Risk Factors for Osteosarcoma in Young People in Cornwall: A Case-Control Study

Mel Wright¹ MSc PGC BSc DipHE RN(A) RN(MH) and Dr Derek Pheby²

- ¹ School of Sociology, Politics and Law, University of Plymouth
- ² Formerly Director, Unit of Applied Epidemiology, University of the West of England, Bristol (retired).

Correspondence: Dr Derek Pheby, Plaishetts House, Hadspen, Castle Cary, Somerset, BA7 7LR. Email: derek@appliedepidemiology.org.uk

Abstract

A case-control study has been carried out in an attempt to identify risk factors that may be implicated in a group of cases of osteosarcoma in young people that have occurred over a nine year period in or near the West Cornish town of Helston. The incidence of osteosarcoma in the study area was substantially in excess of the national rate, but did not significantly exceed that found in the South-West region as a whole (chi² with continuity correction = 0.003; p 0.956).

Data were obtained by postal questionnaire. In addition, domestic radon levels in the homes of cases and controls were obtained by direct measurement. Statistical analyses included tests of association χ^2 tests, or Fisher's Exact Test where appropriate) and the calculation of odds ratios for exposure. For continuous variables, Mann-Whitney tests of rank distribution were undertaken.

Much higher levels of domestic radon were found in the houses of cases compared with those of controls (Mann-Whitney rank distribution test: p=0.000376). Other possible risk factors identified were diphtheria/tetanus/pertussis immunisation, difficulty coping at school, periods of low mood, and previous accidents. BCG immunisation appeared to have a protective effect. However, logistic regression analysis showed that these were unimportant in comparison with radon, and their role as possible risk factors for osteosarcoma is by no means proved.

The strength of association with radon exposure is remarkable and convincing. Other associations which are weaker, but nonetheless statistically significant, are consistent with previous published research. The study should, though, be repeated on a larger scale in order to replicate these findings.

Key Words: Environmental health; osteosarcoma, radon, risk factors.

Introduction

A case-control study has been carried out in an attempt to identify risk factors that may be implicated in a group of cases of osteosarcoma in young people that have occurred within the past decade in or near the West Cornish town of Helston. Recently, concern has been expressed there about the number of cases (BBC News 2004; Guardian Unlimited, 2004, the "Helston Packet", 29th January and 26th February 2004). A total of seven

cases has been identified, which were diagnosed with osteosarcoma in the period 1996 to 2003 inclusive. Data pertaining to six of these cases, three of whom were male and three female, are presented here. In every case, they were aged less than 20 when diagnosed with osteosarcoma. The public perception was that these cases constituted a cluster, and that the incidence of osteosarcoma was in excess of that normally to be expected in that area. There was concern that this might have resulted from exposure to a common risk factor, which remained unidentified.

Osteosarcoma, though a rare form of cancer, is the fourth most common cancer in people under 20 years of age (Homa *et al.*, 1991), and the most common type of bone cancer in this age group. It is derived from primitive bone-forming mesenchyma (Kramarova and Stiller, 1996). It is bimodally distributed by age, with an initial peak at age 15-19 years (Fraumeni and Boyce, 1982).

Table 1.0 summarises the incidence of osteosarcoma in the study area (i.e. an area of radius 13 miles around Helston). It will be noted that, while the incidence of osteosarcoma was substantially in excess of the national rate, it did not significantly exceed that found in the

Table 1.0: Incidence of osteosarcoma in people aged <25 in the UK*, the South West* and the Study Area

| Location | | | | | | | |
|---------------------------------------|------------------|-----------|-----------|--|--|--|--|
| | UK SW Study area | | | | | | |
| No. of cases | 732 | 210 | 7 | | | | |
| Population <25 (100,00s) | 112.03 | 6.94 | 0.14 | | | | |
| Observation | 1995-2000 | 1995-2000 | 1995-2005 | | | | |
| No. of years | 6 | 6 | 9 | | | | |
| Person/years at risk | 672.18 | 41.64 | 1.26 | | | | |
| Annual rate/100,000 population <25 | 1.09 | 5.04 | 5.56 | | | | |
| Rate ratio (UK = 1) | 1.00 | 4.62 | 5.10 | | | | |

^{*} Sources: Office for National Statistics 2000 and Stiller et al.. 2004.

Mel Wright and Dr Derek Pheby

South-West region as a whole (chi^2 with continuity correction = 0.003; p 0.956).

Very little is known about the aetiology of osteosarcoma in humans. Following local representations, the South West Cancer Intelligence Service prepared a report on the alleged cluster (South West Cancer Intelligence Service, 2004). This states that the aetiology of osteosarcoma is generally poorly understood. The only aetiological factor that is unequivocally recognised is ionising radiation (Rowland et al., 1983), having first been described in female factory workers after World War One who were exposed to radium and mesothorium (Patterson and Harman, 2001) Similarly, treatment especially of children with external beam radiotherapy is known to be associated with an increased risk of osteosarcoma (Patterson and Harman 2001, Tabone et al., 1999), particularly in those with an inherited susceptibility. However, as the SWCIS report points out, there has been no published scientific evidence to date linking radon exposure to osteosarcoma in young people, in either the South West (Thorne et al., 1996) or nationally (Cartwright, 2002). It should be noted that parts of Cornwall have some of the highest levels of radon intensity in England and Wales (Green at al., 2002), particularly in Kerrier district, where 48% of properties were found to have unsafe levels (Spear, 2004). Henshaw et al., (1990) identified that, at an indoor level of 110Bg/m³, radon may cause 23-43% of cancer, and noted that the existence of radon hot spots had implications for the clustering of childhood cancer in the UK.

Other factors suggested to be related to osteosarcoma include tall stature, previous bone trauma (Fraumeni, 1967, Miller, 1976, Scranton *et al.*, 1975) and viruses (Finkel *et al.*, 1975). Animal studies have demonstrated an excess risk of bone sarcomas among larger breeds of dogs which suggests that a relationship may exist between human bone cancer and a large body size at the time of diagnosis (Operskalski *et al.*, 1987, Benedict *et al.*, 1988). Genetic factors have also been identified in a small percentage of cases. Hereditary factors are involved in some patients (Hansen MF 1991), and there is an increased risk in siblings of patients (Coley, 1970, Schimke *et al.*, 1974). Genetic mutations, e.g. of the p53 gene, and an increased incidence in children with the Li-Fraumeni syndrome have been reported (McIntyre *et al.*, 1994).

A number of antenatal environmental exposures such as infective agents, drugs and radiation are capable of altering the normal development of an embryo which could contribute to the development of osteosarcoma in

young people. Parental occupation is also of interest because parents can bring home chemicals or dusts from their workplace on their clothes, thus exposing their children. Parental chemical exposures may be associated with increased risk of osteosarcoma in children. Schwartzbaum et al. (1991) identified a statistically significant odds ratio of 2.6 among 78 childhood osteosarcoma patients for parents who reported that they gardened with fertilisers, herbicides and pesticides in the perinatal period, compared with parents of other childhood cancer patients. A genetic predisposition has also been suggested (Buckley et al., 1998, Birch 1999). Earlier occurrence of osteosarcoma in girls than in boys may be associated with their earlier adolescent growth spurt (Price, 1955). Previous trauma is another factor which may predispose to osteosarcoma (Operskalski et al., 1987). Immune status is another factor that has been implicated, with one study finding a highly significant excess of children with osteosarcoma who had not been immunised (Hartley et al., 1988). Finally, psychological factors have been impugned, with another study finding that children who experienced difficulties at school were particularly at risk (Frentzel-Beyme et al., 2004).

The SWCIS report states that there was no statistically significant difference in incidence between West Cornwall and surrounding areas (South West Cancer Intelligence Service, 2004). However, this assessment was made at PCT level, so any local clusters would have been substantially diluted in a larger population. It concluded that there was no evidence of a single environmental or other risk factor causing osteosarcoma in the West of Cornwall Primary Care Trust area, and that public concern was therefore misplaced. However, the study conducted by the SWCIS was a descriptive study utilizing cancer registry data. It was not an analytical study, and was neither designed to establish causation of this group of cases, nor indeed could it as the requisite data on environmental exposures was unavailable. SWCIS itself has commented that the cancer registry does not hold data on lifestyle factors which may affect cancer risk, duration of residence, or occupational or environmental exposures (South West Cancer Intelligence Service Factsheet 18).

The SWCIS report further asserted that any additional investigation of this group of cases would be unethical, as any study of such a small number would necessarily be underpowered (South West Cancer Intelligence Service 2004). This is incorrect. It is true that type 2 errors are more likely in a study of a small sample, but whether or not it is underpowered depends on the effect size of

Risk Factors for Osteosarcoma in Young People in Cornwall: A Case-Control Study

possible risk factors being examined. Thus, our power and sample size calculations indicate that a matched case-control study of six cases, with four controls per case, with an α -value of 0.05 and a power of 0.8, would produce a significant result where the exposure in the control population was 10%, and the odds ratio 15:1. The present study is thus designed to complement and extend the SWCIS study.

Methods

A case-control study was undertaken, in order to identify, where possible, differences in exposure to a range of risk factors between the six cases and a group of matched controls. The specific potential risk factors investigated include radon, antenatal exposures, a family history of cancer or congenital malformation, parental occupation and psychological disturbances. Ethical approval was granted by the Faculty of Applied Sciences Ethics Committee at the University of the West of England, which follows NHS governance procedures.

The cases were identified via third party introductions or from word of mouth and media interest (television, radio and press). A close relationship had developed between locally affected families since the high media interest and as such facilitated the task of case finding. The study area comprised some 531 square miles within a 13-mile radius of Helston. The area extended from Redruth to the North, to the Lizard to the South, and St. Ives to the West.

Four controls were selected per case. Some were neighbourhood controls introduced by cases' families, while others were voluntary participants from local schools who had heard about the study. Cases and controls were matched for age, sex and ethnicity. For both cases and controls, participation was on the basis of informed consent.

There were two sources of information regarding exposures of interest. For most exposures, data were obtained by postal questionnaire. In addition, domestic radon levels in the homes of cases and controls were obtained by direct measurement.

The questionnaire, which was piloted among 25 unaffected families, addressed events during the index pregnancy, past medical and social history of the subject and his or her parents and siblings and other family members. Risk factors examined included length of gestation, place and type of delivery, birth weight,

condition at birth, neonatal events (e.g. phototherapy, breastfeeding), previous illnesses, drug use and abuse, previous medical treatments including radiotherapy, immunisation status, and familial exposure to chemicals.

Domestic radon measurements were made using the Pylon Radon Detector Model AB-5, which is an instrument validated by the NRPB and the United States Environmental Protection Agency. The tool was calibrated for efficiency, and a pilot study undertaken to ensure familiarity with the equipment. In order to ensure comparability of results, families were asked to keep windows and doors closed as much as possible for twentyfour hours prior to, and during, measurements, and to turn off all air exchange systems. Siting of the equipment, well away from outside walls and avoiding draughts, was important, as was avoidance of taking measurements during severe storms or strong winds. Calculations were made using an hourly interval method, and expressed in Bg/m³. All radon measurements were made between 1st September and 1st October 2004. Each assessment was undertaken over an 8 hour period. The first three hourly readings were ignored, allowing the instrument to acclimatise to its surroundings. Cases and controls were assessed sequentially, the sequence being determined by random allocation. The apparatus was set up in a bedroom of the occupants' choosing. Background readings were calibrated after each assessment and programmed into the detector. Because of the possible effect of variations in air pressure on radon measurements, local barometric readings were obtained from Culdrose, Cornwall at 12:00 GMT (at www.metoffice.gov.uk).

Results were calculated using the radon concentration formula:

Where:

C is the concentration (units depend on sensitivity)

CPM is the count per interval value expressed in counts per minute

BG is the background level expressed in counts per minute

S is the counting sensitivity value

Statistical analyses included tests of association (χ^2 tests, or Fisher's Exact Test where appropriate) and the calculation of odds ratios for exposure. For continuous variables, such as radon measurements, Mann-Whitney tests of rank distribution were undertaken.

Mel Wright and Dr Derek Pheby

Table 2.0Domestic radon measurements (Bq/m³)

| Case | Results: Bq/m3 | | | | | | | | |
|-------------------|----------------|-----|------------------|-----|-----|-----------------------------------|--|--|--|
| number | Cases | | Matched controls | | | | | | |
| | | A | В | С | D | Average of matched controls | | | |
| 1 | 260 | 176 | 164 | 120 | 106 | 141.5 | | | |
| 2 | 3484 | 52 | 136 | 198 | 166 | 138.0 | | | |
| 3 | 234 | 98 | 90 | 220 | 122 | 132.5 | | | |
| 4 | 352 | 126 | 144 | 144 | 162 | 144.0 | | | |
| 5 | 200 | 54 | 40 | 108 | 140 | 85.5 | | | |
| 6 | 250 | 26 | 38 | 40 | 40 | 61 | | | |
| All cases average | 796.7 | | | | | 117.1 | | | |

Table 3.0Domestic Radon levels in relation to the NRPB
Intervention Level of 200Bq/m³

| | cases | controls | Total |
|--------------------------------|-------|----------|-------|
| At or above intervention level | 6 | 1 | 7 |
| Below intervention level | 0 | 23 | 23 |
| Total | 6 | 24 | 30 |

Results

Measured radon levels in the houses of cases and controls are summarised in Table 2.0. There was a marked difference, in relation to the NRPB domestic radon intervention level of 200 Bg/m³, between the

homes of cases and of controls (p, by Fisher's Exact Test = 0.0000117). These are summarised in Table 3.0. In order to examine possible meteorological factors that might have influenced radon measurements, possible correlations between radon measurements and wind speed, wind direction, rainfall, humidity, air temperature and atmospheric pressure were examined. The strongest correlation (r = 0.28) was with wind speed, i.e. only 7.8% of variation in radon measurements could be explained by variations in this.

Radon measurements ranged from 26 to 3484 Bq/m³. This latter measurement was very much higher than the second highest radon measurement, which was 352 Bq/m³. Consequently, a Mann-Whitney rank distribution test was conducted in order to avoid the possible distorting effect of this one extreme value. The cases occupied five of the top six ranks, while the remaining case was in rank 7. The probability of this distribution having arisen by chance was 0.000376. Similar tests were

Risk Factors for Osteosarcoma in Young People in Cornwall: A Case-Control Study

undertaken in respect of other continuous variables, but no other significant results were obtained. Clearly, radon levels measured at a particular point in time are not a measure of total exposure. Accordingly, cases and controls were asked about duration of residence in their current addresses, and there were in fact no significant differences between them.

As regards categorical variables, 2x2 contingency tables were examined, and odds ratios and 95% confidence limits calculated. The results in respect of exposures experienced by cases and controls are summarised in Table 4.0.

Cases were much more likely than controls to have had diphtheria/tetanus/pertussis immunisation, and also to have had difficulty coping at school, periods of low mood, or previous accidents. However, BCG immunisation was much more frequent among the controls, and therefore appeared to have a protective effect. A logistic regression analysis was undertaken, which involved construction of a model incorporating those variables which individually were positively associated with the occurrence of osteosarcoma, viz. radon above intervention level, diphtheria/ tetanus/pertussis immunisation, school difficulties, periods of low mood, and previous accidents. The model chi² for this fivevariable model was 30.02 (p < 0.0001). An identical model chi² was found for a two variable model comprising radon above intervention level and diphtheria/tetanus/pertussis immunisation only, indicating that school difficulties, periods of low mood, and previous accidents had no impact at all on the predictive power of the model. For radon above intervention level alone, chi² was 24.64 (p < 0.0001, without continuity correction).

Other possible risk factors enquired about included factors related to the biological mother's obstetric history, i.e. occupation before, during and immediately after the birth, and exposure to X-rays, medication and trauma during pregnancy. Parental occupations (particularly in horticulture and agriculture) were also enquired about, and exposures to chemicals and other external agents (i.e. dusts, fumes, X-rays, fertilisers, herbicides, pesticides, prescription medication, alcohol and tobacco), and serious illnesses in the extended family. Factors concerning the construction of buildings which might affect levels of domestic radon (presence of double glazing, cavity wall insulation, or draft exclusion, and construction date) showed no variation between cases and controls. There were no significant associations involving any of these

factors, or with the existence of serious illnesses in other family members, except for genetic diseases in aunts, which cases reported more frequently than controls (odds ratio = 1.83; 95% confidence interval = 1.14 – 23.83). There would not appear to be any plausible biological mechanism to explain this, and it appears likely that it is simply a chance result arising as a consequence of multiple hypothesis testing.

Discussion

Summary of main findings

The number of cases identified was markedly higher than that which would have been found if national incidence rates applied, but was not significantly higher than the regional rate. The number of cases found in the Helston area during the study period cannot therefore be regarded as a cluster. Consequently, there is no need to postulate a specific localised exposure which may have been a risk factor for osteosarcoma. There is always a danger, in investigating an alleged cluster, of artificially designating a cluster by post hoc rationalisation (the socalled 'Texas sharpshooter' fallacy). We have not done this. Rather, we have investigated a group of cases which occurred in close proximity in space and time. Whether or not these cases constitute a cluster is a matter of semantics, and such labelling is not necessarily helpful in endeavouring to identify possible risk factors.

