



**Grazing animals and the risk of
Cryptosporidium parvum
contamination to the raw water supply
STATUS REPORT 2002**

Mount Lofty Ranges Watershed Protection Office

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**Grazing animals and the risk of *Cryptosporidium parvum* contamination to the raw water supply:
Status Report 2002**

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TABLE OF CONTENTS

ACKNOWLEDGMENTS	I
INTRODUCTION	1
Purpose	1
Project objectives.....	1
APPROACH	2
Literature review	2
Field trial summary	2
FIELD TRIAL DESIGN	4
Sites.....	4
Equipment.....	6
Faeces	7
RESULTS	10
Rainfall.....	10
Concentration of <i>C. parvum</i> in faecal material from calves	10
DISCUSSION	11
Natural conditions.....	11
Approach to the collection of infected faeces	11
Faeces analysis approach.....	12
Data loggers	12
CONCLUSION	13
REFERENCES	14

List of Figures

Figure 1 Design of east sites (20 m buffer and 0 m control buffer)	5
Figure 2 Design of west sites (5 m and 10 m buffers)	5
Figure 3 Rainfall amounts for Flaxley displayed as daily figures (12 June–1 October 2002).....	10

List of Plates

Plate 1 Flume, weir and collection sheet set up	6
Plate 2 BC 150 flume as installed at the Flaxley sites	7
Plate 3 Four-day old calves at Flaxley.....	8
Plate 4 Cages used to house infected calves and designed to collect the infected faeces	8

List of Tables

Table 1 Concentration of <i>C. parvum</i> oocysts collected and stored at Flaxley Agricultural Centre.....	10
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INTRODUCTION

The Environment Protection Authority's Watershed Protection Office (WPO) is developing a Catchment Risk Assessment (CRA) for raw water quality in the Mount Lofty Ranges (MLR). The CRA will identify and prioritise hazards to the raw water supply and, through a risk analysis, highlight those hazards that may require further detailed studies. The studies focus on determining the specific risk factors associated with significant hazards that may threaten the raw water supply. Once the relevance of the risk factors has been determined, mitigation options can be tested in the field to determine their effectiveness.

The WPO and other stakeholders have identified pathogens, particularly *Cryptosporidium parvum*, as one of the most significant risks to raw water supplies. An assessment in the upper Torrens catchment in 2000, and monitoring within sub-catchments, identified that pathogens pose a potential threat to the water quality at Gumeracha weir and thus the Adelaide water supply system.

A detailed understanding of pathogen sources is critical if the risk to the water supply is to be minimised. Grazing animal manure is a known source of pathogens and, therefore, information on pathogens in relation to grazing animals has been identified as a priority. National studies have also identified grazing animal management as a key issue for investigation.

The project 'Grazing animals and the risk of *C. parvum* contamination to the raw water supply' (*Cryptosporidium* project) was identified as a priority project to assess factors that contribute to water quality risk and to identify possible mitigation options.

Purpose

The purpose of this document is to provide internal and external stakeholders with a status report for the *Cryptosporidium* project. The literature review will be reported as a separate document.

Project objectives

The project set out to:

- investigate *C. parvum* risk to the water supply system from grazing animals, and the land management practices which may influence the occurrence of this risk
- highlight potential management techniques that may mitigate the risk
- field test the effectiveness of management techniques.

APPROACH

The project had two distinct phases:

- literature review
- field trials to test mitigation options (namely buffers).

The literature review revealed several mitigation options – for example, calving paddocks, manure management and veterinary options – but there was no capacity to test them in the field. Buffer strips require very little active management compared to other options and little is known about their impact on *C. parvum*, hence they were to be tested in this project.

Literature review

The literature review of information relevant to grazing animals, *C. parvum*, land management practices and catchment characteristics in relation to the impact on raw water quality:

- allowed an understanding of the biology and ecology of *C. parvum* to be gained
- improved the understanding of sources of *C. parvum*
- improved the understanding of infection and prevalence of *C. parvum* between species
- identified the relative concentrations of *C. parvum* produced by high risk animals
- established the highest risk period for high risk animals
- identified the relationship between rainfall and *C. parvum* and what constitutes a high risk period
- identified what instigates a pollution event
- established what work has been done elsewhere and identified how this relates to the MLR watershed
- established what types of management techniques are relevant to the watershed.

