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Cryptosporidium in River Waters: Inception of the Study

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CRYPTOSPORIDIUM IN RIVER WATERS: INCEPTION OF THE STUDY

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EXECUTIVE SUMMARY

The Committee of Experts established after the outbreak of cryptosporidiosis in Swindon and Oxfordshire in 1989 made recommendations for studies to determine levels of oocysts in source waters and to seek to determine the origin of the oocysts. This report describes the initiation of a NRA funded study to ascertain whether or not a link can be established in agricultural areas between livestock farm pollution incidents or farm husbandry and the occurrence of Cryptosporidium oocysts in surface waters. The rationale for the choice of sites, the choice of sampling and analytical procedures and the programme of study are described.

The study will complement the survey of occurrence of oocysts in surface waters and groundwaters, the presence of oocysts in potable water and cryptosporidiosis in the community. Discussions have been held and close liaison is anticipated with a MAFF funded study investigating the survival of oocysts within the farm waste environment.

KEY WORDS

Cryptosporidia, surface waters, pollution.

1. INTRODUCTION

Cryptosporidia, on intestinal protozoan parasite, has only been recognised as a pathogen of animals and humans during the last decade. In normal healthy individuals cryptosporidiosis usually takes the form of an acute self-limiting diarrhoea from which the patient recovers fully. In the immuno-suppressed the disease may be far more serious. The disease is widespread in farmed animals. Infected animals, and humans, excrete large numbers of oocysts, the environmentally resistant form of the protozoan, during the course of the disease. Thus it is likely that agricultural pollution incidents, land run-off or sewage effluents may contaminate surface waters. The size and nature of the oocyst give the potential for small numbers to pass through treatment systems and enter potable water supplies. The oocyst is resistant to disinfection and the infective dose to man is low. A number of waterborne outbreaks have occurred in the US and the UK.

Following an outbreak of cryptosporidiosis in Swindon and Oxfordshire in 1989 the Government established a group of experts under the chairmanship of Sir John Badenoch to advise on the significance of cryptosporidia in water suppliers. Their report (Department of the Environment and Department of Health (DoE/DH) 1990) made a number of recommendations which included (Recommendation 4) "Research should be undertaken to determine the levels of oocysts occurring in different types of water sources in the UK including groundwater and to seek to determine the origin of these oocysts."

The National Cryptosporidium Survey, funded in part by the NRA, as R&D Project 151 has addressed the occurrence of oocysts in surface waters and groundwaters in relation to the presence of oocysts in potable waters and cryptosporidiosis in the community (Miller and Carrington, 1992; National Cryptosporidium Survey Group, 1992; Marshall, Stanwell-Smith and Holmes, in preparation) and the study has also been compared with similar studies in the US (Carrington and Miller, 1993). A study funded by MAFF and SOFAD will investigate the partitioning between liquid and solid fractions of farm wastes and the survival in farm wastes of a number of pathogens including cryptosporidia. This current study will provide the link between farming practices and occurrence of oocysts in surface waters.

This interim report is required to detail the inception of the study and the full programme.

2. OBJECTIVES

The overall objective is to ascertain whether or not a link can be established in agricultural areas between livestock farm pollution incidents or farm husbandry and the occurrence of Cryptosporidium oocysts in samples of surface water.

The specific objectives are:

1. To define a cryptosporidium oocyst sampling methodology.

2. To undertake a sampling programme for oocysts for cryptosporidium within a selected agricultural area.

3. To monitor water quality and pollution incidents during the sampling period.

There is also a requirement that MAFF, SOFAD and DoE are given the opportunity of contributing to the study and that the work is developed in conjunction with their programmes.

3. INCEPTION OF THE STUDY

The NRA have chosen to use the River Torridge catchment for this study because of the large number of cattle and dairy farms and the relatively low human population. The Torridge catchment has been the subject of a number of studies and a considerable amount of data relating to the water quality and condition exists. The NRA South West Region have agreed to co-operate in providing relevant information and data on water quality and pollution incidents as the study progresses.