This study has shown a very strong association between levels of domestic radon and the development of osteosarcoma in the group of cases investigated (p = 0.000376). Radon levels throughout the South West of England are higher than in other parts of the country, and this may account for the relatively high incidence rate for osteosarcoma found in the region. Logistic regression analysis indicated that diphtheria/tetanus/pertussis immunisation may be a relatively minor risk factor for osteosarcoma, but this remains a hypothesis requiring further testing on a much larger scale. Other factors that were weakly associated with the development of osteosarcoma included difficulty coping at school, periods of low mood, and previous accidents, but these had no impact on the logistic regression model, so their possible roles as risk factors should also be regarded as hypotheses to be tested in much larger studies. Paradoxically, BCG immunisation appeared to have a protective effect. In conclusion, the association between domestic radon levels and developing osteosarcoma is very strong, and this suggests a causal relationship.

Mel Wright and Dr Derek Pheby

Table 4.0Comparison of cases and controls in respect of selected exposures

| | Cases | | Controls | | Odds | 95% Confidence |
|---|-------|----|---------------|---------------|-------|-----------------|
| History of: | Yes | No | Category 1 | Category 2 | Ratio | Interval |
| Diphtheria/tetanus/pertussis immunisation | 6 | 0 | 6 | 18 | 18.00 | 1.787 - 181.316 |
| H. influenzae immunisation | 2 | 4 | 15 | 9 | 0.30 | 0.045 - 0.982 |
| Poliomyelitis immunisation | 4 | 2 | 9 | 15 | 3.33 | 0.505 - 22.018 |
| MMR immunisation | 4 | 2 | 18 | 6 | 0.67 | 0.097 - 4.605 |
| BCG immunisation | 2 | 4 | 20 | 4 | 0.50 | 0.013 - 0.745 |
| X-ray exposure | 3 | 3 | 16 | 8 | 0.50 | 0.082 - 3.360 |
| Medication | 1 | 5 | 3 | 21 | 1.40 | 0.119 - 16.459 |
| Bacterial infections | 5 | 1 | 21 | 3 | 0.71 | 0.061 - 8.398 |
| Viral infections | 5 | 1 | 13 | 11 | 4.23 | 0.427 - 41.875 |
| Difficulty coping at school | 5 | 1 | 2 | 22 | 55.00 | 4.128 - 732.747 |
| Difficulty sleeping | 2 | 4 | 4 | 20 | 2.50 | 0.336 - 18.629 |
| Periods of low mood | 3 | 3 | 2 | 22 | 11.00 | 1.271 - 95.181 |
| Swimming | 5 | 1 | 18 | 6 | 1.67 | 1.161 - 17.258 |
| Accidents | 4 | 2 | 4 | 20 | 10.00 | 1.342 - 75.514 |

Risk Factors for Osteosarcoma in Young People in Cornwall: A Case-Control Study

Strengths and the limitations of this study

The main limitation of this study arises from the small number of cases. Nevertheless, the strength of association with radon exposure is remarkable and convincing. Other associations which are weaker but nonetheless statistically significant are consistent with previous published research. The study should, though, be repeated on a larger scale in order to replicate these findings.

Conformity with existing research literature

The strong association with radon exposure is consistent with previous work implicating ionising irradiation in the aetiology of osteosarcoma (Rowland *et al.* 1983), though this particular finding is new information, for, as the SWCIS report (South West Cancer Intelligence Service, 2004) pointed out, there has been to date no published research evidence for the role of radon as a risk factor for osteosarcoma in young people.

Other factors that may constitute risk factors for osteosarcoma include diphtheria/tetanus/pertussis immunisation, difficulty coping at school, periods of low mood, and previous accidents. BCG immunisation, however, appeared to have a protective effect.

A recent collaborative analysis of data from 13 case-control studies of residential radon and lung cancer (Darby et al., 2005), involving over seven thousand cases with approximately two controls per case found a mean domestic radon concentration in the homes of cases of 97 Bq/m³, and in the homes of controls of 104 Bq/m³. This study has found proportionally much larger differences between cases and controls, suggesting that radon is more unequivocally important as a risk factor for osteosarcoma than for lung cancer.

The indication that immunisation status, particularly the apparent protective effect of BCG immunisation, may affect likelihood of developing osteosarcoma is consistent with previous work suggesting that children who had not been immunised were significantly more at risk of osteosarcoma than others (Hartley et al., 1988). As regards accidents, a possible role for trauma as a risk factor has previously been suggested (Fraumeni, 1967; Miller, 1976). In addition, our finding of the possible role as a risk factor of having had difficulty coping at school is consistent with a previous study indicating that children who experienced such difficulties were particularly at risk (Frentzel-Beyme et al., 2004).

The implications for future research and practice

There is a clear need for the findings of this study to be

replicated in larger scale studies, as well as for investigation of the reasons for the demonstrated variations in domestic radon levels, in order to enable effective interventions to be implemented. The risks associated with domestic radon exposure have been so conclusively demonstrated in this study that serious consideration should be given to taking remedial action to reduce the risk at the earliest possible opportunity.

Conclusions

A case-control study of risk factors for osteosarcoma in a group of cases in young people in and around Helston, in Cornwall, indicated a very strong association between domestic radon levels and the development of osteosarcoma. This is consistent with other research, though is the first time that this particular association has been demonstrated in this age group. The strength of the association (p = 0.000376, by Mann-Whitney rank distribution test) strongly suggests a causal relationship. Other associations, e.g. with diphtheria/tetanus/pertussis immunisation, were much weaker, and more research is needed in larger scale studies to elucidate their possible role as causal factors.

Acknowledgements

Our sincere thanks go to the following people who contributed to the study in a number of ways:

Dr. Andy Tubb and Dr. Paul von Dorfman for their continual support and guidance, Mrs. Judy Brown and Ms. Alison McEwen for their expertise and time regarding the radon detector equipment and associated analytical data output, Andrew George M.P. for his mediation between us and significant others, and our respective families who gave us the time and space to conduct the investigation to its conclusion.

Our appreciation also goes to the participants and their families who took part, without which this study would not have come to fruition.

Finally, our thanks go to the Chartered Institute of Environmental Health who funded, and encouraged us to complete, the project.

Mel Wright and Dr Derek Pheby

References

Barometric Readings for Culdrose, Cornwall at 12:00GMT. www.metoffice.gov.uk [Accessed 07/10/04].

BBC News (2004). 'Bone cancer deaths too high'. Available online at: www.news.bbc.co.uk/1/hi/health/3494162.stm [Accessed 22/07/06].

Benedict W F, Fung Y T and Murphree A L (1988). The gene responsible for the development of retinoblastoma and osteosarcoma. *Cancer*, 62, 1691-1694.

Birch J M (1999). Genes and Cancer. *Archives of Diseases in Childhood*, 80 1-3.

Buckley J D, Prendergrass T W, Buckley C M, Pritchard D J, Nesbit M E, Provisor A J, Robison L L (1998). Epidemiology of osteosarcoma and Ewing's sarcoma. *Cancer*, 83, 1440-1448.

Cartwright, R A, Law, G, Roman E, Gurney K A, Gilman E, Eden O B, Mott M, Muir K, Goodhead D, Kendall G (2002). The United Kingdom Childhood Cancer Study of Exposure to Domestic Sources of Ionising Radiation: I Radon Gas. *British Journal of Cancer*, 86, 1721-1726.

Coley BL (1970). Neoplasm of bone 2. Hoeber PB (ed). New York

Darby S, Hill D, Auvinen A, Barros-Dios JM, Baysson H, Bochicchio F, Deo H, Falk R, Forastiere F, Hakama M, Heid I, Kreienbrock L, Kreuzer M, Lagarde F, Mäkeläinen I, Muirhead C, Oberaigner W, Pershagen G, Ruano-Ravina A, Ruosteenoja E, Schaffrath Rosario A, Tirmarche M, Tomá ek L, Whitley E, Wichmann H-E, Doll R (2005). Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies. *BMJ*, 330, 223-8.

Finkel M P, Reilly C A, Biskis B O (1975). Viral aetiology of bone cancer. *Radiation Oncology*, 10, 28-39.

Fraumeni J F (1967). Stature and malignant tumours of bone in childhood and adolescence. *Cancer*, 20, 967-973.

Fraumeni J F and Boice J D (1982). Bone. In Schottenfeld D, Fraumeni J F (eds.). *Cancer*

Epidemology and Prevention. Philadelphia, Saunders, 814-826.

Frentzel-Beyme R, Becher H, Salzer-Kunlschik M, Kotz R, Salzer M (2004). Factors affecting the incident juvenile bone tumours in an Austrian case-control study. *Cancer Detection and Prevention*, pp1-11.

Guardian Unlimited (2004). What is killing our children? Available online at: http://society.guardian.co.uk/cancer/story/0,8150,1092 495,00.html. [Accessed 22/07/06].

Green B M R, Miles J C H; Bradley E J, Rees D M (2002). Radon Atlas of England & Wales. National Radiological Protection Board. NRPB – W26. Oxon.

Hansen M F (1991). Molecular genetic considerations in osteosarcoma. *Clin. Orthop.* 270: 237-246

Hartley A L, Birch J M, McKinney P A, Teare M D, Blair V, Carrette J, Mann J R, Draper G J, Stiller C A, Johnston H E, Cartwright R A (1988). The interregional epidemiological study of childhood cancer (IRESCC): case-control study of children with bone and soft tissue sarcomas. *Brit. J. Cancer*, 58, 838-842.d.

Helston Packet (29th Jan 2004). Cancer cluster fears 'unfortunate coincidence'. http://archive.helstonpacket.co.uk/2004/1/29/21091.ht ml. [Accessed 11/07/06.]

Helston Packet (26th February 2004). Cancer: MP says report is `weak'.

http://archive.helstonpacket.co.uk/2004/2/26/19768.ht ml. [Accessed 11/07/06.]

Henshaw DL, Eatough J P, Richardson R B (1990). Radon as a causative factor in induction of myeloid leukaemia and other cancers. *The Lancet*, 335, 1008-1012.

Homa D M, Sower M R, Schwartz AG (1991). Incidence and survival rates of children and young adults with osteosarcoma. *Cancer*, 67, 2219-2223.

Kramarova E, Stiller CA (1996). The International Classification of Childhood Cancer. Int. J. *Cancer*, 68, 759-765.

McIntyre J F, Smith-Sorensen B, Friend S H, Kassell J, Borresen A L, Yan Y X, Russo C, Sato J, Barbier N,

Risk Factors for Osteosarcoma in Young People in Cornwall: A Case-Control Study

Miser J (1994). Germline mutations of the p53 tumour suppressor gene in children with osteosarcoma. *J. Clin. Oncol.*, 12: 925-930

Miller R W (1976). Aetiology of childhood bone cancer: epidemiologic observations. *Recent Results in Cancer Research*, 54, 50-62.

Office for National Statistics (2000) Cancer Statistics: Registrations (Series MB1), Nos. 28 (1995-7), 29 (1998), 30 (1999) and 31 (2000). London.

Operskalski E A, Preston-Martin S, Henderson B E, Visscher B R (1987). A case-control study of osteosarcoma in young persons. *American Journal of Epidemiology*, 126(1), 118-126.

Patterson A J & Harman D C (2001). Bone tumours and soft tissue sarcomas in *Oncology*, ed. Spence RAJ & Johnston PG, Oxford University Press.

Price C H G (1955). Osteogenic sarcoma: an analysis of the age and sex incidence. *Brit. J. Cancer*, 9, 558-574.

Rowland R E, Stehney A F, Lucas H F (1983). Dose-response relationship for radium-induced bone sarcomas. *Health Physiology*, 44(Suppl. 1), 515-531.

Schwartzbaum J A, George S L, Pratt CB, Davis B (1991). A exploratory study of environmental and medical factors potentially related to childhood cancer. *Medical Pediatric Oncology*, 19, 115-121.

Schimke R N, Lowman J T and Cowan GAB (1974). Retinoblastoma and osteogenic sarcoma in siblings. Cancer, 34, 2077-2079

Scranton P E, de Cicco F A, Totten RS, Yunis E J (1975). Prognostic factors in osteosarcoma. *Cancer*, 36, 2179-2191.

South West Cancer Intelligence Service (2004). Osteosarcoma in children and young adults in the West of Cornwall Primary Care Trust. January.

South West Cancer Intelligence Service. Factsheet No. 18: Cancer Clusters

Spear S (2004). 'Kerrier tests reveal shock radon levels.' *Environmental Health News*, 19(21), p4.

Stiller C, Quinn M, Rowan S Childhood Cancer (2004).

Chapter 13 in The health of children and young people. London, Office for National Statistics. Available online at: www.statistics.gov.uk/children/downloads/child_cancer.pdf [accessed 22/07/06].

Tabone M D, Terrier P, Pacquement H, Brunat-Mentigny M, Schmitt C, Babinboilletot A, Mahmoud HH, Kalifa C. (1999). Outcome of radiation related osteosarcoma after treatment of childhood and adolescent cancer: a study of 23 cases. *Journal of Clinical Oncology*, 17(9), 2789-95

Thorne R, Foreman, N K and Mott M G, (1996). Radon in Devon and Cornwall and Paediatric Malignancies. *European Journal of Cancer*, 32A, no. 2, 282-285.

Psocid infestations and domestic kitchens

Dr Bryan Turner¹

¹ Department of Life Sciences, King's College London

Correspondence: Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH UK. Telephone: +44 (0) 2078484292. Email: bryan.turner@kcl.ac.uk

Abstract

Infestation of dry food products in domestic kitchens by psocids is a common cause of complaint to food manufacturers and retailers. The natural assumption is that the infestation arrived in the contaminated food products. The primary psocid species involved is *Liposcelis bostrychophila*, a 1mm—long brown wingless species of tropical origin. This psocid is parthenogenetic, needs temperatures around 20°C to reproduce and so cannot persist out of doors in the UK and is uncommon in food manufacturing and retail establishments but is frequently found in domestic premises.

To test whether dry goods, specifically flour, is contaminated prior to purchase, pairs of bags of flour were randomly bought from a range of retail outlets in a number of locations in England and Northern Ireland. One of the two bags was immediately sealed inside a plastic bag whilst the other was placed in a typical domestic kitchen in the location. After one week this bag too was sealed in plastic and both bags were sent to King's College where they were "incubated" for three months. Twenty two of the 133 bags that spent a week in kitchen cupboards were infested with psocids. None of the 135 control bags sealed immediately on purchase contained psocids.

Thus, the commonly held idea that bags of flour purchased from shops are sometimes contaminated with psocids is not supported by this study. Psocids already present in the dwelling are attracted to the fresh flour, which then appears to be the source. It is suggested that psocid infestations in sea containers is a potential source, so that anything might carry individuals in its packaging into the home.

Keywords: Domestic kitchens; environmental health; flour; *Liposcelis bostrychophila*; psocids; supermarkets.

Introduction

Infestations of psocids, specifically the booklouse *Liposcelis bostrychophila* (Badonnel), are common causes of consumer complaints to retailers and manufacturers of stored food held in domestic kitchens in the UK (Turner, 1987; Turner and Ali, 1996; Turner and Bishop, 1998). Turner and Bishop (1998) showed that the incidence of psocids in households generally was increasing, almost doubling in England within the 10 years from 1987 to 1997. Previous studies by this author have looked at the range of materials that psocids have infested, the growth

of the problem of psocid related complaints both in the UK and elsewhere (Turner, 1987), factors of their biology (Turner and Maude-Roxby 1988; Turner 1998), population dynamics (Turner 1994), insecticide resistance and genetics (Turner et al., 1991; Ali and Turner, 2001) and relationships with bacteria (Yusuf et al., 2000; Yusuf and Turner, 2004). These studies chart the highly adaptable and resilient nature of the liposcelid and the qualities that make it so successful as a domestic pest. In addition, and in conjunction with NABIM and the Chartered Institute of Environmental Health, the author has produced a pamphlet for the public on the prevention and control of psocids (distributed to manufacturers, retail outlets, EHOs and the public – details from www.fabflour.co.uk or www.kcl.ac.uk/psocids).

Since Liposcelis bostrychophila is parthenogenic (no males), it is capable of very rapid population growth in comparison with similar, native, bisexual species, where typically half the eggs are males. Originally from the tropics, it needs temperatures above 20°C to thrive and for populations to grow. It is not found out of doors in the UK and is typically only found in domestic situations (Turner and Ali, 1996). Egg to adult at 25°C takes one month and below 15°C egg development ceases (Turner, 1994). They are quite tolerant of dry conditions, needing only six hours per day with a relative humidity above 60% (Rudolf, 1982) which is found in most domestic situations during the night time.