The literature review aimed to establish the relative risk of grazing animals for sub-catchments in the watershed.

Field trial summary

Field trials were set up at the South Australian Research and Development Institute's (SARDI) Flaxley Research Centre. The aim of the field trials was to establish the effectiveness of buffer strips in removing *C. parvum* from water. The sites were used to capture paddock runoff samples. Samples were to be taken from four different treatments and analysed for *C. parvum*, nutrients and turbidity. The sites were monitored to establish rainfall quantity and intensity.

The faeces were collected from animals at Flaxley. This eliminated the need to source and manage stock and allowed a known concentration of oocysts to be placed on the ground. It also reduced the risk of introducing disease.

A slurry of faeces containing a known concentration of *C. parvum* was to be placed along the buffer strips, and water samples collected during significant events to allow the effectiveness of buffers in reducing transport and viability of *C. parvum* to be determined.

The Sydney Catchment Authority and the University of New South Wales are simulating the breakdown of faeces and the impact this has on *C. parvum*. These results will be used to ensure faecal consistency is considered.

Runoff from the sites was to be captured and tested for *C. parvum* concentrations under three different buffer distances: 20 m, 10 m and 5 m, with 0 m being the control (Figures 1 and 2). The control was to be monitored without buffers to qualify the ability of the buffer widths to reduce viable numbers of *C. parvum*.

Composite water samples were to be taken to reduce the cost of sample analysis. Sample timing was to be determined once runoff characteristics were established from the first 3–4 runoff events.

At the completion of the buffer trial, monitoring of the control site (no buffer) for *C. parvum* concentrations in runoff water was to continue for a number of events. This would establish how long it took for the risk to be removed.

FIELD TRIAL DESIGN

Sites

Soils

The soils of the Flaxley catchments are dominantly Chromosols ie they have a neutral to slightly acid pH and are characterised by a very strong texture contrast between the A and B horizons. They are formed on a micaceous siltstone parent rock. Profile depth in both catchments is about 1–1.5 m over the weathering bedrock. The soils are relatively uniform between and within the catchments. The exception is the soil in the gully of the eastern catchment, which has a very dark, thick A horizon and appears to be sedimentary in origin. Probably the upper part of the profile (the A horizon) has developed from sedimentary deposits which were eroded from up slope when the catchment was cleared.

Apart from this minor soil type, classified as a Dermosol (ie a thick, dark A horizon high in organic matter), the soils are remarkably uniform. The A horizon thicknesses are 11–15 cm and are of a sandy loam texture (ie 10–15% clay). All soils in the mid and upper slopes have a pale colored leached horizon (the E horizon) immediately above the clay B horizon. The E horizons usually contain a significant amount of ironstone gravel. Variation in the amount of this gravel gives rise to a difference in the soil classification at the subgroup level. Those soils that have a particularly high content of gravel are classified as ferric. The B horizons of all soils are medium to heavy clays but have a strong, angular, blocky structure. This open structure may allow water to penetrate relatively freely through the B horizon to the weathering bedrock (the C or R horizon).

The mineralogy of the soils is very uniform. It is dominated by kaolinite with minor amounts of vermiculite and quartz. The exchange capacity is therefore likely to be low. (Fleming et al. 1996)

Physical features

Each site was surveyed on a 10 x 10 m grid to establish topography, slope, area and to allow the sites to be accurately staked out for installation of the collection sheet and flumes. The survey was then used to design each site and determine which buffer treatment should be applied to which site (see Figures 1 and 2). The treatments were placed on a site taking into consideration the resultant area once the buffer was applied so that the areas were approximately the same. This ensured that each site had a similar catchment area and hence would respond in a similar manner and yield similar amounts of water. Each catchment area was approximately 0.8 ha.

Pasture

The pasture at Flaxley is a mix of clover and rye grass. The age of the pasture is unknown.

