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Short contribution

A quick, semi-quantitative method for recording nematode gut parasite eggs from archaeological deposits

Summary

The current quantitative approach to parasite remains is discussed and found to be overcomplicated in relation to the results produced and in view of the increasing need for rapid recording techniques in environmental archaeology. The simpler, semi-quantitative method presented here has been tested and found to produce adequate results in a short time, using uncomplicated equipment.

Introduction

The aim of this contribution is to examine the quantitative approach to the preparation of soil samples for parasite egg analysis employed by Jones and co-workers, and to suggest a simpler method. The efficacy of the latter is supported by the results of analyses of samples from excavations in Carlisle and York.

As has been suggested elsewhere (e.g. Kenward 1992) it is becoming increasingly necessary to develop rapid recording techniques for biological remains from archaeological sites as funding diminishes in relation to volume of work, particularly as the trend continues towards intensive surveys of sites (as opposed to investigation of a few, possibly

atypical, contexts). Such techniques for recording insect and plant remains are now well established (Hall and Kenward 1990; Kenward *op. cit.*). Parasite egg analysis remains a somewhat laborious task which to date has largely been carried out using relatively specialised techniques and equipment. This is unfortunate in view of the fact that results are rarely precise, often just indicating the likely presence or absence of faeces in a deposit, sometimes indicating a particular host species, generally *Homo sapiens*. It is increasingly necessary to develop a more rapid technique in the light of work discussed by Jones *et al.* (1988, 275-6), which highlights the fact that parasite eggs can be preserved in a much wider range of archaeological deposits than previously expected, including sediments containing little trace of other organic materials.

Currently, results from parasite egg analysis are presented in number of eggs per gram of sample. These counts are obtained using either a dilution method, such as the modified Stoll technique (Jones and Hutchinson 1991; Hall and Kenward 1990, 297) or a salt solution flotation method (Hall and Kenward (*ibid.*), where a saturated magnesium sulphate solution was used). Both techniques stem from methods used by parasitologists to detect worm eggs or larvae in the fresh faeces of humans (Davey 1966, 110-12) and other mammals (MAFF 1971, 1-16).

A wide assortment of both dilution and flotation methods suitable for the concentration of eggs of various or particular species have been brought together by the Ministry of Agriculture, Fisheries and Food (MAFF 1971, 1-16) for use by veterinarians, and these methods have been adopted, with very little modification, by environmental archaeologists studying parasite eggs in samples of ancient deposits. Hence, we have also adopted the clinical parasitologist's practice of estimating numbers of eggs per gram of deposit (originally eggs per gram of *fresh faeces*), a value designed to be one of 'a series of counts or comparison of counts in animals of *known history*' (my emphasis) or to be 'of some help in the diagnosis of helminthiasis provided they [the counts] are interpreted with caution' (MAFF 1971, 1). In the context of veterinary parasitology we are warned that 'the assumption that the size of worm burdens may be accurately deduced from faecal egg counts has not proved

justified'; there is a diurnal variation in the number of eggs released in faeces, eggs are often not evenly distributed throughout the faeces, and the moisture content is variable, thus affecting its density (*ibid.*). Furthermore, in infections where the worms are close together and the parasitized mucosa is badly damaged, egg production can be expected to be reduced (Faust *et al.* 1962, 232). These facts alone render the use of 'egg count per gram' fairly meaningless unless strict control is put on the time of day of faeces collection and on moisture content.

In addition to these unknowns, the variations in egg counts from archaeological contexts will clearly be greatly affected by unknown factors such as dilution by other waste and backfill deposits, biodegradation of organic material (Jones 1982, 68) and the incidence of parasitism in the contributing population.

Considering all these variables, there seems little justification in attempting to estimate egg concentration in archaeological deposits to anything more than a rather crude level. It may be argued that nobody has previously attempted to assess the worm burden of an individual or the proportion of an infected population on the basis of the analysis of archaeological sediments, yet a measurement has been adopted which has little purpose other than to be used in this way. It may enable a comparison between samples to be made, or indicate the presence of faeces, but as a quantitative measurement it has spurious accuracy, is generally unnecessary, and may be misleading.