Although a wide range of materials can be colonised, bagged flour in particular is highly attractive and is targeted. A key reason for this lies in the way flour is manufactured and packaged. To be successfully milled, wheat needs to have a specific moisture content (12-14% ww). This moisture is then present in the final flour. To allow the flour to lose some of this water and prevent the development of musty smells and taste due to fungal contamination, the flour is packed in paper bags rather than sealed in plastic. Thus to a psocid, a bag of flour represents a source both of moisture and food and they can detect the moisture emanating from it and use the moisture gradient to home in on the bag of flour. In time, as the flour ages and desiccates, it becomes less attractive to psocids.

Methods

People were recruited across a number of areas in England and Northern Ireland (Table 1.0) to visit local supermarkets and other retail outlets and purchase pairs of bags of flour (Table 2.0). One of the bags was

Dr Bryan Turner

Table 1.0The geographic areas where bags were purchased

| Belfast |
|---------------------|
| Cumbria |
| Lincolnshire |
| Greater Manchester |
| Northamptonshire |
| Bedfordshire |
| Hertfordshire/Essex |
| NE London/Essex |
| Berkshire/Hampshire |
| Buckinghamshire |

immediately sealed inside a "zip top" plastic bag and the other treated as a normally purchased bag, taken home and placed in a kitchen cupboard for one week. From experience this period will provide time for the bags to be "discovered" if any psocids are in the kitchen. Exactly where in the kitchen the bag should be placed was not defined, although many described whether the cupboards used were head height or floor mounted types, or if the bag was placed in a walk-in pantry. The contents of the cupboards were not defined or mentioned. Importantly, none of the householders' kitchens used was knowingly infested with psocids. After one week the "kitchen" bag was also sealed in a "zip top" plastic bag and both bags were then sent by parcel post to King's College London. Here they were kept in warm conditions (25°C) for three months, this being sufficient time for psocid populations to develop from any single eggs or individuals in the bags. The plastic bags were kept sealed and in separate incubation facilities to ensure no possible contamination in the laboratory. Furthermore the "kitchen" and control samples were kept in separate incubators. At the end of the three month incubation period the bags were disassembled and any psocids present noted. For logistical reasons the survey took place during the autumns of 2003 and 2004, this being the season of the year when the greatest numbers of complaints about psocids are received (Turner and Ali, 1996). There was no significant difference between the findings for the two years and so the data for both years were combined.

Results

A total of 268 bags of flour of 16 different types from 25 trade labels was purchased during this study. Of those 135 bags were controls, bagged as soon as purchased and 133 bags were located in kitchens for one week. In the kitchens 51% of bags were in eye level cupboards, 30% in below the counter cupboards, 9% in pantries and the rest in a variety of other locations. In total 22 bags were infested with Liposcelis bostrychophila after the three months incubation period, all of which were bags that had been exposed in kitchens. None of the control bags showed any sign of psocid presence. Although the numbers of psocids were not counted, in all but one of the 22 cases the infestations were very obvious with psocids running over the outside of the paper flour bags within the plastic sealed bag. There were no trends as to the types of flour that were infested (8 Plain flour, 7 Self-raising, 7 speciality and bread flours). The greatest number of infestations was in the South Midlands – but this area was best represented in the study (Table 1.0). Infestations were also found in Carlisle, Stockport, South Essex and North London. Analysis of infestations by geographic area, however, is unlikely to be of any great significance since the study's coverage of the country was very patchy and uneven.

A χ^2 association analysis was carried out to see if there was a significant association between the psocids and the kitchen samples and the converse, no psocids with the control samples. The null hypothesis being that the infested bags would be equally spread between the control and the kitchen samples. This analysis gave a χ^2 value of 22.23, greatly in excess of the 95% probability value of 3.84, indicating a strong association between psocids and kitchens and not with bags of flour at the point of purchase.

Discussion and Conclusions

These findings are in accordance with other, largely unpublished, work carried out by the author in which there is a partitioning of psocid species found in homes compared to warehouses (Turner and Ali, 1996). *Liposcelis bostrychophila*, although ubiquitous, needs warm conditions, in excess of 20°C, to reproduce. It cannot live outdoors throughout the year in the UK and is the normal species found in domestic property. Most if not all situations of consumer complaints of psocids on new flour and other farinaceous products are caused by the attraction and concentration of undetected scattered

Psocid infestations and domestic kitchens

psocids in kitchens onto the product. This occurs rapidly within a few days. If a reasonable number of psocids colonise the new bag by the time it is used, it will be seen to be infested and the natural explanation is that the product arrived in the house already infested. If that scenario was an option, then at least some of the control bags in this small study would also have been infested.

This study does not help to answer the wider question as to how the psocids spread from one household to another – how did the primary infestation of the property occur if not via contaminated foods? In addition there is the unanswered question as to why the numbers of houses that have psocids is increasing. It is highly likely that almost anything that is bought into a home may carry psocids. In the case of *L. bostrychophila* it is important to note that it is parthenogenetic so a single egg or a juvenile or adult can found a new population. This idea that they may be on anything derives from an unpublished study made by the author for a company that makes plastic packaging, who were finding their product contaminated with psocids. The psocids in this case were moving on to pallets of cardboard boxes of product during overseas transportation in sea containers. Every one of five sea containers sampled contained psocids, living in the crevices of the floor of the container and feeding on spillages of powdered food from previous transhipments which had been swept into the cracks between the floor boards between uses. The plastic packaging is totally inedible and its only attraction to wandering psocids within the sea container may have been the insulation effect on temperature changes. As the sea containers are shipped into warmer conditions, the metal containers quickly heat up, whilst conditions in the depths of the boxes of packaging will stay, at least for a time, relatively cooler.

Commercial shipping containers carry all sorts of products including foods all over the world. The containers used by this specific packaging company are now pre-treated with an insecticide fog to prevent contamination of their product but that is not a standard practice, and therefore the possibilities for cross contamination of the type described are very high.

Acknowledgements

Thanks to all those who helped with this survey and my commiserations to those who through the study discovered they had psocids in their kitchens.

Aldi Asda 14 Caulfields Co-op 10 Costcutter 16 Kwiksave 4 Lidl **Morrisons** 20 Netto Safeway 2 Sainsburys 56 Salisbury Healthfoods 6 Sawers 2 Somerfield 6 Spar Tesco 94 Waitrose 16

Table 2.0The retail outlets where the bags of flour were purchased

^{*} number of bags of flour

Dr Bryan Turner

References

Ali N and Turner B D (2001). Allozyme polymorphism and variability in permethrin tolerance in British populations of the parthenogenetic stored product pest *Liposcelis bostrychophila* (Liposcelididae, Psocoptera). *Journal of Stored Products Research*, 37, 111-125.

Rudolph D (1982). Occurrence, properties and biological implications of the active uptake of water vapour from the atmosphere in Psocoptera. *Journal of insect Physiology*, 28, 111-121.

Turner B D (1987). Forming a clearer view of *L. bostrychophilus. Environmental Health*, 95, 9-13.

Turner B D (1994). *Liposcelis bostrychophila* (Psocoptera, Liposcelididae), a stored food pest in the UK. *International Journal of Pest Management*, 40,179-190.

Turner B D (1998). Psocids as nuisance problem in the UK. *Pestcide Outlook*, 9, 27-30.

Turner B D and Ali N (1996). The pest status of Psocids in the UK. 2nd International Conference on Insect Pests in the Urban Environment, Edinburgh, UK.

Turner B D and Bishop J (1998). An analysis of the incidence of psocids in domestic kitchens: the PPFA 1997 household survey (What's bugging your kitchen). *Environmental Health Journal*, 106, 310-14.

Turner B D and Maude-Roxby H (1988). Starvation survival of the stored product pest *Liposcelis bostrychophilus* Badonnel (Psocoptera, Liposcelidae). *Journal of Stored Products Research*, 24, 23-28.

Turner, B D, Maude-Roxby, H and Pike V (1991). Control of the domestic insect pest *Liposcelis bostrychophila* Badonnel (Psocoptera): an experimental evaluation of the efficiency of some insecticides. *International Pest Control*, 33,153-157.

Yusuf M, Turner B D, Whitfield P, Miles R and Pacey J (2000). Electron microscopical evidence of a vertically transmitted Wolbachia-like parasite in the parthenogenetic, stored-product pest *Liposcelis bostrychophila* (Psocoptera: Insecta). *Journal of Stored Products Research*, 36: 169-175.

Yusuf M, and Turner B (2004). Characterisation of Wolbachia-like bacteria isolated from the parthenogenetic stored product pest psocid *Liposcelis bostrychophila* (Badonnel)(Psocoptera). *Journal of Stored Products Research*, 40, 207-225.

Environmental factors associated with the presence of *Vibrio parahaemolyticus* in sea products and the risk of food poisoning in communities bordering the Gulf of Mexico

Glennda Lucía Charles-Hernández, MSc12, Dr Enrique Cifuentes2, Dr Stephen J. Rothenberg2

- Public Health Laboratory Tamaulipas (LESPT), Cda de las Flores 44. Bldg., Minister of Health, México.
- $^{2}\,$ Environmental Health, Center for Population Health Research, National Institute of Public Health (INSP), Mexico.

Correspondence: Dr Enrique Cifuentes, INSP, Av Universidad 655. Sta. Maria Ahuacatitlan, Cuernavaca, Morelos, CP 62508, México. Telephone: 777 329 3000 ext 3300. Fax: 777 101 2937 Email: ecifuent@correo.insp.mx

Abstract

The objectives of our study were to evaluate: (1) the effect of water temperature and salinity on the presence of *Vibrio parahemolyticus (Vp)* in seafood, (2) the association between the presence of this bacterium in sea products and number of food poisoning cases, (3) the virulence of isolated *Vp* strains, and (4) the appropriateness of risk management measures.

We conducted an ecological study (2001-2003) in coastal districts in the Gulf of Mexico. Data included seawater temperature and salinity, the presence of Vp in seafood and cases of human infection – the Vp strain and virulence were tested only in positive samples. We conducted time-series analysis and used Generalized Linear Models with binomial family, and a logit link to construct multivariate models.

The prevalence of Vp was 11.3% in seawater samples and 20% in oysters. We observed a significant association between the presence of Vp in seafood and human positive samples (p= < 0.05). Higher seawater temperature was followed by a 1.29 times increase of Vp detection in seafood (p=0.05); the prevalence of this bacterium in sea products increased 18.3 times during the warmer season (p=0.06); higher water salinity (1%) was followed by a 3.46 times increase of Vp positive results from seafood samples (p=0.01). Kanagawa tests showed positive results in 84.6% of human samples and 72.7% of seafood samples, respectively.

Given the seasonality of seafood poisoning outbreaks, health protection policies should integrate various sources of information (for example, environmental, epidemiological and food surveillance) into risk communication.

Key words: Climate; environmental health; food poisoning; Gulf of Mexico; marine products, *Vibrio parahaemolyticus*; water salinity.

Introduction

Food safety is a global health concern. Seafood, particularly raw or insufficiently cooked, may be a vehicle for pathogens responsible for disease outbreaks, trade conflict and economic losses. The list of infectious agents involved in food poisoning (FP) is long. Among the bacteria, the genus Vibrio includes a dozen species, from which Vibrio parahaemolyticus (Vp) has received only partial attention. Vp however, is one of the major causal agents of both

documented outbreaks and sporadic cases of food poisoning (Merson et~al., 1976). The incubation period is usually brief (12 – 24 hours), although it may extend up to 3 days or more. Symptoms include severe and sometimes bloody diarrhea, accompanied by abdominal pain, nausea, vomiting, headache, fever and chills that may or may not indicate septicaemia (Baross et~al., 1968). The virulence of Vp can be assessed in the laboratory by the Kanagawa hemolysis test. Detection of these bacteria in seafood samples usually implies risk management and health protection policy (e.g. FP outbreak control).

Vp is a hallophylic bacterium inhabitant of estuaries and coastal zones (Oliver et al., 1997). During hot season periods it is relatively easy to detect Vp in seawater, sediments, plankton, fish, oysters and other marine products that constitute reservoirs of the bacterium in the ecosystem (Baross et al., 1968). Numerous cases of seafood poisoning show a similar seasonal pattern (Fujino et al., 1953, Daniels et al., 2000, DePaola et al., 2000, Kraa, 1995).

Credible epidemiological information related to seafoodborne diseases from Mexico is scarce; reported prevalence of Vp in sea products ranges between 6 – 70% (Franco etal., 1991, Torres and Fernandez, 1993) but potential lessons are not being translated into risk communication programmes focusing on uninformed consumer groups (eq. the local population and tourists).

An increasing number of FP outbreaks has been reported from the coastal zone of Tamaulipas (Gulf of Mexico, Figure 1.0) where Vp has been implicated as the causal agent; the bacterium is often detected in food street vendors and restaurant workers. No data was found concerning alternative sources of transmission (e.g. seawater).

In this study we evaluated (1) the effect of sea temperature and salinity on the presence of Vp in sea food, (2) the association between the presence of Vp in environmental and seafood samples and number of FP cases among consumers, (3) the virulence of isolated strains of Vp, and (4) the appropriateness of risk management as well as health protection measures.

Materials and Methods

We conducted an ecological study in the coastal region of Tamaulipas (Gulf of Mexico, Figure 1.0) over 2001–2003. We obtained environmental records for sea temperature (°C) and salinity (%) from the Oceano-

Glennda Lucía Charles-Hernández, Dr Enrique Cifuentes, Dr Stephen J. Rothenberg

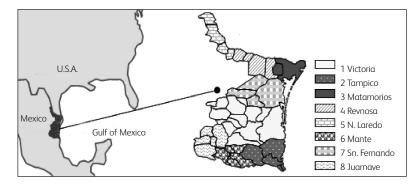
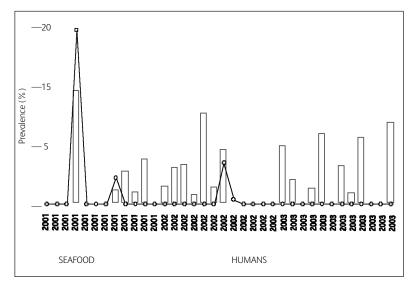


Figure 1.0 Study Area

graphic Research Center of the Navy Secretariat files (CIOSM), whose staff use the direct method (1 metre depth, using a thermometer with a rank of -100 to 100°C) for superficial sea temperature measurements, and refractometry procedures for water salinity.

We obtained data on the presence of Vp in sea products and human stool samples from the Public Health Laboratory in the state of Tamaulipas (LESPT). Food products files included data on type of product (e.g. oyster, fish, shrimp), sanitary jurisdiction (SJ) providing the sample, dates of collection, analysis, as well as the corresponding results. Human case records for positive Vp tests contain demographic data for each individual. We obtained the Vp strain only from food and human samples with positive results, and evaluated the virulence using the Kanagawa test i.e. heamolysis (Okuda and Nishibuchi, 1998), for which we used the agar Wagatsuma (with human erythrocytes, Group "O").

Figure 2.0 Prevalence of *Vibrio* parahaemolyticus in seafood and humans (2001-2003)



Data analysis

We defined three coastal districts (CDs): Victoria, Tampico and Matamoros. For the purpose of analysis, non–coastal settlements were assigned to their common source of seafood (e.g. coastal regional markets). Independent variables were the monthly sea superficial temperature (°C) averages and water salinity (%). Dependent variables were the presence of the bacterium (Vp) in sea products and human stool samples.

We conducted time-series analysis, and defined a lag time ranging from 0 to 2 months for independent variables in relation to the presence of *Vp* in seafood. Our univariate analysis included the averages of water temperature and salinity, as well as Vp presence both in sea food and human samples. The bivariate analysis allowed detection of statistical associations: (1) between the presence of Vp in sea products and environmental variables and (2) between isolations of Vp in sea food and human samples. We conducted the multivariate analysis by using Generalized Linear Models (GLM) with progressive incorporation of variables, while evaluating the influence of potential confounders. Given that the prevalence of Vp was restricted to fall between 0 and 1, we used a GLM with binomial family and a logit link to construct multivariate models over time, correcting for autocorrelation of the time series with a robust calculation of standard errors (Hardin and Hilbe, 2001). We used the statistical software package STATA version 8.2 (Stata Corp., College Station, TX).

Results and discussion

Food poisoning cases were restricted to four out of the 36 months (Figure 2.0). Seawater sample prevalence of Vp was 11.3% (CI 95%: 6.0, 18.9), while prevalence of Vp in seafood was 3.1% (57/1861) which was concentrated mostly in 20 out of the 36 analyzed periods. The bacterium was detected predominantly in oysters (Table 1.0; prevalence = 19.9% and CI 95%: 13.0, 28.3).

Data on positive human cases included a total of 162 individuals, all of whom required urgent medical attention. The general prevalence of *Vp* in stool samples was 2.2% (87/4022) with the largest proportion of cases reported in people aged 21-30 years; we detected no significant differences between sexes.