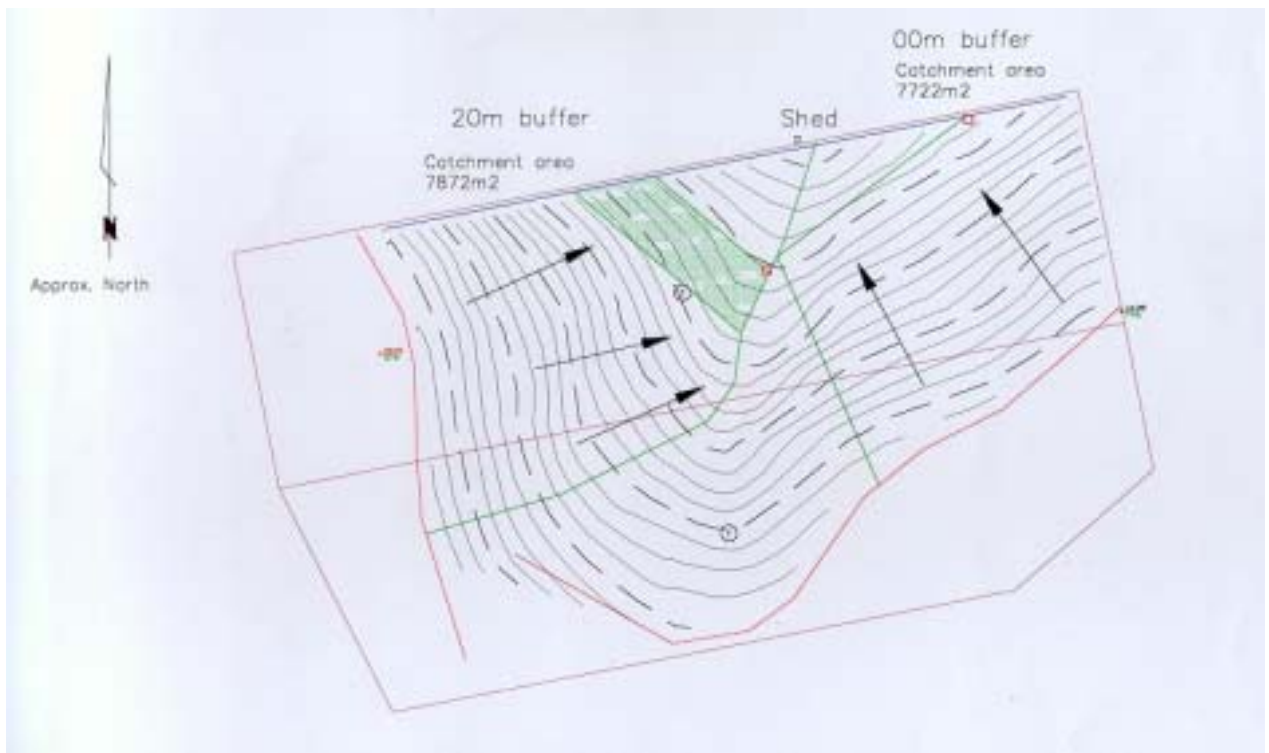


Figure 1 Design of east sites (20 m buffer and 0 m control buffer)