The alternative method proposed in this paper still provides an indication of the presence of ancient faeces in a deposit, but is more rapidly employed and requires less specialised equipment and procedures, and is thus more suited to current needs. Quite simply, it involves using 'squashes' of raw sediment.

The method given below was used for the present experiments. It may be desirable to change the scanning magnification (Kenward *et al.* (1986, 246), for example, used x120). The magnification used here was adopted because of the equipment available, rather than for good theoretical reasons. Clearly if *measurement* of eggs is to be attempted, a higher magnification should be used (Kenward *et al.* (*op. cit.*) suggest x400).

Methods

In the 'squash' technique small lumps of raw sediment (approximately 3 mm diameter) were taken from three separate points within the sample (to take account of heterogeneity) and homogenised in a little water by shaking. After allowing coarse particles to settle for a few moments, a drop of the supernatant was removed using a Pasteur pipette and placed on a 76 x 22 mm glass microscope slide and covered with a 22 x 50 mm cover slip (an approximately 1 cm diameter drop on the slide being sufficient). With a little practice it became fairly easy to ensure that the mixture was not too flooded with water or so thick that large particles were incorporated in the 'squash'. The whole mount was then scanned rapidly using a magnification of x60 and the abundance of eggs was recorded semi-quantitatively on a six-point scale: one; 'trace' (estimated as 2-5); 'few' (6-10); 'some' (11-20); 'many' (21-100) and very many (probably more than 100).

This method was first carried out on 70 samples, mostly from Roman deposits, from excavations at Old Grapes Lane A (OGLA), Carlisle, Cumbria, U.K., provided by the Carlisle Archaeological Unit for insect and parasite analysis (results are presented by Allison *et al.*, forthcoming).

In order to check the reliability of the results obtained, it was decided to test a number of samples using the modified Stoll technique (MAFF 1971, 3-4), in the form used by Jones and Hutchinson (1991, 68-9). Eighteen samples were checked: six for which no eggs had been found in the squashes, six for which small numbers had been noted, and six for which they had been recorded as numerous.

Briefly, a subsample of 6 g was taken from each sample and placed in 42 ml of sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) solution, in which it was disaggregated by shaking. Each subsample was left for 2 days before pouring it through a freshly flamed 250 μm sieve to remove coarse particles and adding a further 42 ml of water. A 0.15 ml aliquot of the resulting suspension was then placed on a 76 x 25 mm microscope slide and covered with a 22 x 50 mm cover slip. The mount was then scanned under a transmission microscope at a magnification of x60, and all the eggs seen were identified and counted.

Following the modified Stoll method it would then have been a simple matter to convert the number of eggs counted into an estimate of eggs per gram by multiplying by 100 (Jones and Hutchinson 1991, 70; Jones 1985, 109). This has not been done here for two reasons: firstly, in order to facilitate comparison with the 'squash' technique results and, secondly (and more importantly), because this 'multiplying up' exaggerates the errors inherent in the counting technique. It would not be difficult to believe that a lump of deposit with an actual concentration of, say, 300 eggs per gram might give an estimate of between 0 and 1000 or more.

Table 2 shows the results of this comparison for the 18 samples from OGLA.

It was immediately clear from the results that only samples with a low concentration had been tested and it was deemed necessary to test some samples with a known, higher, concentration.

Seven samples from Anglo-Scandinavian deposits from excavations at 16-22 Copper-

gate, York, previously examined using the modified Stoll technique by A. K. G. Jones (Kenward and Hall, forthcoming) were re-examined using the squash technique. Two counts had originally been made on some of the samples and both have been given.

Unfortunately the unused portions of the parasite subsamples chosen had become desiccated, so it was first necessary to rehydrate a small portion of each. Again, three small lumps were taken from different areas of each sample. These were then soaked in a little water for 3 days. At this stage it was still not possible to disaggregate the samples by shaking, so it was decided to boil each for a few minutes. All but one of the samples then broke down fairly well, but an initial examination of sample 3000 showed that it had not fully disaggregated. A further subsample of it was therefore soaked in a little sodium pyrophosphate solution for 2 days before re-examination.