CD-2 had the highest water temperature (25.6°C), whereas CD-3 had the lowest (24.7°C). We observed the highest average sea salinity concentration (3.5%) in CD-

Environmental factors associated with the presence of Vibrio parahaemolyticus in sea products and the risk of food poisoning in communities bordering the Gulf of Mexico

| Samples | Total samples analyzed | Total positive samples of <i>Vp</i> | Prevalence | 95% CI |
|----------------|---------------------------|--|------------|-----------|
| Fish | 634 | 2 | 0.3 | 0-1.1* |
| Oysters | 116 | 23 | 19.9 | 13.0-28.3 |
| Crabs | 19 | 1 | 5.3 | 0.1-26.0 |
| Shrimps | 230 | 8 | 3.5 | 1.5-6.7 |
| Squids | 29 | 0 | 0 | 0-11.9* |
| Octopus | 30 | 0 | 0 | 0-11.6* |
| Others | 23 | 1 | 4.3 | 0.1-21.9 |
| Seawater | 106 | 12 | 11.3 | 6.0-18.9 |
| River water | 538 | 7 | 1.3 | 0.5-2.7 |
| Lagoon | 112 | 3 | 2.7 | 0.6-7.6 |
| Pristine water | 24 | 0 | 0 | 0-14.2* |

Table 1.0Prevalence (%) of *Vibrio*parahaemolyticus (*Vp*) in seafood and environmental samples. Tamaulipas, Mexico (2001-2003).

3, whereas the lowest (1.9%) was found in CD-2; CD-1 was intermediate for both variables (24.9°C and 3.3%).

Table 2.0 shows the results of the multivariate analysis. The prevalence of Vp in sea food increased 18.3 times during the summer months (July, August and September) when compared with the winter months (January, February and March) (p=0.06). Water salinity was a strong predictor of the detection of Vp in seafood. In the final model, every 1% increase in water salinity was followed by a 3.46 times increase in Vp prevalence in sea products (p=0.01). The model also showed that the increase of 1°C in sea temperature was followed, a month later, by a 1.29

fold increase of Vp in seafood samples (p=0.05). Virulence tests were positive in 84.6% of human samples (22/26) and in 72.7% of seafood samples (16/22).

One of the original goals of this work was to model the association between Vp in seafood products and the occurrence of Vp among individuals with symptoms of FP. Figure 2.0 shows the prevalence of Vp in humans and in seafood products over the three year study period. Only three outbreaks of Vp in humans were detected, the first in April 2001, the second in August 2001, and the third during the July-August period of 2002. Although the Vp data was insufficient to perform a formal analysis of the

^{*}One-tailed test, 97.5 % Confidence Interval.

Glennda Lucía Charles-Hernández, Dr Enrique Cifuentes, Dr Stephen J. Rothenberg

Table 2.0GLM accounting for the effect of covariates on the prevalence of *Vp* in seafood (n= 27).
Tamaulipas, Mexico (2001 – 2003).

| Prevalence Vp | Odds ratio | P-value | IC 95% Robust CI | |
|---|------------|---------|------------------|---------|
| | | | Lower | Upper |
| Mean salinity (%) at one month lag | 0.28 | 0.01 | 0.110 | 0.760 |
| Mean water temperature (°C) at one month lag | 0.77 | 0.05 | 0.596 | 1.005 |
| Season 2 (April, May, June)* | 3.63 | 0.12 | 0.690 | 19.106 |
| Season 3 (July, August, September* | 18.25 | 0.06 | 0.877 | 380.000 |
| Season 4 (October, November, December)* | 5.67 | 0.19 | 0.416 | 77.453 |

^{*}Omitted referent season was season 1(January, February, March)

seafood-human relationship, some characteristics of this data should be noted: the first human outbreak was the largest and was also coincidental with the highest prevalence of Vp in seafood during the study period. The second outbreak coincided with a very low prevalence of Vp in seafood several months after the first FP event. In the third outbreak, the prevalence of Vp in stool samples approached 5%, but the prevalence of Vp in seafood reported that month, the seventh highest in the record, was not remarkably high. In fact, we observed a higher Vp prevalence during four different months i.e. after the event, but without a single Vp case in humans reported.

To our knowledge, this is one of the few epidemiological investigations conducted in Mexico linking climatic factors and seafood poisoning. We confirmed the presence of *Vp* detection in seawater and seafood (especially oysters), during the warmest months of the year. We also confirmed the association between water salinity and the presence of the bacterium in sea food. These observations are consistent with the main findings reported by other authors (Hlady and Klontz 1996, CDC 1997, CDC 1998, Jiang and Chai 1996, Doyle and Brian 2000, Levine and Griffin 1993, Kelly 1982, DePaola *et al.*, 2000 and 2003, Kaneko and Colwell 1973, Daniels *et al.*, 2000).

The strengths of this investigation included both the confirmation of the strains of *Vibrio parahaemolyticus* and virulence of *Vp* using Kanagawa tests, because they illustrated the link between the isolated strains of *Vp* in humans and food samples, which is not commonly reported (Levine and Griffin, 1993, Okuda and Nishibuchi, 1998).

Limitations of the study included potential information bias, particularly regarding water salinity and sea superficial temperature; some of the environmental data suggested technical inconsistencies (e.g. timing of measurements). Additional shortcomings reflected shortage of data (lack of logistical support or unfavourable climatic conditions) and lack of consideration to further combination (e.g. temperature and salinity). It was not possible to incorporate other variables into the final model, due to the limited number of observations (positive human cases). Although we may with some success have predicted high prevalence of Vp in seafood from environmental variables in certain regions, we are still unable to determine the critical conditions leading to FP outbreaks in humans in this region, given the presence of Vp in seafood.

Environmental factors associated with the presence of Vibrio parahaemolyticus in sea products and the risk of food poisoning in communities bordering the Gulf of Mexico

The links between enteric diseases and consumption of sea products during the hot season of the year are commonly known. Prior to the birth of the microbiological theory of food borne diseases, fishermen and merchants have known about seasonal health hazards and, traditionally, they adopted restrictive harvest practices, sale or consumption of certain sea products. The current global demand for seafood, in synergy with institutional weakness (commerce, public health and environment) in some regions, has resulted in marine overexploitation and excess fishing, and has increased population vulnerability for disease epidemics (Garret 1994).

Recommendations

- First, operational links between public health surveillance systems, food safety and environmental monitoring must be reinforced, with emphasis on risk communication and management integrated strategies.
- Second, environmental measurements must be made by trained personnel, using calibrated instruments, and under standard conditions (day of the week, hour, place and depth).
- Third, laboratories may include Vp in epidemiological surveillance, in addition to V. cholerae and enterobacterias during outbreak investigation (FP), and, if possible, complementing with Kanagawa tests.
- Finally, public health policies should translate various data into risk communication (e.g. to consumers and tourists) by using more efficiently regional media (Radio, TV). Food-borne outbreaks, trade conflict and economic losses are likely to reoccur otherwise.

Acknowledgements

The Oceanographic Research Center of the Navy Secretariat (CIOSM) who provided climatic information.

The Public Health Laboratory from Tamaulipas State (LESPT), Mexico who provided laboratory results.

The Mexico US Foundation for Science and Technology (FUMEC) who provided partial financial assistance.

Leticia Hernández and Carlo E Medina- Solis for help with the initial analysis.

References

Altekruse S, Bishop R, Baldy L, Thompson S, Wilson S and Ray, B (2000). Vibrio gastroenteritis in the US Gulf of Mexico region: The role of raw oysters. *Epidemiol Infection* 124: 489-495.

Baross J and Liston J (1968). Isolation of *Vibrio parahaemolyticus* from the Northwest Pacific. *Nature*, 217: 1263-1264.

Centers for Disease Control and Prevention (1998). Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters, Pacific Northwest, 1997. *Morb Mortal Wkly*, Rep 47: 457-462.

Centers for Disease Control and Prevention (1999). Outbreak of *Vibrio parahaemolyticus* infection associated with eating raw oysters and clams harvested from Long Island sound, Connecticut, New Jersey, and New York, 1998. *Morb Mortal Wkly Rep*, 48: 48-51.

Colwell R and Anwarul H (1994). Environmental Reservoir of *Vibrio cholerae*. In: *Disease in Evolution*. *Annals of the New York Academy of Sciences*. New York. Editorial Staff. Volume 740: 44-52.

Daniels N, Mackinnon L, Bishop R, and Altekruse S (2000). *Vibrio parahaemolyticus* infections in the United States, 1973-1998. *J Infect Dis*, 181: 1661-1666.

DePaola A, Kaysner C, Bowers J, and Cook D (2000). Environmental investigations of *Vibrio parahaemolyticus* in oysters following outbreaks in Washington, Texas and New York (1997, 1998). *Appl Environ Microbiol*, 66: 4649-4654.

DePaola A, Nordstrom J, Bowers J, Wells J and Cook, D (2003). Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters. *Appl Environ Microbiol*, 69: 1521-1526.

Desmarchelier P (1997). Pathogenic Vibrios. In: Foodborne microorganisms of public health significance, 5th Ed, pp 285–312. Senate Journal.

Doyle T and Bryan R (2000). Infections Disease Morbidity in the US Region Bordering Mexico, 1990 – 1998. *J Infect Diseases*, 182: 1503-1510.

Franco, M., Flores, A., and Suárez, H (1991). The prevalence of *Vibrio parahaemolyticus* and its antibodies in seafood handlers in Mérida, Yucatán. *Salud Pública de México* 33: 173-177.

Fujino T, Okuno Y, Nakada D, Aoyama A Fukai K and Mukai T (1953). On the bacteriological examination of Shirasu food poisoning. J *Jpn Assoc Infect Dis*, 35: 11-12.

Garret L (1994). The coming plague: newly emerging diseases in a world out of balance. Farrar, Straus and Giroux. New York. USA.

Hardin J and Hilbe J (2001). The binomial – logit family. In: Generalizad Linear Models and Extensions. Texas: Editorial State Press, College Station.

Hlady, W., Klontz, K (1996). The epidemiology of Vibrio infections in Florida, 1981 – 1993. *J Infect Diseases*, 173: 1176–1183.

Jiang X and Chai T (1996). Survival of *Vibrio* parahaemolyticus at low temperatures under starvation conditions and subsequent resuscitation of viable, nonculturable cells. *Appl Environ Microbiol*, 62: 1300-1305.

Kaneko, T, Colwell, R (1973). Ecology of *Vibrio* parahaemolyticus in Chesapeake Bay. *J Bacteriol*, 113: 24-32.

Kelly M (1982). Effect of temperature and salinity on *Vibrio* (Beneckea) *vulnificus* occurrence in a gulf coast environment. *Appl Environ Microbiol* 44: 820-824.

Kraa E (1995). Surveillance and epidemiology of foodborne illness in NSW, Australia. *Food Australia*, 47: 418-423.

Levine W, Griffin M (1993). Vibrio infections on the gulf coast: results of first year of regional surveillance. *J Infect Diseases* 167: 479-483.

Matsumoto, C, Okuda, J, Ishibashi, M, Iwanaga M, Garg P and Rammamurthy T (2000). Pandemic spread of an O3:K6 clone of *Vibrio parahaemolyticus* and emergence of related strains evidenced by arbitrarily primed PCR and tox RS sequence analysis. *J Clin Microbiol*, 38: 578-585.

Merson, M, and Morris, G (1976). Traveler's diarrhea in México. A prospective study of physicians and family members attending a congress. *N Engl J Med*, 294:1299-1305

Okuda J and Nishibuchi M (1998). Manifestation of the Kanagawa phenomenon, the virulence-associated phenotype of *Vibrio parahaemolyticus* depends on a particular single base change in the promoter of the thermostable direct haemolysin gene. *Mol Microbiol*, 30: 499-511.

Oliver J and Koper J (1997). Vibrio species. In: Food Microbiology: Fundamentals and Frontiers. Washington DC: Editorial ASM Press, 228-264.

Ramírez H, Charles G, Guerrero E, Chuc, B, and Becerra E (2003). Possible *Vibrio parahaemolyticus* endemic source from Tamaulipas. III Congreso Nacional de Químicos Clínicos y Medicina de Laboratorio. Cancún, Quintana Roo.. Clave QC6.

Senate Journal (1999). Senate Journal State of Texas pp. 2226-2232. Available online at *www.capitol. state.tx.us/sjrnl/76r/pdf/5-21.pdf*. [accessed 22/07/06].

Simon J (1997). Endangered Mexico: an environment on the edge. A Sierra Club book. San Francisco, USA.

Srutala W, Stiegel M, and Sarrubbi F (1982). Decontamination of laboratory microbiological waste by steam sterilization. *Appl Environ Microbiol*, 43: 1311-136.

S.S.A (Ministry of Health IV (1993). Enteritis due to *Vibrio parahaemolyticus* CIE-9 005.4; CIE-10 A05.

Torres V and Fernández E (1993). Incidence of *Vibrio parahaemolyticus* in raw fish, oysters and shrimp. *Rev Latin Microbiol*, 35: 267-272.

Twedt R (1989). Vibrio parahaemolyticus. In: Foodborne Bacterial Pathogens. New York: Editorial Marcel Decker Inc.: 543-568.

Palm kernel waste management through composting and crop production

Mr O O. Kolade¹, Dr A O Coker¹, Professor M K C. Sridhar² and Professor G O Adeoye³

- ¹ Department of Civil Engineering, University of Ibadan, Ibadan, Nigeria
- ² Division of Environmental Health, University of Ibadan, Ibadan, Nigeria
- ³ Department of Agronomy, University of Ibadan, Ibadan, Nigeria

Correspondence: Prof M K C Sridhar, University of Ibadan, Ibadan, Nigeria. Telephone: 234-803-7273836; Email: mkcsridhar@yahoo.com

Abstract

Palm kernel wastes produced from small and medium-scale industries pose a serious environmental problem in Nigeria. A portion of these wastes is used as feed supplements for livestock but most are disposed off by burning in the industry for heating purposes. This practice is an environmental concern and the byproduct ash is also a problem which needs to be addressed. Alternative economic disposal methods are necessary and one potential method is to convert the wastes into compost and put them back on the farm.

This paper describes a study in which palm kernel waste was converted into compost using goat manure and poultry droppings as nitrogen supplements. The compost was assessed for crop yield using green amaranth as a test crop under greenhouse conditions. The composting process was complete within 42 days and the quality was found to be good.

The results indicate that the composts can be applied at 4 tonnes/Ha to obtain yields comparable to those of organo-mineral fertilizer and chemical fertilizer which are popular among Nigerian farmers.

This composting process has the potential to address the environmental health concerns arising from the current way of dealing with palm kernel wastes.

Key Words: compost; chemical fertilizer; environmental health; livestock wastes; oil palm wastes; organo-mineral fertilizer.

Introduction

The oil palm (*Elaeis guineensis* Jacq) is a native of the humid tropics of West Africa. Cultivation originated where oil palm trees were interplanted in traditional agricultural production systems along with other annual and perennial crops. The tree flourishes where the annual rainfall is 2,000 mm or more. It occurs wild along the banks of rivers and streams in the transition zone between rain forest and savanna.

Palm kernel oil is obtained from the seed (the kernel or endosperm) which contains about 50 per cent oil. When the oil has been extracted, the residue known as "palm kernel cake" (PKC) is rich in carbohydrate (48%) and protein (19%) and is used as cattle feed (Onwueme and Sinha, 1991). The ash contains large amounts of

potassium. When the PKC is further solvent extracted to remove oil, it becomes "palm kernel de-oil cake" which has no nutritional value (carbon 42.73%, nitrogen 0%, volatile matter 67.71% and calorific value 4031 Kcal/Kg) and is mostly used as fuel source in industry. Thus in the palm oil industry the wastes arise from palm fruits and the seeds and is a growing problem. Using them as fuel is not environmentally sound and alternative methods are to be sought. One potential method is to convert the wastes into compost and put them back on the farm.

PKC is generally deficient in nitrogen and to make it into compost needs supplementary nitrogen rich materials. In the Nigerian communities livestock wastes are readily available. The most common livestock that are reared around the farms and residences are goats and sheep, poultry and, to some extent, pigs. There are also many goat farms in virtually all major cities and many households in Nigeria keep poultry. This could be the source of the nitrogen.

In the last 10 or more years interest in compost making and utilization among Nigerian communities has been revived as a result of increasing wastes and environmental degradation in both rural and urban areas. Added to that, Nigerian farmers use agrochemicals to the barest minimum due to non-availability, unequal distribution or high cost of procurement. The Federal Government of Nigeria is focusing more on sustainable agriculture and economic development programmes which in turn demand an inward looking approach for agricultural inputs. Recycling wastes for farm inputs is a viable option as evident from earlier reported work (John et al., 1996, Egbewumi et al., 1997, Sridhar and Adeoye, 2004).

This paper describes the conversion of PKC into compost using goat manure and poultry droppings and evaluating the product using green amaranth (*Amaranthus* spp) as a test crop.

Materials and Methods

Materials

The waste materials used are palm kernel cake (PKC), goat dung and poultry manure. PKC was obtained at a palm kernel processing unit near Bodija market in Ibadan. Fresh poultry manure and goat/sheep manure were obtained from the University of Ibadan Research Farm. The organo-mineral fertilizer was obtained from Oyo State Pace Setter Organic Fertilizer Plant, Ibadan.