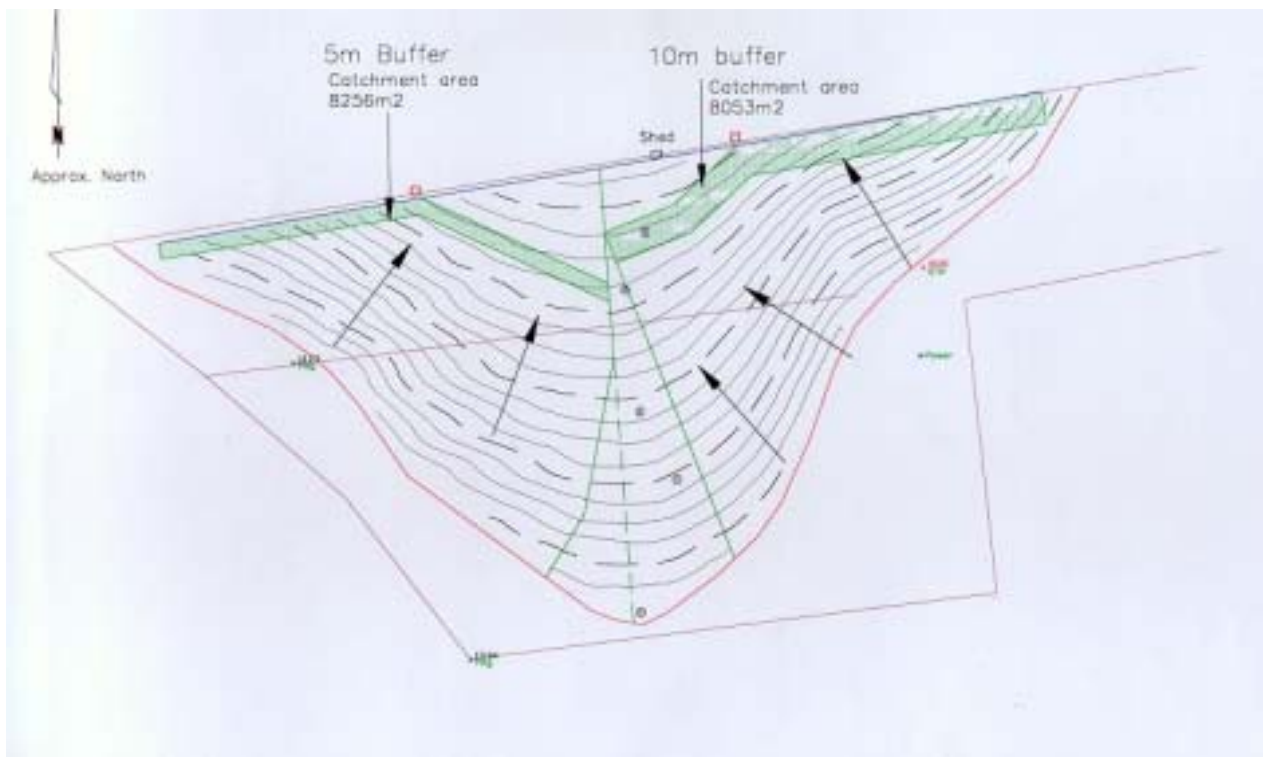


Figure 2 Design of west sites (5 m and 10 m buffers)

Equipment

Collection sheets

To collect the water and direct it to the flumes, 300 mm stainless steel and galvanized sheet metal was used (see Plate 1). The sheet was placed 50 mm below the surface and held in place with wooden stakes or sand bags. The sheets followed contours established from the survey so that overland flow would be efficiently directed to the flumes. The stainless steel sheeting can be seen coming into the picture from the left

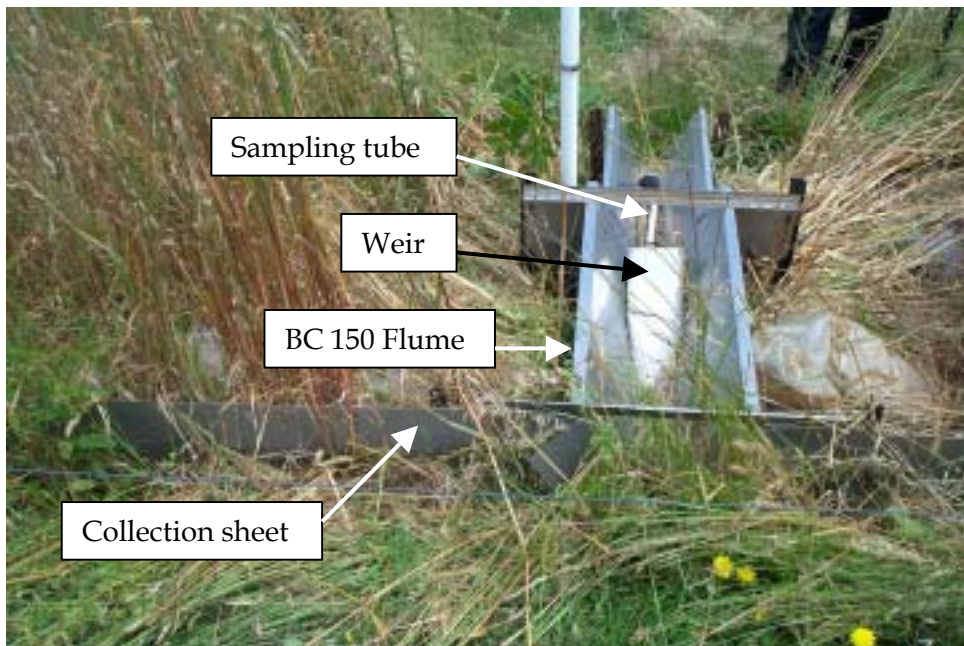


Plate 1 Flume, weir and collection sheet set up

Flumes

Stainless steel flumes were used to allow water level to be converted to a flow rate. BC 150 flumes were used, and were made from 1.6 mm stainless steel with a weir (see Plate 2). The flumes were made and supplied by Metal Form Industries. These flumes have facilities for suction lines to collect the sample and bubbler lines to measure the water level (which is converted to flow).