The scanning procedure was carried out in the same way as for the OGLA 'squash' samples. The eggs were, however, recorded both semi-

Sample	Stoll		Squash	
	<i>Trichuris</i>	<i>Ascaris</i>	<i>Trichuris</i>	<i>Ascaris</i>
3	15	1	few	trace
7	0	0	trace	0
14	3	0	one	0
15	5	0	one	0
17	0	0	0	0
24	1	1	0	0
26	0	4	trace	few
35	0	0	0	0
39	0	1	0	0
42	0	0	one	0
48	1	1	trace	trace
50	0	4	0	0
59	0	0	0	trace
64	1	0	0	one
67	0	2	0	0
76	0	0	few	one
77	0	0	one	0
85	0	0	0	one

Table 2. Parasite eggs from 18 samples from the Old Grapes Lane A site, Carlisle, recorded using the modified Stoll technique (quantitative) and the squash technique (semi-quantitative).

quantitatively and as number of eggs per slide (the latter in order to test the ratio of *Trichuris* to *Ascaris* eggs in the sample and to compare this with the same ratios from Jones' results). The counts are recorded in Table 3, and Table 4 compares the *Trichuris/Ascaris* ratios.

Results and discussion

In examining the results obtained from the OGLA samples, it is firstly important to note that in all cases the concentrations of eggs were quite low, the highest apparently being that from sample 3, where 15 *Trichuris* eggs were counted using the modified Stoll technique. Given the method suggested by Jones (1985, 109) this would indicate a concentration of 1500 eggs per gram, which Jones tentatively suggests is indicative of a layer 'probably containing a substantial amount of faeces; (Jones 1985, 112). The squash technique showed a 'few' *Trichuris* eggs. The techniques were thus in broad agreement. Sample 76, on the other hand, also gave a 'few' *Trichuris* eggs but the subsample examined by the modified Stoll technique was apparently barren. Presumably this was the result of sample heterogeneity.

In all other cases, it appears the results from the squash and modified Stoll methods are

similar where egg concentrations are low. Low numbers of eggs may be missed by either technique, but the archaeological significance of low concentrations is doubtful. As suggested by Allison *et al.* (forthcoming) these variations are not really surprising considering the likely patchy distribution of eggs in any archaeological deposit.

The results obtained from the Coppergate samples using both techniques (see Table 3) clearly indicate higher concentrations of eggs and thus provide a further proof of the effectiveness of the squash technique. In cases where a substantial number of eggs had been revealed by the Stoll technique, the squash method invariably indicated a similar high concentration. In fact, in most cases the results were remarkably close, considering the lack of precision involved in the squash preparations. There are some inconsistencies in the data, however, such as the number of *Ascaris* eggs recorded from sample 1913, though this hardly detracts from the validity of the squash technique. The results for sample 3000 are at first sight less consistent in that, whilst considerable numbers of both *Trichuris* and *Ascaris* were apparent in the squash, these values were still significantly lower than those obtained using the Stoll method. There are two possible explanations for this. Firstly, an examination of the written record for worm

Sample	Stoll		Squash	
	<i>Trichuris</i>	<i>Ascaris</i>	<i>Trichuris</i>	<i>Ascaris</i>
1353	25	4	24 (many)	5 (trace)
—	20	5		
1732	19	15	28 (many)	19 (some)
1907	132	43	105 (v. many)	28 (many)
—	131	48		
1911	142	28	270 (v. many)	41 (many)
—	149	21		
1913	37	0	72 (many)	10 (few)
2135	469	10	282 (v. many)	16 (some)
—	157	12		
3000	117	81	51 ^x (many) 49 ^y (many)	42 ^x (many) 42 ^y (many)