Mr O O Kolade, Dr A O Coker, Professor M K C. Sridhar and Professor G O Adeoye

The chemical fertilizer, NPK 15-15-15 was obtained from the local fertilizer supplier.

Composting process

Composting was carried out using combinations of PKC and poultry manure (3:1 ratio) and PKC and goat/sheep manure (3:1 ratio). The composting was carried out according to the methods standardized in our laboratory in locally made woven baskets which facilitate natural aeration of the composting material (Sridhar and Adeoye, 2003). The amount of waste in each basket was 10 Kg (7.5 Kg PKC and 2.5 Kg livestock waste) and kept in a greenhouse at the university. A clean plastic sheet was spread under each basket to collect the leachates and these were put back into the composting material. The composting was carried out for six weeks. At daily intervals, temperatures of the contents were measured and pH values were determined at weekly intervals. To facilitate optimal aeration of the compost mixtures, the basket contents were turned once each three days for two weeks, and subsequently once a week for the remaining period of the process. The final composts were weighed at the end of the composting period to assess the per cent degraded, dry weights and chemical composition.

Greenhouse experiments

The matured composts were tested in a greenhouse experiment to assess their nutrient levels and growth of a test crop green amaranth (*Amaranthus* spp). The choice of the test crop was based on the fact that the vegetable is widely consumed, economical for large-scale cultivation throughout the year, and rich in minerals and vitamins. Besides, the growth is faster in the five ecological zones in Nigeria and is resistant to common pests. Kogbe (1976) extensively studied the manurial requirements of the leafy vegetable *Amaranthus* in evaluating the various manures. Makinde (2006) evaluated the effects of organo-mineral fertilizer on growth and yield of *Amaranthus caudatus* and *A. cruentus* in two locations in Lagos State.

The composts prepared were also compared with a locally available organo-mineral fertilizer (OMF) developed by the authors which is being marketed under the trade name "Pace Setter Organic Fertilizer" and is popular among farmers in the region. This OMF was prepared on a commercial scale using slaughterhouse wastes and market wastes and enriched at the factory with supplemental nitrogen (urea) and phosphorus (single superphosphate) at levels 3.5% and 1.0%, respectively. In addition, NPK (15-15-15) chemical fertilizer, and control soil were also used in the experiments.

Black polythene bags holding 2Kg of exhausted soil (composition: organic matter 6.2g kg¹, total kjeldahl nitrogen 0.30 g kg¹, available phosphorus 2.43 mg kg¹, sand 9.38 g kg¹, silt 20 g kg¹, and clay 41 g kg¹) were used, and two rates of each manure and fertilizer were applied, using 2000 Kg/Ha and 4000 Kg/Ha for the compost applications, and 300 Kg/Ha and 600 Kg/Ha for the NPK application. The pots were irrigated with tap water to the field capacity.

Methods of analysis and evaluation

The parameters measured to assess yield were plant height (in cm), stem girth (in cm) and number of leaves. The yields were measured weekly over a period of five weeks and the results obtained at the end of the experiment are given here. The five-week period was chosen because of the economic yield at this period of growth before the flowering and seed formation starts.

Raw wastes and the composts made from them were analyzed for various physico-chemical parameters according to standard methods. Organic carbon was determined using wet digestion method as described by Walkey Black (Allison, 1973). Each sample was ignited slowly in a muffle furnace to a final temperature of 550°C. The loss in weight and the residue left were weighed and calculated for carbon.

Total nitrogen was determined by the Micro-Kjeldahl method (Jackson, 1962). The technicon AALL method was used for determination of phosphorus (Technicon Instrument Corp.,1992). The pH of the samples was measured using a pH meter with glass electrode. The mean ambient temperature during the experiment was 29°C.

Results and Discussion

Changes during composting

The chemical composition of the raw materials used in the experiment is given in Table 1.0. The results show that PKC has the highest carbon content of 96.21 whilst goat dung has the highest per cent of nitrogen (3.62%). The phosphorus content of poultry manure (3.29%) is very much higher than the respective values for PKC and goat dung.

From the experiments, a 3:1 ratio of PKC and livestock wastes is a good mix for fast thermophilic degradation in

Palm kernel waste management through composting and crop production

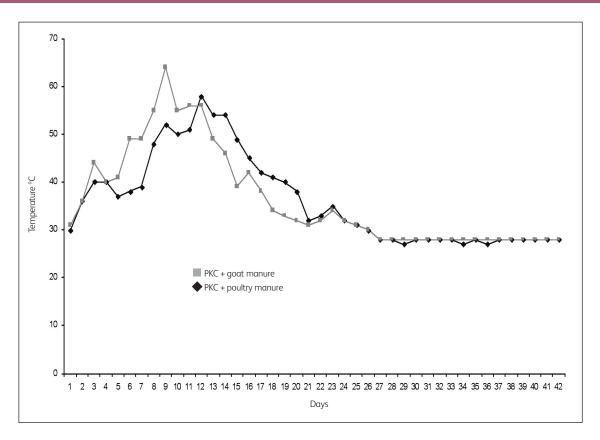


Figure 1.0Temperature changes during composting process

the composting process. The temperature changes during the process of 42 days are shown in Figure 1.0. Temperature was 30°C for PKC and poultry manure mixtures at the beginning of the composting which continued to increase until it reached a peak of 58°C on the 12th day, when the increase was gradual until it stabilized at 28°C at the end of fifth week of composting. In the case of PKC and goat manure mixtures the temperature rose from 31°C at start and progressively

increased until it reached the peak of 64°C on the ninth day, and thereafter it stabilized at 28°C by the end of fifth week and remained until the end of the experiment.

The quality of the finished composts was assessed by following the nutrient levels, C:N ratio, moisture level and texture. The values were within the acceptable limits (Table 2.0). The compost made from PKC and goat manure, however, showed higher nitrogen and

Moisture Carbon **Phosphorus** Potassium (K) Waste Nitrogen (N %) (C %) (P) content, % Palm Kernel Cake 58.92 96.21 2.88 0.60 0.19 75.94 0.18 Goat dung 29.79 3.62 0.51 Poultry droppings 49.11 78.83 2.83 3.29 0.16

Table 1.0Composition of wastes used in the composting (dry weight basis).

Mr O O Kolade, Dr A O Coker, Professor M K C. Sridhar and Professor G O Adeoye

Table 2.0Composition of the composts prepared from palm kernel cake. (Composting period of 6 weeks).

| Treatment | pH value | Carbon (C %) | Nitrogen (N %) | Phosphorus (P) | Potassium (K) |
|---------------------|----------|-----------------|----------------|----------------|---------------|
| PKC+ poultry manure | 7.35 | 81.56 | 3.52 | 0.188 | 0.277 |
| PKC + goat manure | 7.46 | 80.64 | 4.63 | 0.195 | 0.149 |

Table 3.0A comparative assessment of various composts and rates of application on the growth response of Amaranthus sp (5 weeks after planting, mean of 3 replicates).

| Compost type and rate of application | Plant height, cm | Number of leaves | Stem girth, cm |
|--|------------------|------------------|----------------|
| Control (Soil only) | 6.0 | 7 | 0.07 |
| PKC + Poultry Manure, 2 tons/Ha | 15.2 | 8 | 2.90 |
| PKC + Poultry Manure, 4 tons/Ha | 21.5 | 10 | 3.10 |
| PKC + Goat Manure, 2 tons/Ha | 11 | 8 | 1.75 |
| PKC + Goat Manure, 4 tons/Ha | 20.1 | 8 | 2.80 |
| Organo-mineral fertilizer, 2 tons/Ha | 23.5 | 11 | 0.35 |
| Organo-mineral fertilizer, 4 tons/Ha | 30.0 | 10 | 4.90 |
| NPK (15:15:15) Chemical fertilizer, 300 Kg/Hα | 22.0 | 10 | 4.20 |
| NPK (15:15:15) Chemical fertilizer, 600 Kg/Ha | 28.5 | 11 | 5.50 |

phosphorus levels which are needed in the composts prepared in Nigeria. Increasing the nitrogen levels in the natural composts is a challenge and supplementing with natural sources of nitrogen is more environmentally friendly than opting for mineral sources (Sridhar *et al.*, 2001; Adeoye *et al.*, 2005).

Assessment of compost quality using a test crop

The results of the assessment of growth of the test crop

Amaranthus spp (Table 3.0) indicate that the performances of composts prepared from PKC + poultry manure and PKC + goat manure were comparable with those of OMF and NPK particularly when applied at 4 tons/Ha. The peak heights for PKC and poultry manure were 21.5cm, PKC and goat manure 20.1cm, and the respective values for OMF and NPK were 30cm and 28.5cm. The maximum stem girth for PKC and poultry manure was 3.1cm, PKC and goat manure was 2.8cm,

Palm kernel waste management through composting and crop production

and OMF was 4.9cm, while NPK produced a peak girth of 5.5cm. PKC and poultry manure produced a maximum of 10 leaves, PKC and poultry manure produced eight leaves, while OMFA and NPK produced 10 and 11 leaves respectively.

It was noted that composts cannot outperform chemical fertilizers in terms of yields, particularly with respect to green vegetables. Traditionally, the recommended levels of application are 10 tons/Ha or more. In the Nigerian situation, most of the farmers preferred to have a product suitable for single application once before planting. Organo-mineral fertilizers meet such demand. The OMF used in the experiments performed better than the PKC composts because it was supplemented with additional nitrogen and phosphorus. However, the advantages of using organic manures lie in the fact that they release the nutrients judiciously and promote stable soil aggregates with residual fertilizing effects for subsequent crops. Furthermore, they are environmentally friendly.

Conclusions

- The management of palm kernel wastes is a serious environmental problem in many Nigerian cities as it is produced in large amounts by a vibrant industry and mostly disposed off through burning in industry, a process which causes environmental problems.
- It was observed that by mixing palm kernel wastes with livestock wastes good compost can be prepared which is comparable to other organo-mineral fertilizers and chemical fertilizers in terms of the plant growth parameters – plant height, stem girth and number of leaves.
- An application rate of 4 tonnes per Ha was found optimal for the desired yields of vegetable crops as tested with green amaranth.
- This composting process has the potential to reduce the environmental health problems created by the existing method of dealing with palm kernel wastes.

References

Adeoye G O, Sridhar MKC, Akinsoji N A and Adeoluwa O O (2005). Evaluation of naturally decomposed solid wastes from municipal dump sites for their manurial value in South West Nigeria, *Journal of Sustainable Agriculture,*

26 (4), 143-152.

Allison F E (1973). Soil organic matter and its role in crop production. Elsevier, Amsterdam, pp 417-444.

Bates R G (1954). Electronic pH determination. John Wiley and Sons. Inc .New York.

Egbewumi, O O, Sridhar, M K C and Asuzu, M C (1997). Composting through community action: A rural experience in Nigeria, *Journal of Appropriate Technology*, UK, 23: 34-35.

Federal Ministry of Agriculture (1981). A Report, p.33. Ibadan, Nigeria.

Jackson M E (1962). Soil Chemical Analysis. Prentice Hall, Englewood Cliffs, N.J.

John N M, Adeoye G O and Sridhar M K C (1996). Pelletiization of compost: Nigerian Experience, *Biocycle*, U.S.A., 37: 53-54.

Kogbe J O S (1976). Studies on manorial requirements of Nigeria leafy vegetables, 1. Effect of poultry manure on the yield and component of *Amaranthus (Amaranthus caudatus)*, *The Nigerian Agricultural Journal*, 13, (3):84-87.

Makinde E A (2006). Preliminary evaluation of the effects of organo-mineral fertilizer on growth and yield quality of *Amaranthus caudatus* and *A. cruentus* in two locations in Lagos State, Ph.D. Thesis, Agronomy Department, University of Ibadan.

Onwueme I C and Sinha T D (1991). Field crop production in tropical Africa. CTA (The Technical Centre for Agricultural and Rural Co-operation), Ede, The Netherlands, pp. 1-480.

Oyenuga V A (1968). Nigeria's Foods and Feeding-Stuffs, Ibadan University Press, pp. 1-99. Ibadan, Nigeria.

Sridhar M K C, Adeoye G O and AdeOluwa O O (2001). Alternate nitrogen amendments for organic fertilizers, in optimizing nitrogen management in food and energy production and environmental protection: proceedings of the 2nd International Nitrogen Conference on Science and Policy, The Scientific World, 1, pp. 6.

Sridhar M K C and Adeoye G O (2003) Organo-mineral fertilizers from urban wastes: developments in Nigeria, *The Nigerian Field*, 68: 91-111.

Technicon Instrument Corp (1972). Industrial Methods. No144-71A, Tarry Town, New York.

No144-71A, Tarry Town, New York. <<JEHR 5(2) Marked up Corrections (full journal)(15th August). pdf>>

Housing as a health determinant: is there consensus that public health partnerships are a way forward?

Jill Stewart¹ MSc BSc MCIEH FRSH PGCE ACIH, Professor Annmarie Ruston² MSc PGCE, Julie Clayton³ MSc BA

- ¹ Senior Lecturer, School of Health and Social Care, University of Greenwich
- ² Professor of Health and Social Care Studies, Faculty of Health and Social Care, Canterbury Christ Church University
- ³ Research Fellow, Centre for Health Research and Evaluation, School of Health and Social Care. University of Greenwich

Correspondence: Professor Annmarie Ruston, North Holmes Campus Canterbury, Kent. CT1 1QU. Telephone: 01227 782676. Fax: 01227 784408. Email: a.ruston@blueyonder.co.uk

Abstract

Policy makers have been increasingly interested in measuring the health effects of housing and in gathering evidence to shape policy and recent policy changes in private sector housing have once again brought housing and health issues to the fore. Partnership working is seen by the Government as a way forward to reducing inequalities in health and developing sustainable communities. Seventeen people who are responsible for private sector housing in one area of the South East of England were interviewed to ascertain their role in partnership working and the extent to which evidence-based housing was being promoted within these partnerships. Four main themes were explored: two at the level of the individual practitioner - housing as a health determinant and applying evidence to practice and two at the partnership level - current partnerships and measuring health gain.

The findings suggest that it will be quite some time before those responsible for private sector housing will be in a position to influence the cross agency, cross sector public health agenda within local partnerships. In particular, they need to develop their analytical capacity and refocus their partnership arrangements to move beyond the purchaser/provider relationships.

Public health partnerships were reported as variable in effectiveness. Respondents, although positive about the future contribution of such partnerships to positioning housing as a central health determinant, suggested that there was a need to more clearly define the roles, responsibilities and objectives before they could become really effective.

Key words: Environmental health; health determinants; housing; partnership working; public health.

Introduction

Housing and health

The association between housing conditions and physical and mental ill health has been empirically established through epidemiological studies (Thomson et al., 2001). The relationship is, however, complex comprising of the interaction of poverty, inequality, access to housing and housing as an internal and external living environment. Aspects of housing that affect health outcomes are often described in relation to the internal and external environment. Factors influencing the quality of the internal environment include

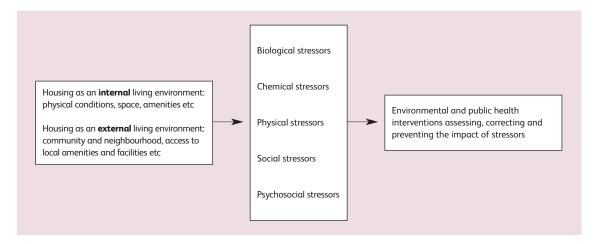
indoor pollutants, cold and damp, hazardous internal structures and fixtures and noise. Those affecting the external environment include neighbourhood quality, infrastructure deprivation, neighbourhood safety and social cohesion (Taske et al., 2005). The causal links between these different dimensions of housing, neighbourhood, environment and health can operate at a number of interrelated levels. Additionally, as poor housing is also linked to other forms of deprivation, such as poor education, unemployment, social isolation etc, it is difficult to isolate, modify or assess the overall health impact of housing conditions (Taske et al., 2005).

Thus, it can be seen that housing should have a prime place on the health inequalities agenda. It also has wider importance because small health effects can have a large impact at the population level (Thomson et al., 2001). Policy makers have been increasingly interested in measuring the health effects of housing and in gathering evidence to shape policy and recent policy changes in private sector housing have once again bought housing and health issues to the fore for those involved in delivering housing improvements. For example, there has been an increased emphasis on sustainable housing and communities across tenures. Also a growing interest in meeting housing related need at a strategic level e.g. tackling tuberculosis, home safety or fuel poverty, as well as, at a more individual level by, for example, meeting the needs of an older home owner through services delivered by a Home Improvement Agency. More recently, health impact assessments, which have become more important in accounting for health gain arising from policy success, are starting to find their way into the housing arena. It is now recognised that those responsible for improving housing conditions will need to recognise and respond to environmental stressors (see figure 1.0) in order to be able to promote healthier housing and environments (Burke et al., 2002).