The NRA propose that seven sites in the catchment are sampled on three occasions in Spring 1993 and three occasions in Autumn 1993. This arrangement would take into account seasonal variation in flow, weather conditions and likely levels of oocysts in the environment. WRc would use subcontractors, Hallsannery Field Centre, Bideford, to carry out the sampling and the analyses would be performed at Medmenham. The Principles of Hallsannery Field Centre are qualified biologists and geographers who live and work in the Torridge area.

WRc has held a series of meetings from which the programme of work was developed. These were:

- 30 November 1992 with the Project Leader to confirm the detail of the contract.
- 12 January 1993 with the Pollution Inspector for the Torridge Catchment (C Westcott) to confirm the availability of water quality data and pollution incidents and to discuss likely sampling sites.
- 12 January 1993 with Institute of Grassland and Environmental Research (IGER) (Drs Paine, Merry and Maudsley), the main contractors for the MAFF study to discuss areas of mutual interest.
- 13 January 1993 with IGER to reconoitre likely farms for their study to maximise the co-operation between the MAFF Study and the NRA study.
- 13 January 1993 with Hallsannery Field Centre for briefing on project requirements, sampling procedures and sampling sites.

The Drinking Water Inspectorate (DWI) have expressed interest in the study and the information it can provide in relation to water quality at Torrington Water Works and a meeting has been arranged between DWI, NRA, WRc, South West Water and IGER for 24 February 1993.

4. SAMPLING AND ANALYSIS

4.1 Sampling sites

4.1.1 Rationale for choice of sites

The original concept was that the same body of water would be sampled as it moved downstream and the analyses carried out sequentially from the lower to the upper but ceasing when the first negative was reported. This strategy was intended to reduce costs. However, data provided by NRA South West Region shows that under low flow conditions the time of travel from Black Torrington (approximately 2½ km upstream of Point 3, Figure 4.1) to Newbridge, Torrington (Point 7, Figure 4.1) a distance of 35 km to be about 73 hours. Reliable data upstream of Black Torrington is not available.

Flow conditions within the R. Torridge can change rapidly during stormy conditions. Thus, difficulties would be experienced in sampling the same body of water and the increased cost of sampling over three days would exceed the savings on analyses. Local anecdotes suggest that there is considerable back and forward mixing during low flow conditions, but this is not borne out by flow data provided by the NRA. The Torridge basin comprises of a number of subcatchments. Data provided by NRA suggests that the activities and the level of pollution incidents differs between them. Sampling sites were chosen in conjunction with the local NRA to determine the influence of the subcatchments upon the water quality of the main river.

4.1.2 Location of sites

The following sites have been selected, their approximate location is shown in Figure 4.1. The sampling team will determine the exact location depending upon local conditions such as ease of access, permission of land owner or the possibility of vandalism of equipment during sampling.

Site 1, Gidcott Mill, grid reference 422094, R. Torridge upstream of confluence with R. Waldon. The catchment drains an area comprising mainly of isolated farms or very small communities. The NRA have identified a number of farms where pollution incidents have occurred.

Site 2, unnamed bridge, grid reference 4105080, R. Waldon upstream of confluence with R. Torridge. Some small villages drain into this catchment. The NRA have identified only one farm where a pollution incident has occurred.

Site 3, Sheepwash, grid reference 486057, R. Torridge below confluence with R. Waldon. Sheepwash is just downstream of two small streams where previous pollution incidents have occurred. South West Region NRA have a permanent automatic water quality monitoring station at this site.