Table 3. Parasite eggs from 7 samples from the 16-22 Coppergate, York, site recorded using the modified Stoll technique (quantitative) and the squash technique (quantitative and semi-quantitative). x/y: before/after treatment with sodium pyrophosphate solution.

egg analyses held in the Environmental Archaeology Unit suggested that this sample may have received non-standard treatment because of its proportionally high *Ascaris* content. A second possibility is that the squash subsample was not sufficiently disaggregated even using sodium pyrophosphate (the two results obtained with and without pyrophosphate use are very similar), which may indicate the need for a more effective disaggregating agent. When the sample was originally examined it was described as 'moist to wet', so disaggregation may have been more easily accomplished than for the dehydrated material used here.

A further important question answered by the Coppergate data is whether or not the squash method is equally effective for different parasite species. Table 4 shows a comparison of the ratios of *Trichuris* to *Ascaris* eggs in all seven samples. It is clear that in all cases the two methods have given a similar ratio.

Sample	Stoll	Squash
1353	6.25 4.0	4.8
1732	1.27	1.47
1907	3.07 2.73	3.75
1911	4.89 7.09	6.59
1913	—	7.2
2135	46.9 13.08	17.6
3000	1.44	1.21 ^x 1.17 ^y

Table 4. Ratio of *Trichuris* to *Ascaris* eggs in samples from 16-22 Coppergate tested using the modified Stoll and 'squash' methods. x/y = before/after treatment with pyrophosphate; No *Ascaris* eggs were recorded from sample 1913 by the Stoll method so no ratio can be given.

The squash technique has clearly been vindicated by these results for use on archaeological deposits suspected of containing parasite eggs. It will certainly provide an adequate and rapid check, where a large number of samples are being examined, in order to isolate those samples where significant numbers of parasite eggs are present. In addition it also appears suitable for more thorough and systematic examination, where measurements of individual eggs are required. If necessary, subsamples could be disaggregated using chemical means and even sieved prior to measurement. However it is the view of the author that it would have been possible to measure the eggs in the samples discussed here without further processing, which is useful in view of both the cost of specially made sieves and the time involved in the preparation of Stoll samples.

Further, by recording semi-quantitatively, the results obtained are explicitly seen to be estimates, perfectly adequate for establishing the presence or absence of significant amounts of faecal material. The recording scale used is open to discussion and it may be thought necessary to revise it. It should be borne in mind, however, that an accurate measurement of faecal concentration is never likely to be obtained using parasite eggs for the reasons discussed in the introduction. From the data obtained by Jones (1985, 112-13) it could, very cautiously, be suggested that 'trace' amounts probably represent the background level for many urban occupation deposits in the British Isles. 'Few' possibly indicates some faecal contamination in a deposit, 'some' may indicate a substantial amount of faeces, 'many' a probable faecal layer and 'very many' a deposit consisting primarily of faeces.

It is hoped that this technique will aid the future study of parasites, especially in areas where analysis has been dismissed previously as too complicated, time-consuming or expensive. The squash method is fairly easily adapted to surroundings, requiring little specialised equipment (except a transmission microscope), and could probably even be attempted in a site hut during excavation. This study will also have been of some use if it provokes the examination of methods used in other archaeological disciplines, especially where they have been borrowed from related fields and perhaps applied indiscriminately.

Acknowledgements

The author would like to thank Harry Kenward, Allan Hall, Annie Milles, Keith Dobney, John Carrott and Colin Nicholson for their time and advice in both my initiation into work on parasites and in the preparation of this contribution. This note is based on a project carried out in the Environmental Archaeology Unit, University of York, during a period of undergraduate work experience.

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Disk copy received: July 1992

Book notices

These two books both have something to offer the palaeo(ethno)botanist and may well be of interest to all environmental archaeologists.

de Rougemont, G. M. (1989). *A field guide to the crops of Britain and Europe*. London: Collins. 367 pp., numerous colour pls., line drawings, maps. ISBN 0 00 219713 8. £14.95

Although published in 1989, this useful addition to Collins' generally excellent *Field*