Housing policy has increasingly recognised the need for decent housing for all in sustainable communities, with priority given to the most marginal social housing estates (DETR, 2000a; ODPM, 2003a; ODPM, 2004a). There has been an accelerated emphasis on local strategic solutions to housing issues, notably in ways of delivering private sector housing renewal (DETR, 2000b; Stewart *et al.*, 2005). In addition, the Housing Act 2004 introduces a completely new way of assessing housing conditions across tenures through the evidence based Housing Health and Safety Rating System (HHSRS) (ODPM, 2003b; ODPM, 2004b).

Jill Stewart, Professor Annmarie Ruston, Julie Clayton

Figure 1.0 Health impacts on housing as a living environment (adapted from Burke et al., 2002)



The Government has set a Public Service Agreement (PSA) target of bringing all social housing into a decent condition — with most improvement taking place in deprived areas and for vulnerable households in the private sector — and increasing the proportion of people who live in homes that are in decent condition by 2010 (ODPM 2003a, 2005a, 2005b). This represents a considerable task given that in 2001 there were 6.7 million households living in non-decent homes, of which 5.2 million households lived in the private sector and 1.5 million were social tenants. Much of this work will be of concern to those environmental health and/or housing officers who have responsibility for private sector housing.

These policy changes present challenges for local authorities that are seeking to ensure that housing stock in their area is contributing to the overall health of their population. However, local authorities should no longer be alone in meeting these challenges. The public health agenda – with its emphasis on partnership working to address health inequalities – now offers the potential for joint working to help meet the complex interrelationships of health and housing. Indeed, public health is an integral concern within urban planning processes relating to the physical and social environment (Taske et al., 2005). Partnership arrangements including Local Strategic Partnerships, Community Strategies and Local Delivery Plans should help those charged with delivering healthier housing to access a wider range of resources to meet their targets. However, although the new partnerships are well established in theory, concerns have been expressed about whether the partners are really working together and sharing values, focus, vision, direction and objectives (Evans, 2004; Hunter and Sengupta, 2004; Wills and Woodhead, 2004). Barriers include unresolved issues in

partnership working, capacity problems, organisational changes and differing performance management regimes. Indeed, it has also proven difficult, so far, to assess health gain arising from some local partnership strategies, such as fuel poverty even where the strategy itself has received national recognition in its development and implementation (Stewart and Habgood, work in progress).

It is within this context that the research reported here sought to explore, in particular, private sector housing managers/officers perceptions of housing as a health determinant, the extent to which they felt able to promote housing on the public health agenda within public health partnerships and whether partnerships result in cohesive, evidence-based, joint strategies for private sector housing renewal.

Methods

The overall objectives of the study reported here were to:

- Ascertain private sector housing managers/officers perceptions of housing as a health determinant;
- Examine the extent to which private sector housing mangers/officers feel they influence joint planning through public health partnerships;
- Ascertain the extent to which joint plans influence private sector housing renewal;
- Investigate the extent to which the HDA/NICE Evidence Base informs practice and strategy development.

One county in the South East of England provided the geographical parameter for the study. Each local authority in the County, the County Council, the Unitary

Housing as a health determinant: is there consensus that public health partnerships are a way forward?

Authority and a sample of housing associations was contacted to identify who within the organisation was responsible for private sector housing renewal. This task proved to be difficult because in some organisations the responsibility was with the environmental health department, in others it was the housing department and in some it was not clear who had responsibility for the private sector. Additionally, the people identified as having responsibility for private sector housing were not necessarily the same as those that engaged in partnership working. Tracking down suitable respondents took a considerable degree of persistence and time. In total 20 organisations were contacted and interviews eventually secured in 17 of these.

Two sources of data were accessed. First, each organisation that was contacted was asked to provide its policy documents relating to public health and housing. Once assembled, these were read and analysed in order to examine the extent to which housing was identified or raised as a health determinant, which aspects of housing were covered within policies and strategies for dealing with problems identified.

Second, semi structured telephone interviews were held with key representatives within the organisations selected. An interview guide was used and questions covered the nature of the public health partnerships that respondents were part of, how health determinants were categorised and prioritised in the partnerships, their views on housing as a health determinant, housing-related strategies delivered by the partnerships, how housing was promoted within the partnerships, whether they were evidence-based and any barriers to joint working.

The interviews were tape recorded and transcribed verbatim. Analysis was conducted using a cut and paste technique for content analysis (Kippendorf 1980). The content analysis utilised four separate passes through the transcripts to cover all evolving categories. Patterns and meanings emerging were then identified.

The data from the semi-structured interviews and analysis of publications have been synthesised to address the study objectives.

Results

The major focus of public health is on improving health and reducing inequalities and the public health agenda has been concerned with organisational change to the NHS to

deliver public health through PCTs' partnership arrangements with other organisations, notably local authorities (Stewart, 2005). In order to examine the potential of those with responsibility for private sector renewal to be able to effectively influence the public health agenda within these partnerships, it is useful to explore a number of factors which might affect the way in which they engaged with the public health agenda. Factors include first, those specific to the individual; that is the way in which housing is perceived as a health determinant and their use of evidence to inform practice. Second, those relating to partnership working; that is the way in which housing is prioritised on partnership agendas, the extent to which partnerships have cohesive, shared objectives and the measurement of health gain from housing interventions.

Individual level – housing as a health determinant Health and housing were described as being very much interrelated by most respondents with the majority referring to the internal housing environment as a main source of problems that could potentially affect the health of residents:

"My personal opinion is that housing has a very detrimental effect — if we have poor housing conditions yes; that has a very direct correlation with health. In particular damp housing, houses that are not easy to heat, fuel poverty, all have a direct effect — there is no doubt in my mind that this is the case and that is one of the reasons why we try and deal with all of those issues in the various bits of grant money that we do have." Int 5

A smaller proportion also identified external environmental factors that were considered to potentially influence health:

"Yes, basically things like rubbish accumulation, nuisance from noise, problems with burglary – home safety really." Int 10

In line with research evidence about the relationship between housing and health, respondents described physical and mental health consequences for residents of living in poor housing:

"I think there is a clear link...obviously it's around the physical connection in that poor standard accommodation links directly to illnesses and problems that are easily and readily identified – whether it's dampness and chest infections and those sorts of things that arise from that, or whether

Jill Stewart, Professor Annmarie Ruston, Julie Clayton

it's around the other areas which aren't often readily identifiable, about the effects that poor quality housing has on people's own aspirations and their mental health." Int 17

They also generally reported that the new Housing Health and Safety Rating System (HHSRS) would shift the emphasis away from housing defects towards dealing with people's health and safety needs – moving away from bricks and mortar to the occupiers. Thus respondents' descriptions revealed a public health perspective of the relationship between housing and health and a clear understanding of the evidence of housing factors that could affect health. However, housing was only specifically mentioned in relation to health in two of the policy documents analysed.

Individual level – applying evidence to practice

The written policy documents revealed that some organisations had carried out local surveys of the state of their housing stock and the existence of evidence of the effects of poor housing on health. A number of respondents also mentioned the existence of research evidence relating the effects of poor housing on people's health but they were considerably less confident about the use of evidence to underpin practice. In order to tease out the extent to which evidence was informing their individual practice respondents were asked if they used the public health evidence provided by the Health Development Agency (now part of the National Institute for Health and Clinical Excellence). Although respondents reported that they had not themselves accessed this type of information they felt that it may be possible that others within their organisation would have done so:

"It's not something that I have referred to, but it could be something that my officers who are responsible for the strategy may well have referred to. We certainly refer to health data in terms of prevalence around learning disability and mental health in order to develop needs analysis which reflects national or regional prevalence, so that we can try and direct our resources appropriately to the service user groups that need it most." Int 15.

Clearly, when needing to produce policy documents respondents would expect to include health statistics but not necessarily be aware of any evidence of what constitutes effective housing interventions or routinely apply it to practice.

Partnership level – current partnerships

Respondents were asked about the public health partnerships that they and their private sector housing colleagues were involved in. Three respondents reported being unsure about whether there were any partnerships and a further three reported that they thought there were partnerships but that they themselves were not involved:

"I don't know that there are any formal ones whatsoever. Our role is very much a reactive role. The section that I look after is very small – myself and two other officers, field officers and half an admin person – so private sector housing is three and a half people basically all together. So we are almost always reacting to complaints and investigating complaints and there is really very little, if any, proactive work going on." Int 8

The remaining respondents (11) were able to identify at least one public health partnership that they were involved with. However, respondent's descriptions of the nature of these partnerships varied from partnerships that were perceived as a form of monitoring of services,

"At the moment the only formal agreement we have is with 'Supporting People' which is obviously a government initiative – it comes out of the Deputy Prime Minister's Office. Those are our regulators if you like. They monitor our service delivery and make sure that what we are providing is adequate and is also value for money and needed in the local area." Int 4

to partnerships where respondents (3) described their umbrella organisation taking a lead role:

"I mean the chief executive – he actually chairs the Health Policy Board which comprises of all the local authorities, the PCTs and they are sort of fully engaged with the health agenda and are members of the Teenage Pregnancy Group. We have a multiagency joint assessment and referral procedure for social housing etc., which is under the Supported Housing Needs Scheme." Int 6

Partnership working was reported as a political necessity but it was clear from respondents that they perceived the effectiveness of public health partnerships in relation to achieving a shared vision and objectives to be variable. Respondents views varied from one extreme where partnerships were seen as successful in meeting the public health agenda and containing housing targets,

Housing as a health determinant: is there consensus that public health partnerships are a way forward?

"We have input in the local strategic partnership and we have actually got some indicators in there of how well we are doing and we have taken that a step further with the County Council because they are an 'Excellent Authority'. They are able to exhibit some freedoms if you like from control from central government, and they have come up with agreement where they have agreed with all local authorities that they will be delivering on certain issues, and there are a couple of housing targets we have put in there." Int 5

through a middle ground perspective where respondents were positive that in future partnership working would become more effective from a housing perspective,

"I think the other element that will work when it's a bit more defined, but it's certainly a start, is the Community Plan, where we have fed in elements of our housing strategy, the PCT have fed in elements of theirs etc., but at the moment it's quite a cumbersome document and as a result I think the danger is it will gather dust unless it can, in that sense, be made a bit more relevant. But I think there is real potential there for working that up, so that there will be some better defined common outcomes and aims and objectives basically." Int 13

to the other extreme where housing was seen as incidental to the main business of the partnership:

"Well, we tend to have meetings about different subjects but in terms of housing, I have to say that the meetings are limited to my knowledge and as I say it's because of the fact that it's not... high on the agenda. If it's an opportunity to develop housing, fine, or opportunity to purchase land or something of this nature, so that we can move forward with other strategic objectives." Int 9

Partnership level – measuring health gain

One of the ways of showing the effectiveness of joint strategies would be to ascertain whether health gains arising from the joint plans, and housing interventions in particular, are considered and measured. Generally speaking, respondents considered the area of achieving and measuring health gain to be problematic and alien to their sphere of work. For example, the following respondent felt that the collection and analysis of that type of data was beyond their skills. Respondents reported being more likely to make assumptions about the effectiveness of any intervention rather than obtaining definitive evidence:

"We have got a renewal area here and the idea is to try and improve the area, to improve the conditions for the people, in part, who are living there now. So yes, it's always going to have an effect on them and we are aware of that, but for us it's a slightly different thing. Because we are not looking at the collection of, say, health statistics the way people like the PCT are, we are not aware of what we might be doing having a direct effect on an individual's health or even the health statistics in the area. Somebody else would probably look at that because we wouldn't necessarily comprehend the statistics... we are not really competent to say whether or not the statistics were collected properly or not, so we wouldn't know if it was actually having a long term effect – we would just believe it would. We wouldn't be able to prove it, to be fair, because it would take too long for somebody to collect the statistics to prove it." Int 3

Respondents reported being in favour of being able to provide evidence of effectiveness, particularly in light of the requirements of the new Housing Act, but again felt that it was very difficult to achieve:

"This has proved difficult because it would be ideal, particularly with the falls prevention to see if housing intervention has decreased the hospital admissions ...unfortunately we haven't been able to do that as yet, but it is something that would be really great, particularly with the new legislation coming on board sort of thing. It is part of the Falls Strategy Action Plan to gain the evidence because obviously a lot of funding rests on evidence base etc and particularly with health it is quite difficult sometimes to actually gain that evidence base that ...as a result of housing action has actually contributed to that health effect sort of thing. It is quite difficult. So yes, it is quite difficult to back up – even though you know it's commonsense really that you are sure it's true." Int6

Ensuring effectiveness of joint plans and housing interventions was, however, considered to be dependent on the setting of clear strategies and defined outcome measures and partnerships were considered to be some way off achieving the required common strategies. Nevertheless, the future potential was reported:

"There is a lot of scope for better joint working, common strategies and input into one another's strategies and defining outcomes. The end result of that, of course, is that the impact of programmes on health gain isn't best defined or evaluated because

Jill Stewart, Professor Annmarie Ruston, Julie Clayton

that's the end product and I think the sooner we move to having better defined outcomes at the start and commonality of data collection in terms of targets and objectives to say the least then I think the faster we will move to a clear assessment of 'OK, what have these programmes achieved in terms of health and health improvement?'" Int 13

Partnership level – how is housing promoted within partnerships?

To further explore the extent to which housing was seen as a health determinant within partnerships, respondents were asked how housing was promoted and prioritised. A dichotomous response emerged: either respondents were not aware of any means by which housing was promoted as an issue:

"It's not really. Again, I think it's probably incidental rather than actively promoted." Int 8

"I guess it's promoted really directly at the meetings that the various officers have. I am obviously involved in all of them, but I think really we are always – when we come across primary care personnel – we are always beating the drum about housing because it's something that they come struggling to. As I say they are not really necessarily seeing that they need to do anything." Int 9

"That's a hard one, really. Other than through representation... I think the process is more trying to be involved with other agencies, where it seems appropriate to be involved and through that, in a sense, to champion the housing issues when it is right to do so. So our Housing Strategy Development as an example started off with involving all agencies in what the issues are. So part of that process is to get everybody else involved in the housing issues, so that the actual development of the strategy itself is done across agency and with a large number of people." Int 11

It was evident from respondents' discourses that partnership working was valued but that as yet it was not fully developed in relation to tackling housing as a public health issue. The majority of respondents described partnerships in terms of groups or organisations working together out of necessity. Successful partnership working was, therefore, localised and was more akin to joint working to meet individual organisations' objectives or to meet individual needs:

"Well, a lot of the time it is essential; we wouldn't have got anywhere with our grants if we hadn't had Care and Repair. I mean, we have worked with them for a long time...and we were able to get them to do an awful lot—or they can do an awful lot for us. If they hadn't been there; we wouldn't have spent the money, we wouldn't have got the work done to be honest." Int 3

"The Supporting People – local plan – that's been very effective. Basically, that is for people with housing needs that have some Supported People input, whether it be as a result of mental health conditions etc. or they are vulnerable etc; then all the parties come together and actually sit down and discuss their housing needs and where it would be perhaps suitable." Int 6

The data suggests that partnerships could potentially provide the means by which housing is promoted as an important health determinant and by which evidence of the effectiveness of housing interventions to reduce inequalities could be collected and disseminated. However, partnerships need to develop further before this becomes a reality and before housing is proactively tackled as a major cause of health inequalities. Respondents reported reacting to policy requirements as the focus of their work rather than being in a position to drive the public health agenda forward – partnership working has not fully facilitated this move.

Discussion

The Government has set out its vision for creating genuinely sustainable communities through Community Strategies and Local Strategic Partnerships (ODPM, 2005). Local Strategic Partnerships are expected to develop a common vision for a more sustainable future which is based on an in-depth analysis of the specific needs of the area and which results in priorities that can be translated into meaningful outcomes. The Sustainable Community Strategy is envisaged as performing an over-arching role to ensure effective and transparent allocation of resources in the locality. They will set out a vision of an area and coordinate and drive the delivery of local services leading to improved outcomes for citizens that go beyond the remit of any one partner. Key to this will be the ability to influence the polices and structures of partner agencies. This is expected to address some of the problems created by individual agencies or thematic partnerships that have developed local plans entirely separately (ODPM, 2005).

Housing as a health determinant: is there consensus that public health partnerships are a way forward?

In particular, it is expected that housing strategies should be developed with reference to the Sustainable Community Strategy.

Given the central position of housing in the health inequalities agenda it will be important for those who are responsible for housing to be able to ensure that local housing evidence is translated into potential health outcomes and that these are articulated within the key public health partnerships by influencing the policy and practices of partner organisations.

However, the data from the study reported here suggest that there is some way to go before this can be fully realised. A number of themes emerged which are of importance. First, respondents tended to view partnerships as an essential means of delivering individual housing-related policies rather than integrated public health policies across partnerships. Thus, partnerships were successful where good relationships had been established between the housing staff and those who were delivering services on their behalf. Thus, narrowly focused views about the role of partnerships tended to limit the potential for respondents to articulate a clear housing/health vision.