Auto samplers

ISCO 3700 portable automatic samplers were used to collect water samples at preset times. A 9.5 mm suction line was connected to the flume. A standard ISCO communication cable was used to connect the sampler to the flow meter. The sampler was housed in a garden shed immediately adjacent to the flume.

Flow meters

ISCO 4230 bubbler flow meters were used to measure flow and to trigger the samplers. The flow meter was calibrated so that zero flow was the top of the weir. The flow meter was housed in a garden shed immediately adjacent to the flume.



Plate 2 BC 150 flume as installed at the Flaxley sites

Pluviometer

A 0.2 mm tipping bucket rain gauge was connected to the flow meter at site 2 to measure rainfall amount and intensity at the sites.

Faeces

The faeces slurry with a known concentration of *C. parvum*, which was to be placed above each buffer strip, was to be sourced from infected calves housed at the Flaxley site in sheds. Calves are used as they excrete large quantities of *C. parvum*.

Calves

To ensure infected faeces could be sourced for the project, calves were infected with *C. parvum*. 1–16-day old male *Bos taurus* species, Holstein/Friesian strain, were used in the experiment (see Plate 3). Animal ethics approval was sought and obtained for this component of the project.

Infection

Two animals were orally given 10^6 viable *C. parvum* oocysts sourced from Murdoch University, Perth. The oocysts were bleached and sterilised to ensure no other diseases or viruses were introduced to the animals. The oocysts were orally administered to the calves using a plastic disposable 1 mL pipette. Other calves were introduced to the infected calves so that they would naturally contract *C. parvum*. All infected calves were given electrolytes and access to water to limit the impact of the pathogen on their welfare.



Plate 3 Four-day old calves at Flaxley

Cages

The calves were placed in purpose built cages so that faeces could be easily collected. The cages were elevated from the ground surface and had metal sheeting attached to the under side to collect the faeces passed by infected animals. The metal sheeting drained the faeces to a plastic container at a single point. The cages were located in a shed (see Plate 4).



Plate 4 Cages used to house infected calves and designed to collect the infected faeces

Management

The collected faeces were transferred from the containers attached to the collection sheet to a 60 L storage barrel after 2% potassium dichromate was added to ensure it was preserved.

Storage

The barrels were stored in a coolroom where the temperature was kept below 4°C.

Testing

Initially, direct smears were used to establish if calves were infected and shedding oocysts. This proved too insensitive and the Australian Water Quality Centre (AWQC) was engaged to determine the concentration of oocysts. The AWQC used a microscopic enumeration of enteric protozoa method.

The method involves the addition of a known amount of faeces (usually 1 mL or 1 g depending on the consistency of the sample) to 9 ml of sterile water in a faecal particle separator. The separator has two interconnected tubes with a straining device in between. A small amount of detergent (used to emulsify the sample) and 3 ml of ethyl acetate is added to the sample which is then shaken for 30 seconds. The sample is allowed to drain through the straining device (to remove coarse debris) and then centrifuged for 5 minutes. The supernatant is discarded including the top layer of ethyl acetate which has extracted the fatty material in the sample. The resulting pellet is washed by centrifugation, re-suspended, stained and counted in a haemocytometer.

RESULTS

Rainfall

All four treatments were monitored to establish the volume of flow leaving the treatments from 12 June 2002 to 1 October 2002. Total rainfall for that period was 253 mm (see Figure 3). Sites 3 and 4 had no runoff for the period of monitoring. Site 1 had 225 L runoff and site 2 had 271 L runoff.