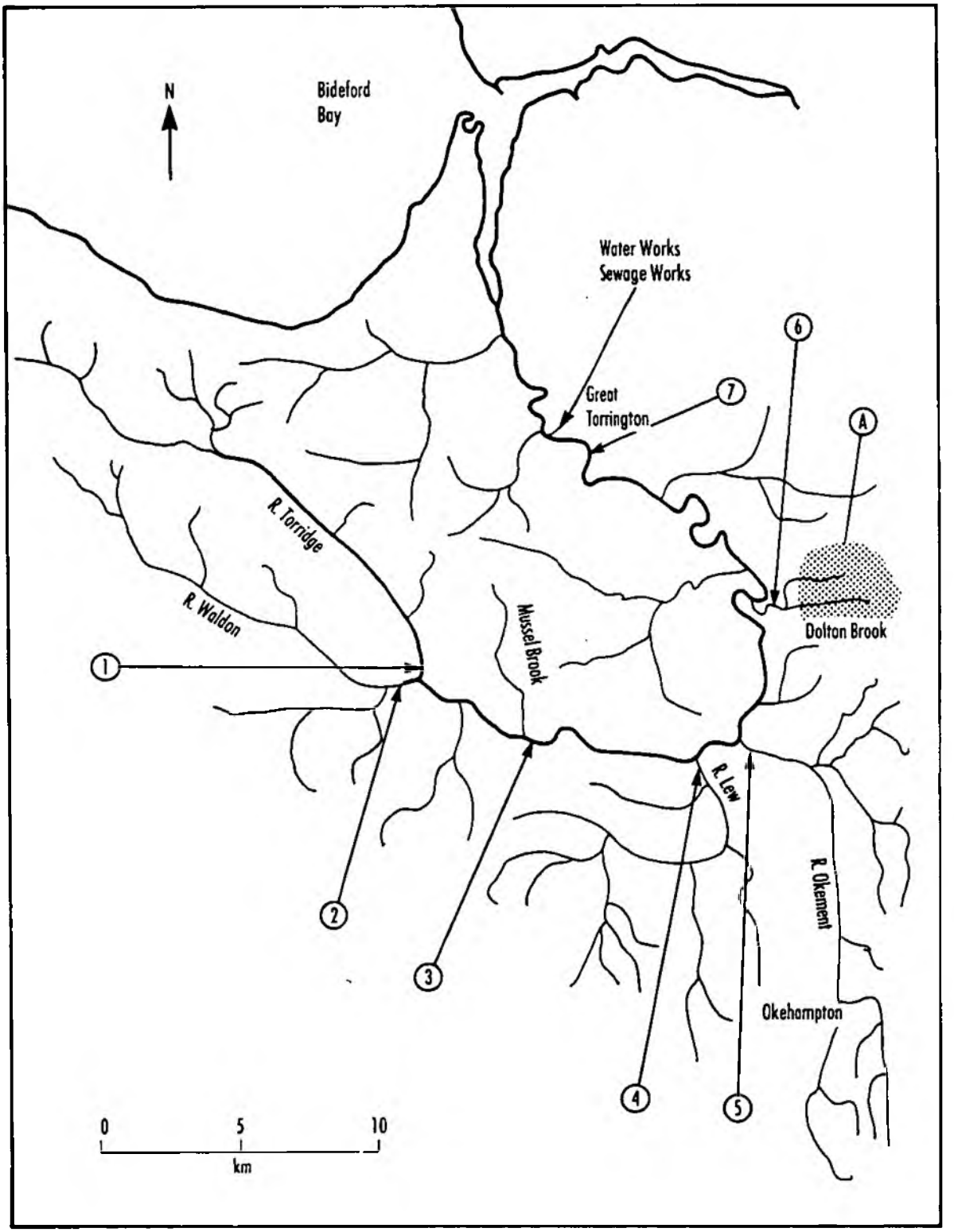

Figure 4.1 Torridge catchment showing sampling points

Site 4, unnamed bridge near Strawbridge, grid reference 533054, R. Lew just above confluence with R. Torridge. This subcatchment drains on an area containing a large number of isolated farms but few pollution incidents have been recorded.

Site 5. The choice of this site on the R. Okement will depend upon local access, it will either be near the bridge on the footpath between Arnold's Fishleigh and Nethercott Barton (grid reference 559063) or adjacent to the road bridge at Week (grid reference 467058). This subcatchment includes the northern part of Dartmoor and the town of Okehampton.

Site 6, unnamed bridge, grid reference 560117 Dalton Stream above confluence with R. Torridge. This is a relatively small catchment but a number of potentially problematic farms in the area. These and a number of other farms identified during the recognoitre of the area, together with the ease of access from North Wyke prompted IGER choose this area for the MAFF study. These farms are within the shaded A, on Figure 4.1.