Second, respondents were unclear about how they could contribute to both the assembly and implementation of evidence-based housing strategies to improve the health of the population. In a situation where they were unclear how to action this in relation to their own work, it was unlikely that they would be able to articulate and strongly influence the agenda of other organisations, particularly the health services. Indeed respondents indicated that not only did they not have the requisite skills or analytical capacity but they did not see it as part of their remit. In these circumstances, the partnership agenda may well be medically dominated in relation to health and health inequalities.

Third, it was evident both from the data and from the range of individuals and organisations that it was necessary to access to determine who was responsible for private sector housing that this is not a homogenous group of professionals. Therefore, it would be difficult for them to collectively influence the public health agenda. The majority of respondents indicated that the only way in which they contributed to the strategic development of public health was through representation on various partnerships but they were not necessarily achieving their aim of influencing health care or primary care trusts about the importance of housing and health.

Thus, although the majority of espondents considered that housing represented a key health determinant, they were not necessarily being successful in raising its profile on the public health agenda within partnerships. Nevertheless, partnerships were viewed positively and expected to be of value in the future providing there were clear and measurable objectives set. There would need to be joint plans which were monitored within each organisation in relation to the overall public health strategy set by the partnership and each partner would need to have a mutual understanding of how each organisation worked. This again presents a problem in relation to private sector housing as the role is not clearly defined, nor is the organisational structure within which it sits.

Thus, although partnerships were viewed as the way forward in the future, it was clear that there is a need to develop the public health role of private sector housing officers before they will be in a position to effectively promote housing as a health determinant within partnerships. In particular housing officers' analytical capacity, use of evidence-based housing solutions and strategic approach to problem solving needs to be further developed.

Conclusions

Housing conditions have been associated with both physical and mental health problems, housing variables make deprivation indicators a good proxy for morbidity and it is clear that tackling inequalities in housing also addresses health inequalities (Smith *et al.*, 1997). Partnership working is seen as the way forward in devising cross-agency and cross-sector solutions to health inequalities though the sharing of resources and plans. However, if housing is to take centre stage in this process, the impact of private sector housing needs to be acknowledged and those responsible for this area of work need to be able to influence the public health agenda more effectively within partnerships.

Jill Stewart, Professor Annmarie Ruston, Julie Clayton

Acknowledgements

This research was part funded by a research grant from the Chartered Institute of Environmental Health.

References

Burke S, Gray I, Paterson K and Meyrick J (2002). Environmental Health 2012: A key partner in delivering the public health agenda. London, Health Development Agency.

Department of Environment, Transport and the Regions (DETR). (2000a). A Decent Home: the revised definition and guidance for implementation. Sections 1&2. London, DETR.

Department of Environment, Transport and the Regions (DETR). (2000b). The Housing Green Paper. Quality and Choice: A decent home for all. London, Joint publication from the Department of the Environment, Transport and the Regions and the Department of Social Security. Available online at www.communities.gov.uk [Accessed 20/07/06].

Evans D (2004). 'Shifting the balance of power?: UK public health policy and capacity building' in *Critical Public Health*, 14, issue 1: 63-75.

Hunter D J and Sengupta S (2004). Editorial: 'Building multidisciplinary public health' in *Critical Public Health*, 14, issue 1: 1-5.

 $\label{eq:Kippendorf K} \textbf{K (1980)}. \ Content analysis: an introduction to its methodology. Beverly Hills Sage.$

Office of the Deputy Prime Minister (ODPM). (2005). Local Strategic Partnerships: Shaping their future – A consultation paper. London

Office of the Deputy Prime Minister (ODPM). (2005a). Sustainable Communities: Homes for all. London, ODPM

Office of the Deputy Prime Minister (ODPM). (2005b). Sustainable Communities: People, Places and Prosperity. London, ODPM

Office of the Deputy Prime Minister (ODPM). (2004a). A Decent Home: the definition and guidance. London, ODPM.

Office of the Deputy Prime Minister (ODPM). (2004b). Housing Health and Safety Rating System Guidance (Version 2). London, ODPM. Available online at www.communities.gov.uk [Accessed 20/07/06].

Office of the Deputy Prime Minister (ODPM). (2003a). Sustainable Communities: Building for the future. London, ODPM. Available online at www.communities. qov.uk/ [Accessed 20/07/06].

Office of the Deputy Prime Minister (ODPM). (2003b). Housing Bill – Consultation on draft legislation, London: HMSO. Online. Available www.communities.gov.uk [Accessed 20/0706].

Smith S, Alexander A and Easterlow D (1997). Rehousing as a health intervention: miracle or mirage? *Health and Place*, 3 No. 4, 203-216.

Stewart J, Bushell F and Habgood, V (2005). *Environmental Health as Public Health.* London, Chadwick House Group Ltd.

Stewart J and Habgood V (work in progress) The benefits of a Health Impact Assessment in relation to fuel poverty: assessing Luton's affordable warmth strategy and the need for a national mandatory strategy.

Stewart J and Gray I (2005). Health and Housing, *Environmental Health Journal*, 113 (06) pp. 24-25. Available online at *www.ehj-online.com/archive/2000/june2005/june6.html* [Accessed 20/07/06].

Stewart J (2005). A review of UK housing policy: ideology and public health. *Public Health*, 119, 525-534

Taske N, Taylor L, Mulvihill C and Doyle N (2005). Housing and public health: α review of reviews of interventions for improving health. *Evidence Briefing NICE*.

Thomson H, Pettigrew M and Morrison D (2001). Health effects of housing improvement: systematic review of intervention studies. *British Medical Journal*, 323, 187-190.

Wills J and Woodhead D (2004). 'The glue that binds ...: articulating values in multidisciplinary public health'. *Critical Public Health*, 14, issue 1: 7-15.

Health and Fear: a study of the use of fear in promoting healthy behaviours among 18-25 year old students in relation to smoking.

Kevin Maguire¹ MSc BSc BA CPsychol MCIEH and Dr Kevin Love¹

¹ Division of Criminology, Public Health and Social Policy, Nottingham Trent University.

Correspondence: Kevin Maguire, York House, Mansfield Road, Nottingham NG1 33A, United Kingdom. Telephone +44 (0)115 848 5540. Email: kevin.maguire@ntu.ac.uk

Abstract

Fear has long been used as a technology of governance, often in a very crude form. In the modern governance of the public's health, however, it is argued that fear is deployed only paternalistically, to secure changes of behaviour that are clearly in the best interests of the individuals concerned Accordingly, fear continues to play an important role as a motivator in contemporary health communications, which now aim to 'market' healthy behaviour. In looking at the use of fear, the current paper begins with a short review of marketing processes, and social marketing in particular, and follows it with a theoretical introduction to evaluation. A multi-phased qualitative study is then described consisting of a focus group, a series of reflexive interviews, and two triangulating interviews; the first two phases included the showing to the participants of health promotion video clips on the effects of smoking. An analysis of data generated points to a psychological effort by the participants to distance themselves from the frightening contents of the video clips; it also identified a series of thought tactics used to achieve this. The theoretical and practical implications of the findings for devising health promotion messages are discussed.

Key words: environmental health; evaluation; public health; health promotion; social marketing

Authors' note

Qualitative research points up the role of the researcher in the research process accepting, in its reflexive nature, that different researchers affect the nature of the data in different ways (the matter is discussed further in the body of the text). In view of this fundamental principle, parts of the paper will be written in the first person (singular and plural), rather than the convention of third person accounts.

Introduction

Social Marketing

Mass-mediated health communication enjoys a history almost as long as the mass-media itself. From their earliest days, radio and television, for instance, have been used by the state to convey health information to the populace. Early health education films such as *Coughs and Sneezes* (National Archives, 1945), illustrate the simple fact that health communications of this sort were (and are) conceived as an attempt to influence individual behaviour, in order to protect the wider public health. Traditional mass-media health communications of this type, along with their contemporary equivalents, are often concept-

ualised methodologically using the metaphor of the "hypodermic": the public body, inoculated by an injection of information that dissuades individuals from engagements in risky health behaviours. The readiness of this clinical analogy is not without significance, and testifies to the powerful post-war trend toward medicalisation, even in the less obviously clinical arena of health education, which until recently all but eclipsed the broader social understanding of health. In these communications, it is as though the public body is itself conceived as a "physiological entity," where injection of carefully prescribed doses of information is expected to lead, in a "linear sequential" fashion (Hastings and MacFadyen, 2002), to the activation of specific processes within rational actors, from which follow the remediation of identified behaviours. The fact that many contemporary health communications perpetuate this approach (albeit with varying degrees of sophistication) is indicative of a continuing reliance on socially decontexualised methods of health intervention.

The recent trend toward the adoption of social marketing techniques in health communication aims to ameliorate some of the inadequacies of traditional, mass-media, health education in this regard. Social marketing has been defined as:

"the application of commercial marketing technologies to the analysis, planning, execution and evaluation of programmes designed to influence the voluntary behaviour of target audiences in order to improve their personal welfare and that of society" (Andreasen, 1995).

The principles of this method have, to some extent, implicitly underpinned health communication initiatives from the beginning. Nonetheless, these principles remained unexplored and undeveloped until the 1970s, when Kotler & Zaltman (1971) first coined the term. In brief, social marketing conceives health communication along commercial lines. Proponents argue that the highly successful, promotional marketing techniques, which have been used to such obvious effect in encouraging certain purchasing behaviours, can similarly be used to encourage desirable health and social behaviours. The method appropriates the '4 Ps' of marketing practice and redefines them in line with its own agenda.

The first of those Ps, product, might thus relate to a physical, health-related article (a condom for instance), but might equally refer to health services (e.g. screening), health practices (e.g. breastfeeding), or indeed, less tangible ideas about lifestyle and identity. *Price* can similarly

Kevin Maguire and Dr Kevin Love

be a simple monetary cost, but more commonly refers to the time, effort or perceived level of "sacrifice" a particular health behaviour requires. The perceived level of social risk is also an important factor in this respect, especially where particular behaviours touch upon issues of identity. The third P, *place*, rather misleadingly refers to the means employed to bring the product to the individual, which for the purposes of mass-media health communication comprises mediating channels such as broadcasting, newspapers and the like. Finally, promotion relates to the mode and scale of visibility appropriate to the communication. The essence of the social marketing technique is to tailor the communication to maximum effect for a given "audience." To do so, it utilises a range of demographic (gender, age, ethnicity, cultural factors, socioeconomic status, sexual preference, level of education), psychographic (aspirations and lifestyles, neophobia and risk aversion, cultural role-models, degree of social integration/ introversion) and geographic data. On the basis of this data, the broad heterogeneous audience is segmented into smaller homogenous units, and the communication then developed in a targeted way. Musham and Trettin (2002) provide an example relating to the promotion of breast-feeding in the low-income, Hispanic population of South Florida. Having previously established the target group's perception that "the primary benefit of motherhood is being loved by the child" (Musham and Trettin, 2002: 284), the television commercials utilised during the intervention emphasised how breast feeding strengthened the mother-infant bond. It takes little analytical prowess to appreciate the fact that social marketing is able to generate significantly more coercive force than traditional, broad-brush methods of mass-media health education.

The UK has recently witnessed television campaigns of a similar style in respect of smoking, clearly targeted at a specifically segmented audience of 18-35 year olds. In the first (NHS, 2005a), a girl is rejected by a prospective suitor under the unremittingly direct strapline, "If you smoke, you stink"; in the second (NHS, 2005b), a rather limp-looking cigarette butt masquerades as a penis, while the commentary informs the listener of the relationship between smoking and impotence. Neither of these cases intends to provoke a consideration of the health risks associated with smoking. Instead, they exploit what is undoubtedly a culturally more important issue for the target audience, the better to sell the product: quit smoking — you will have a more successful sex-life.

The consequent ethical issues are legion¹. For some, utilisation of coercive power in this way exceeds any

accepted notion of state paternalism, far surpassing the strictures of the "harm principle."² For others, the advanced marketing antics of, for instance, the tobacco industry (nicely exposed by a number of authors: Dewhirst & Sparks, 2003; Ernster et al., 2000; Gonzales et al., 2004) warrant similar action from the other side. Yet others, given the seriousness of the consequences of particular health behaviours, concentrate exclusively on questions of effectiveness and fail to even acknowledge that the issue of cultural coercion is at all significant (for example, Biener & Taylor, 2002). We lack the space to explore the panoply of emergent issues that attend the efforts of health communicators to now conceive of health in commercial terms. Concentrating instead on only one aspect of social marketing – the use of fear as a tool for behavioural change – the paper hopes to indicate that studies using rational cognitive processing models may indeed suggest that the persuasive power of massmedia communications increases with increasing arousal of fear (Biener & Taylor, 2002), but that such models are unable to capture the subtle interplay of a subject's conscious and unconscious reactions, which thus result in a more complex and equivocal pattern of response. In short, efforts to "bring the message home" with force and persuasion using fear-based conduits of communication, often result in the entrenchment of an underlying, psychological distance between the intended message and particular audiences. As a consequence, even those mass-media, health communications that employ social marketing techniques remain insufficiently contextsensitive, since they fail to account adequately for the nuanced complexity of target subject responses.

Evaluation

We consider this paper to be a form of evaluation since we are interested in an output (people's behaviours) following an input (the campaigns that use fear to promote health). There are, however, many theoretical pitfalls in thinking about evaluation, some of which we cannot ignore, especially within the frame of "social marketing", since essential to good marketing is good evaluation. Here lies the major challenge in evaluation, one which is encapsulated in the old joke of the marketing director who declares that 10% of her marketing effort works; "but I don't know which 10%".

Books on evaluation will list its types (see, for example, Pawson and Tilley (1997) or Patton M, 1997)) but it is helpful, for this study, to be aware of the major division into two: empirical (or instrumental) and theoretical (or

Health and Fear: a study of the use of fear in promoting healthy behaviours among 18-25 year old students in relation to smoking.

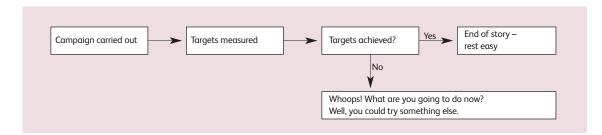


Figure 1.0Black box illustrating empirical evaluation

theory-based). The more empirical approach is a black box of inputs and outputs where the nature of the link between the two is not important (see diagram 1). In this approach, if a particular effort (e.g. healthy eating campaign) is regularly associated with a desired behaviour change (e.g. more vegetables are sold in the shops), then that is all there is to be concerned about. This kind of evaluation is sometimes referred to as goal-driven³ since the achievement of the goal is all that is required. It is the evaluation of our marketing director above. The evaluation of an alcohol drinking campaign among US college students by Clapp et al., (2003) exemplifies such an approach where their post-campaign evaluation found improved knowledge of alcohol drinking but found no changes in drinking behaviour other than increased drinking in one group. Their subsequent discussion in the paper was largely about how they had been very careful in the design and that, therefore, their results were genuine. Only one sentence of the paper contained any consideration of the "why?" question.

Despite the recency of the Clapp *et al.*, (2003) paper, most evaluators view the empirical/instrumental approach as belonging to a former age. Bonner (2003) labelled it as "traditional" and "naïve", and, referring to a 1999 publication, pointed out that the UK's National Health Action Zone Evaluation Team was not content

with this form of evaluation; rather they advocated theory based-evaluation "to promote policy and practice learning for future planning and development" (Bonner 2003:78). Theory-based evaluation tries to identify and explain the connection or mechanism between the effort and the outcome (see Figure 2.0). As its name suggests, this approach relies on various theoretical approaches to knowledge since it looks for theory that explains the link between the input and output. It answers the question implicit in our marketing director's statement, i.e. it tries to find which 10% of her marketing works and why. Put another way by Pawson and Tilley, it can help to explain "what works for whom in what circumstances?" (Pawson and Tilley 1997: 85 quoted in Van der Knaap 2004). Such an approach is particularly helpful where the targetbased evaluation tells us that our effort did not result in the desired outputs. Jason et al., (2003) exemplify theory-based evaluation in looking at the effects of enforcement activity (including fines) on under-age smoking in Illinois in the US. The campaign outcomes and explanations fitted together to make a useful addition to knowledge and understanding which would help with future campaign design and running.

Van der Knaap (2004) would appear largely to concur with much of the import of Pawson and Tilley (1997) (see below) but he does seem to distance theory-based

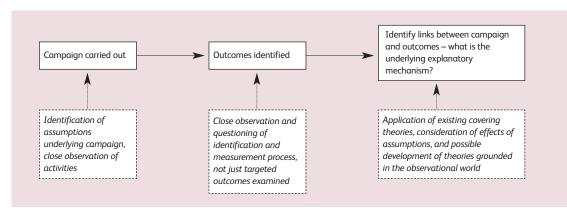


Figure 2.0 Theory-based evaluation

Kevin Maguire and Dr Kevin Love

evaluation from "the positivist ideal" (van der Knaap 2004:17). In contrast, Pawson and Tilley defensively write "evaluation needs another round of positivism bashing like a hole in the head" (1997: xv). There are other differences in their critical realist approach.