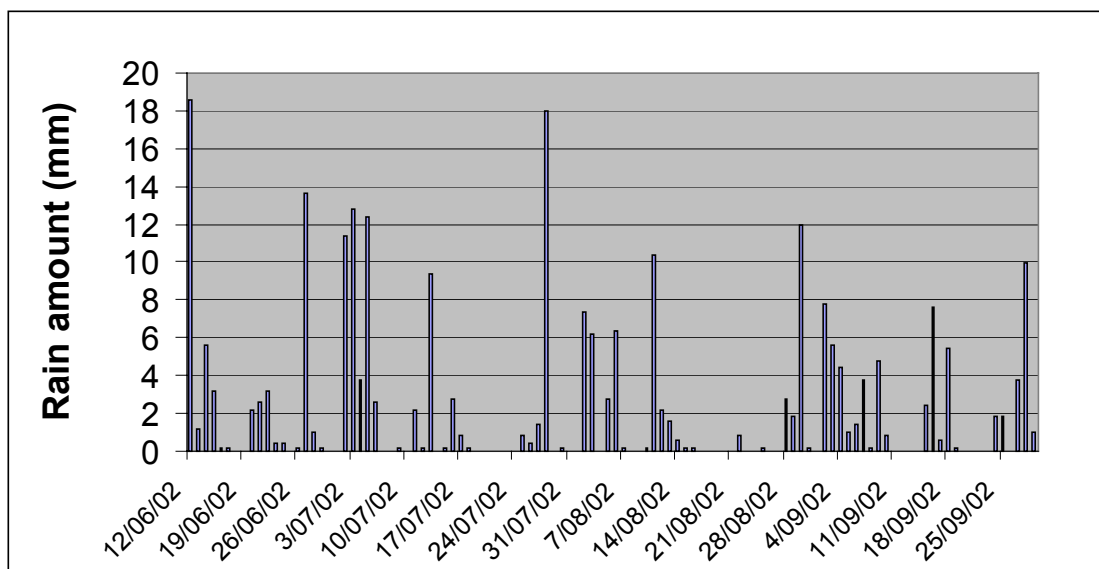


Figure 3 Rainfall amounts for Flaxley displayed as daily figures (12 June–1 October 2002)

Concentration of *C. parvum* in faecal material from calves

Aside from the rainfall and runoff results, much was learnt about *Cryptosporidium* infection and the collection of faeces, and this is reported here. A total of 110 L of calf faeces was collected. This was stored in two separate 60 L barrels each containing approximately 55 L of faeces (see Table 1).

Table 1 Concentration of *C. parvum* oocysts collected and stored at Flaxley Agricultural Centre

Barrel	Date	Concentration*
1	3 June 2002	5.4×10^6 /mL
1	9 July 2002	1.4×10^6 /mL
1	8 August 2002	2.0×10^6 /mL
1	10 September 2002	4.46×10^6 /mL
2	3 June 2002	1.2×10^7 /mL
2	9 July 2002	1.4×10^6 /mL
2	8 August 2002	3.3×10^6 /mL
2	10 September 2002	3.14×10^6 /mL

* Concentration reported as number of oocysts per mL of faeces

DISCUSSION

Natural conditions

Lack of rainfall during 2002 effectively prevented the project from meeting its objectives. The low rainfall and long dry periods of warm weather between rainfall events resulted in the soil drying out to a point where an appropriate volume of runoff did not occur. The ability of the buffers to remove *C. parvum* could not, therefore, be tested.

At the development stage of the project it was anticipated that there would be 6–8 runoff events, based on data collected at the sites in 1996–1999. The plan was to monitor the first 3–4 runoff events from the sites so that a suitable sampling strategy could be developed. This would allow the identification of any background *C. parvum* on the sites and would also provide hydrological data that could be used to refine a sampling strategy. When it became obvious that there may be only one chance to run the project, historical data were used to develop a contingency plan (sampling strategy). The strategy was not implemented because no runoff event ensued.

The sites did not receive enough rainfall to trigger runoff events. Most rainfall events were interspersed with a number of days of no rainfall, allowing the surface soil to drain and dry out to a point where follow up rainfall needed to be significant enough (probably greater than 20–25 mm) to 'wet up' the subsurface soil. The surface soils at Flaxley are light (sandy loams) and need follow up rainfall quickly after receiving 20–25 mm to ensure runoff occurs.