Site 7, River Torridge at Greater Torrington. The exact site will depend on conditions at the time of sampling but it will be between Newbridge Torrington (grid reference 500184) and Taddiport (grid reference 497186). This point will be sufficiently upstream not to be influenced by the sewage works.

By the use of delay timers on the sampling equipment *vide infra*, it should be possible to sample the same body of water at site 1, 2 and 3 and at sites 4, 5 and 6.

4.2 Method of sampling

Two methods of oocysts isolation and recovery are in general use. One, developed at the University of Arizona from methods orginally used by virologists, has been adopted as a provisional standard by the Standing Committee of Analysts (SCA) (1990). In this method volumes of water, 100-400 litres, are filtered on site through a wound polypropylene filter with a nominal retention of 1 µm, over a period of 1-4 hours. The filters are returned to the laboratory for processing. The second method, developed at the University of Seattle (Ongerth and Stibbs, 1987), utilises a grab sample of 20 l, the whole of which is returned to the laboratory for processing.

In the context of this study the SCA method has a number of advantages over the Ongerth and Stibbs method. The sampling equipment including the battery to operate the pump can be carried to the sampling site by one person. The inclusion of delay timers in the battery circuit means that using one or two staff and one vehicle, the sampling at several sites can be arranged to coincide with a particular slug of water. The use of delay timers and valves means that the sample can be taken over a period of hours, or a sample can be representative of a large body of water. Depending upon the water quality large volumes up to 400 l, can be sampled. The filter elements can be packed in to waterproof bags and transported to the analytical laboratory easily and cheaply. The Ongerth method has the disadvantages that it is in essence a grab sample taken over a short period of time. Heavy containers need to be manhandled to the samplers vehicle, possibly over difficult terrain. Large volumes of water need to be transported to the analytical laboratory. Not only is

this costly but commercial carriers usually require a guarantee that no problems will arise from any spillage.

4.3 Analysis of samples

4.3.1 Analytical method

The Ongerth method filters the collected volume of water through a large membrane filter and then the retained material is scraped off, purified and examined microscopically. The SCA method requires that the filter is cut washed and a longer purification procedure applied. The Ongerth method is less tedious and quicker but because of the capital cost of the membrane filter holders generally only one or two samples can be processed at one time. The SCA method is more tedious, but requiring no expensive specialised equipment means that several filters can be processed simultaneously. Some reports in the literature suggest that better recovery of oocysts can be achieved with the Ongerth method. The experience of WRc is that the recovery rates using either method is similar (Whitmore and Carrington, 1992).

Examination of the final deposit will be by examination of a number of small aliquots (usually 20 µl) in discrete wells on a microscope slide. Wells will be examined completely until at least 5 oocysts have been observed or at least 5 wells have been examined.

The identity of observed oocysts will be confirmed by microscopic examination to determine their size and shape and the presence of the 'suture' using differential interference contrast (Normaski) microscopy and by their reaction with fluorescent tagged monoclonal antibodies.

4.3.2 Quality assurance

The NRA requires that a quality assurance programme is in place but suggest that it is carried out internally by WRc. The principles adopted for the National Cryptosporidium Survey will be applied. The Samplers will place a safety pin in the filter to indicate where the water entered the filter holder. On predetermined filters, on receipt at Medmenham, the filter will be slit lengthways through the point of entry and the opened filter will then be quartered. The two pairs of diagonally opposite quarters will be processed independently by two analysts.

4.3.3 Other determinands

The NRA, South West Region, will provide data on dissolved oxygen, temperature, conductivity and pH from the automatic monitoring stations at Sheepwash and Torrington. Measurements of the river flow will be made by the sampling team. It is hoped to have access to a particle size distribution counter.

4.4 Programme of work

It is planned that samples will be taken during the last week of February, March and April 1993. The results of each sampling exercise will be reported informally by letter to the Project Leader, an interim report covering this study period will be presented to the NRA in July 1993. A meeting will be held between WRc and NRA after the second sampling exercise to discuss progress and any possible modification in the programme, particularly in the light of discussions to be held with DWI. A second series of sampling exercises are provisionally arranged for late September, late October and late November.

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