A’s’ model of health behaviour (Penchansky and Thomas, 1981). This supply side psycho-social model, evaluates service provider availability, accessibility, affordability and acceptability (Figure 4.3) and can incorporate the transaction costs of accessing medical services (i.e. the costs involved in making an economic exchange). Kruskal–Wallis tests were used to measure impact after testing showed the assumptions of ANOVA were not met. Evaluation of the effectiveness was experimental and some data was missing. Four out of the original 24 paravets were not selected by their communities for a second training, because they were judged not to have been effective. Hence, the long term effectiveness of training (at ten months) is based on successful paravets and as such may have a bias towards over-estimation. Evaluation of the impact of paravet training was largely based on observational studies, and as such, missing data was not a problem.

Figure 4.3 “Four A” model of health care provision



The evaluation of training service providers had a multilevel design: randomisation was by sub-prefecture, the next level was service provider and the unit of analysis was transaction or the diagnosis/treatment nested within the transaction. Studies with more than two levels require special techniques. We used Generalised latent linear and mixed modelling (GLLAMM), a class of multilevel latent variable models for (multivariate) responses of mixed type (Rabe-Hesketh, 2005). As for GEE, the conditional distribution of the responses given the explanatory variables and random effects is specified via a family and a link function.

Sample size was less of an issue as all veterinary transactions by all service providers were included in the study. Problems with missing data were minimised by providing incentives for record compilation, and by regular (monthly) data collection.

Evaluation of Benefit-cost ratio of interventions

Comparative benefit-cost ratios (BCR) were calculated for the four interventions over ten years using a discount rate of 10%, as recommended in evaluation of AAT control (Shaw, 2003). Data on costs was based on the actual expenditures and, in the case of trypanotolerant cattle, the differential in price between breeds reported by Kamuanga *et al.* (2001). Sensitivity analysis was carried out to allow for plausible increases in price and savings due to economies of scale in large projects. Benefits were assessed in terms of reduction in mortality, as data on this was available for all interventions. The more detailed benefit evaluation in Burkina Faso showed this was the single biggest benefit from trypanosomosis control and constituted around half the total benefits of control. Sensitivity analysis was carried out on the 95% confidence intervals of the estimates of the reduction of mortality for each strategy, to account for imprecision in the estimates. The value of an average bovine was calculated from data on the prices of different categories of animals in the study area and their relative proportions. All calculations were made with Excel®.

4.6 Mathematical model

Modelling was performed using Simile® 3.2 software. This is a software environment for building and running simulation models in ecology, biology, environmental science and related disciplines. It is based on system dynamics, whereby a system is conceptualised in terms of storages and flows, corresponding to underlying differential or difference equations (Forrester, 1971). Simile supports object-based modelling and modular construction; modules can be nested to any depth. We used separate sub-modules to represent tsetse fly and alternative host populations.

Inputs for the mathematical model were derived from published literature and the field studies. The starting parameters represented that of a “traditional west African village”, that is with a population of trypanotolerant cattle. Values for village herd number, rates of entry (birth, purchase) rates of exit (sales, death) and AAT related morbidity and mortality were taken from the epidemiological and KAP surveys. System constants were adapted from Rogers (1988).

The model was built in Tool Command Language and run for 600 time units at steps of 0.1 unit for each scenario and display set to one unit. The model was run under four scenarios: all trypanotolerant cattle, some trypanosusceptible cattle, majority trypanosusceptible cattle and trypanosusceptible plus vector control. The first scenario used AAT prevalences from the survey, in the other scenarios prevalences were predicted by the model and compared to survey findings.