Historical data shows that there are several differences between sites that allow the eastern sites to respond more quickly and yield higher volumes than the western sites, probably because of soil type. The eastern sites are underlain by clays at shallower depths (approximately 25 cm). The western sites are underlain by clays at approximately 31 cm. The percentage of clay in the top 30 cm of soil is also higher on the eastern side (32%) than on the west (16%). It is thought that the soils on the eastern side reach field capacity quicker than those on the west and also dry out more slowly. This means the eastern sites contribute to overland flow more quickly and for longer periods than the western sites.

Approach to the collection of infected faeces

Parasitologists and epidemiologists from SARDI Livestock Systems Division suggested that the calves at Flaxley would become infected with *C. parvum* naturally. This did not occur so a plan to infect calves was developed to ensure infected faeces could be collected. A proven strategy to infect calves and collect infected faeces has been developed and includes measures to ensure the impact on calves is limited. *C. parvum* can be fatal to young calves and steps were taken to ensure their welfare was not compromised. Calves were periodically dosed with electrolytes to limit the impact of *C. parvum* and secondary infections on calf welfare. Calves infected with *C. parvum* that die probably do so from secondary infections caused by rotavirus. The use of electrolytes probably reduces the risk of calves dying.

The infected faeces were stored to be used later when rainfall and runoff occurs. Calving starts at Flaxley in April and can continue through until mid-June. Most runoff at the sites occurs between July and October, therefore a gap exists between faeces collection and application to the sites. The 2% potassium dichromate added to the collected faeces before storage reduces the presence of organisms such as bacteria and fungi that may decrease the viability and concentration of *C. parvum* oocysts. This ensures the faeces containing viable *C. parvum* oocysts can be stored for extended periods. The faeces were tested monthly to establish the survival of the oocysts in the storage barrels. It was found that the concentration remained very stable over time (see Table 1) and it is likely that the stored faeces could be used in 2003 if the project continues.

Faeces analysis approach

The sensitivity of the test used to identify oocysts in faeces is important to the success of *C. parvum* projects. Direct smears were found to be too insensitive and this method caused problems early in the project when attempting to establish if calves were infected. The testing method was changed to the microscopic enumeration of enteric protozoa method when direct smears returned negative results 4–14 days after calves were infected. Faeces collected 12 days after infection were tested using both methods. The results were considerably different with approximately 10^6 oocysts per gram of faeces found using the microscopic enumeration of enteric protozoa method and a oocyst negative result using direct smears. It is suggested that a reliable laboratory that is familiar with sensitive testing methods be used to ensure reliable results in the future.

Data loggers

Early in the project when flow meters were initially set up, there were problems with the data from site 2. The flow meter was logging large volumes of runoff at times when there was no rainfall and logging rainfall that did not occur. The program was checked and appeared to have no problems. The meter was eventually 'reset' when it was determined that the problem was not program or data related.

It is still not clear what caused the problems with the data. There are three possible explanations of the interference with the flow meters:

- electromagnetic radiation from a nearby overhead power line transformer
- electromagnetic radiation pulses from a portable electric fence close to the site
- electrical storms around the time phantom data was logged (at the start of the project people at Flaxley witnessed lightning strikes close to site 2 on numerous occasions).

CONCLUSION

The uncharacteristically dry season experienced during 2002 effectively prevented the original objectives of the project from being met. Flaxley, and the Mount Lofty Ranges in general, experienced drought conditions throughout 2002. Rainfall well below average led to insignificant amounts of runoff, well below that required to initiate a sampling response.

There were complications in obtaining *C. parvum* infected faeces. Calves at Flaxley did not naturally contract *C. parvum*. A strategy was developed to infect the calves and collect the infected faeces.

Many of the unanticipated problems of the project have been rectified or strategies developed to overcome them in the future. For example, the infection of calves and collection of infected faeces can now be done relatively easily and a strategy to do this has been developed.

A considerable amount of information has been gained on the ecology and parasitology of *C. parvum* and how to successfully implement this project.

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Fleming, N., Cox, J.W., Chittleborough, D.J. and Nash, D. 1996. *Optimising phosphorus fertiliser use on dairy farms*. Preliminary report, Dairy Research and Development Corporation, South Australia.