As critical realists, Pawson and Tilley (1997)⁴ have a particular perspective but they also take a most rigorous approach to evaluation theory. Their analysis of evaluation and its place in a knowledge framework (its epistemology) is very useful in considering notions of evaluation since they ask the question "what is the deepseated and underlying mechanism in this particular, observed regularity?" Within their own theoretical approach, they give examples of questioning, not only the use of regularities noted between inputs and outputs, but also the very nature of the observations and what they are thought to mean. In this way they can explain why the same campaign will work in one situation but not another. For them, the context is allimportant but for each local situation, their approach remains realist rather than idealist and thus might still be seen as looking from an empiricist vantage-point.

Study Method

Methodology

The process of getting hold of ideas and thoughts regarding the use of fear to promote health is, almost by definition, not an approach which lends itself to the use of number. Rather, in a survey of ideas, thoughts, and attitudes, a wider trawl is required which avoids preconceptions or attempts to drive people's thought patterns into a set of pre-determined categories. Thus, a qualitative interpretative approach became essential (see Maguire 1997). This does not deny the validity of the use of number; nor does it preclude its use from following on from this study (see Gouldner 1953). In the spirit of Strauss's notion of progressive focusing (see Atkinson 1979), and trying to answer the question "what is the main story here" (Wiseman 1974), a phased approach appealed, especially if the method used in each phase built upon the findings of the previous phase and method.

Within this qualitative approach, three phases (primary sorting phase, secondary focusing phase, tertiary confirmatory triangulation phase) were considered to be needed and three methods identified (focus group, interview, second interview).

- 1: The value of focus groups is increasingly recognised in gathering a pool of ideas (see Bloor 2000); for example, a wide variety of views can be brought together and be built upon from individual comments. Our study used it to identify potential areas to explore during the second (and main) phase of the study.
- 2: The interview is not one method but spans many styles from the most directive and structured (where specific closed questions are asked) to the reflexive and unstructured, the latter end of this continuum being more phenomenological and interpretative: in view of the needs of the study, the latter end was selected as of more use for the study.
- 3: While it might be overstating the situation to talk of hypothesis generation (at least in the normal scientised use of the word), the tentative nature of discovery or conjecture⁵ in any study (positivist or relativist, realist or idealist) is such that it should be subjected to some sort of testing. In our study, the possible knowings identified from the interviews (the "hypotheses") seemed best to be tested by their presentation to further members of the study area not previously concerned with the study. Such people, with some independence and freshness, could shine further light and give further view upon the validity of the ideas. Testing or examination from another angle in qualitative work is often referred to as triangulation, an analogy being drawn with the surveyor or geometrist who use an additional (vantage) point to confirm the location of the point of interest.

Method and materials

The study area consisted of all students between the ages of 18 and 25 at the Nottingham Trent University who regularly read their emails (see below). A multiphase qualitative approach was adopted in the following phases: a focus group consisting of 12 people; 10 interviews that had both a semi-structured part and an unstructured reflexive part; 2 final interviews used for triangulation.

For all three phases, self-selected volunteers were sought via several pan-university mails, which requested an hour or two of their time, offered a £20 gift voucher for a local shopping centre as a reward for taking part, and explained in simple terms the intentions regarding the study. Volunteers were then sent documents giving more detailed explanations of the intention and method of the study plus an explanatory consent form, which included assurances of confidentiality. This was signed by the volunteers and returned either before or, at the time of, the particular phase in which they were participating.

Health and Fear: a study of the use of fear in promoting healthy behaviours among 18-25 year old students in relation to smoking.

Focus group. The volunteers for this group consisted of three men and nine women. After initial explanations and further assurances of confidentiality, the group were shown, at intervals, a data-projected digital version of four public Government-sponsored information broadcasts, each lasting between 20 and 40 seconds down-loaded from the UK's Department of Health (DoH) Website (see DoH undated). After each an unstructured discussion was opened by a facilitator, the intention being to give voice to and identify as many as possible different thoughts and ideas held by the focus group members about health and fear. After showing all 4 broadcasts, a more general discussion was held about health and fear.

The proceedings of the focus group were audio-recorded via a ceiling suspended microphone onto standard audio-tape and were then transcribed by an audio-typist employed specifically for the purpose. The transcription was used to identify any themes running through the discussions. In turn, the themes (identified in the results section) fed into the next phase.

Main interviews. 10 interviews (of four men and six women) were carried out, each lasting about 40 minutes, in a neutral area (university meeting rooms containing only table and chairs) with the interview being recorded onto mini-disc. Before the recorded interview began, the interviewee was reminded of the confidentiality of the interview and therefore the importance of not giving their name or other identifying aspects of their life (e.g. the name of a school or a workplace). They were also reminded that they could terminate the interview at any time that they wished without losing their entitlement to the £20 gift voucher. There were five parts to the recorded interview, namely: an additional reminder of confidentiality; an invitation to give a short (about two minutes) summary of the interviewee's career; an unstructured reflexive part exploring the interviewee's schema and attitudes towards health, fear and the use of fear to "promote" health; a semi-structured section which centred upon the themes identified in the focus group; a closing opportunity for the interviewee to revisit previous parts of the interview or to make any additional comments.

For technical reasons, each mini-disc recording was transferred to conventional audio-tape, which was then used by the typist to transcribe the interview. From the transcriptions the research team again looked for regularities across the separate interviews (see results and discussion).

Confirmatory Interviews. Two confirmatory interviews (of a man and a woman) were held in a method similar to that of the interviews above with a more structured content aimed at exploring the validity of the findings from the main interview phase.

Findings

The use of fear in promoting health, in this study, would seem to be less effective than would have been expected, as will become apparent in this part of the paper where the emergent ideas are discussed without attempting to set them into a theoretical context; this will come later.

Focus group themes

The findings from the focus group are not the main concern of the current paper, only the emergent themes which are pursued in the semi-structured section of the next (and main) phase of the study. The themes were: use of statistics, use of children, use of real people rather than actors, feelings of disgust and fear, aesthetics and beauty.

Main and confirmatory interview emergent themes

The following themes were noted by the researchers:

- Notions of fear
- Notions of health
- Fear about health and reactions to that fear
- Other emotions involved as identified by interviewees
- Ethical considerations regarding the use of fear

These themes are elaborated further below and used to develop a proto-model intended to describe the emergent conceptual structures:

Notions of fear

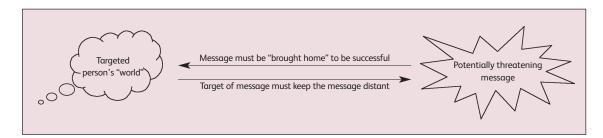
Feeling fear was best articulated by an interviewee as follows:

"Fear ... is wanting not to be there ... if you can get away, you will, or deal with it in some way. The feeling, the actual feeling in the body is perhaps is one of – er – being completely on edge and like wanting to get away and perhaps in some circumstances, not wanting to let yourself down and – er – deal with that, deal with that fear ..."

Some variation was shown in the response of others with associated feelings including shock, being scared, being frightened, being on edge, cringing, and feeling numb. There was not universal agreement, however; for example

Kevin Maguire and Dr Kevin Love

Figure 3.0
Proto-model of use of tactics used by interviewees to distance themselves from a health message



one of the triangulating interviewees considered that "numbness was more of a sensation" rather than feeling fear. This interview also placed the terms on a continuum as follows (mildest first) on edge – numbness – shock – frightened – scared. While such an ordering may not accord with others, it is helpful for evaluating the effects of the use of fear (see later).

Notions of health

It is not surprising that, from a pool of interviewees which included students studying a health-related topic, some of the replies regurgitated the World Health Organisation (WHO) definition of health (Ewles and Simmett1992) but further probing soon dissolved such a coherent face. Health, for many, reverted back to not being unhealthy and then to unhealthy behaviours including smoking, drinking alcohol, and poor diet. The nearest that interviewees came to a positive aspect to health was in their use of the word "fitness". For one interviewee this became synonymous with sport and then linked to the "health-fitness-beauty" triad and thence to ideas of disqust (see later).

Fear about health and reactions to that fear

Linking the two not only illuminated interviewees' understandings of the two separate ideas further but also showed how they were linked in their minds. Of use here was an interviewee who saw fear as not being able to move or act. This person talked of finding a lump on their body when they were 17 and their being "too frightened to do anything". The reactions to the fear were both distancing (see proto-model below) and annoyance: "you can't do that really; it's not your place to make me feel like that".6"

Other emotions involved

As well as fear, other emotions that interviewees considered to have been used in health messages were: shock, disgust and revulsion, shame, and loneliness. While seen as separate from fear, all were seen as negative.

The ethics of using fear

Participants generally felt uncomfortable, with the use of fear; for example:

"I felt it was very er, like I was being manipulated".

An ambivalence arose, however, in that while a respondent thought it was wrong to use fear generally in selling commodities, it was legitimate where health was concerned. For one interviewer, his discovery of his ambivalence surprised himself:

"... I'm sounding like a Marxist here. If awareness should be briefed so people are looking after themselves and leading better lives, then, I thought I'd never say that, but erm perhaps it's a good idea, but I mean the fear factor of it ... I don't know if people should be imposed, there should be an imposition on people to make them scared to stop them doing something which erm an expert says they shouldn't be doing..."

Proto-model

The model developed from the interviews centres on distance (see Figure 3.0). If we see a space between the individual and a potentially threatening message, then the person will try to distance themselves from the threat, i.e. open up the space. The challenge, in the words of another interviewee, is for the promoter to

"... bring it [the message] home ...".

Distancing techniques

Techniques that create distance between an individual and the health message (the perceived threat) were also recognised by interviewees as being used by health messages to reduce that distance. Within the strategy of distancing, the techniques employed were mainly the making of attributions to the message which removed the relevance of the message to the individual. They are summarised in Table 1.0.

Health and Fear: a study of the use of fear in promoting healthy behaviours among 18-25 year old students in relation to smoking.

| Theme | Tactic | | | | |
|---|---|--|--|--|--|
| Television related | They are similar to advertisements: we ignore Ignoring advertisements in general so it easy to ignore those with health messages (3.11) They are often intermingled with other advertisements They can be got rid of by changing the TV channel TV makes anything it transmits unreal (it is often a medium of fiction) | | | | |
| Making of negative attributions regarding the messages: | They are not real The advertisements are not very good advertisements The situations presented are not normal The people in them are old The messages are manipulative and therefore unethical/immoral (and therefore should be ignored) | | | | |
| Others reasons to distance the message | Smoking is comforting: we don't want that comfort disturbed If they stopped people smoking, the Government would lose the tax revenue upon which it depends. Smoking is a habit Smoking has other associations: it is part of a set of activities (e.g. food, drink, and smoking or smoking after sex). | | | | |

Table 1.0Summary of thought tactics used by interviewees to distance themselves from a health message

For one of the triangulating interviewees, habit was seen as something far more powerful than, and different to, creating distance. Another who saw himself as "addicted" did, however, use the distancing technique by seeing his addiction as being taken advantage of:

"just cos I am addicted, you see".

According to the interviewees, health promoters need to shorten that distance by "bringing the message home", an expression used by one of the interviewees which encapsulates the idea of reduction of distance: the message has to mediate the full length of the space between person and message in Figure 3.0. "Bringing the message home" involves working on the boundaries and helping to reshape not only behaviour but, depending on the degree to which the health message accords with the individual, may need to change mental schemata, perhaps having to address deep-seated unconscious aspects of the person. Doing this, however, may not always work in the desired way (see below in discussion). Some of the techniques suggested by interviewees are listed in Table 2.0.



Discussion

The model identified by the interviewees was an implicit psychological model, i.e. it aimed to get a message from the transmitter to each individual's mind with the premise that this would change their behaviour – if it reached home. The implicit model could be interpreted in a variety of cognitive models such as Festinger's (1957 see, for example, Hayes 1994) famous cognitive dissonance model where the dissonance between two cognitions (thoughts)⁷ create such discomfort that one has to change; either to give up smoking or to deny the message. Other psychological models, upon which

Table 2.0Ways suggested by interviewees that might "bring the message home"

Kevin Maguire and Dr Kevin Love

marketers (and presumably social marketers) tend to rely, involve a causal link where thought and or feeling drive behaviour as in traditional attitudinal models (something questioned by various authors, e.g. Ajzen and Madden 1986). Such is the approach in rational choice models, where a knowledge message is supposed to lead to a change in behaviour. It is also the basis for attempts to change feelings in order to change behaviour and for the use of short-term changes in behaviour with the hope that they change thoughts and feeling and thence to longer-term changes in behaviour.

Even psychological models however can point up problems with the distancing - bringing home opposition. Anna Freud's (see Stevens, 1983) models have a special place for resistance and how they are formed and used; these, in particular, explain the problem of 'comfort' removal. Sigmund Freud's concept of *Thanatos*⁹ (Stevens 1983) can also explain activities that result in harm; coming from the unconscious, this drive is particularly difficult to identify and resolve. Sometimes described as an economic model, the constituents of the Freudian model of the psyche may move but the overall energy (the libido) remains the same. Bearing in mind these psychodynamic models, our interviewees provide evidence contrary to the hypodermic model (see above). Thus rather than having enough information injected into a person to inoculate her or him against the undesired behaviour, the 'affective charge'10 it initiates is subverted into building up defence against the initiant force (here the health message).

Of course an individual model is not the only approach to promoting health. Indeed, a more socially embedded approach would appear to overcome many of the problematic responses to health communication highlighted here. A wider social model would accordingly consider not simply the health communication message, but also issues concerning empowered choice and the relative economic power of the various actors (not only the resourcing of promotion activities but also such matters as the power of tobacco companies).

Conclusion

The study has looked at artefacts and outcomes in a campaign to encourage people to quit smoking. It has identified a largely individualistic approach to the desired outcome and also that it often falls at an early hurdle, even within its own methodological approach. While the approach may induce fear, it may not have the desired change in behaviour. If this is the case, it will not

be the first time that a health promoter has damaged rather than helped his or her charge: the history of medicine is often a history of dangerous quackery.

The immediate implication from the study is that care has to be taken when designing and delivering messages so that a counter and resistant psychic force is not induced by the message and its medium. It was heartening to find from the research that the appeal to altruism, inspired by the video clip of children 'smoking', had an effect on smokers and non-smokers alike.

The study does not, however, overcome the important emergent issues of dealing with the habituated addicted smoker through these campaigns and, more fundamentally, redressing the strong social/political oppositions to quit smoking in the form of the tobacco industry's efforts to promote smoking. The ethical issues of what is acceptable in campaigns to reduce unhealthy behaviour also remain unresolved.

Finally, while some parts of public health have only recently become aware of social marketing as a concept (see for example Spear, 2005), this preliminary study throws up questions about an individualistic approach to promoting health; an approach found by the Royal Society (1993) in its report on risk not to have delivered. Rather, they advocated more social and cultural avenues to explore in changing behaviours and outcomes. Such an approach, characteristic of the health development movement, requires a more serious examination by all public health practitioners.

Acknowledgements

We would like to acknowledge the great help given to us in this study by our colleague Andrea Lyons, also from Nottingham Trent University, who organised the successful call for volunteers, helped to facilitate the focus group, and interviewed some of the participants.

We would also like to acknowledge the financial support given to the study by the Chartered Institute of Environmental Health via its research funding initiatives.

Notes

- ¹ Musham and Trettin (2002) consider a number.
- ² In his famous, On Liberty, JS Mill establishes what has

Health and Fear; a study of the use of fear in promoting healthy behaviours among 18-25 year old students in relation to smoking.

become known as the harm principle. Under this principle, if an agent is both competent (i.e. an adult in full control of his/her faculties) and sufficiently well informed, coercion to prevent that agent choosing to engage in behaviour that risks self-harm cannot be justified.

- ³ And no doubt those goals should be as the management books tell us SMART, i.e. Specific, Measurable, Achievable. Reasonable. and with Time scales.
- ⁴ In fact he refers positively to their work on several occasions.
- ⁵ Hence the 'hypo' prefix in hypothesis.
- ⁶ Such annoyance might, of course, also be a thought tactic for distancing themselves from the message see below.
- ⁷ Here between the implications of a health message (the need to give up smoking) and enjoyment of the current activity (the pleasure etc. from smoking).
- ⁸ The latter being exemplified by newspaper campaigns involving free newspapers to build the habit.
- ⁹ Sometimes reduced to "death wish."
- ¹⁰ i.e. a "charge" attached to particular ideas (Evans and Tsatsaroni 1996). This is also referred to as cathexis which Stevens translates as "psychic energy invested in a desire, relationship or object" (Stevens 1983: 48).

References

Ajzen, I and Madden, T J (1986). Prediction of goal-directed behaviour: Attitudes, intentions and perceived behavioural control. *Journal of Experimental Social Psychology*, 22, 453-474.

Andreasen, AR (1995). Marketing social change: changing behaviour to promote health social, development, and the environment. San Francisco: Jossey-Bass Publications.

Atkinson P (1979). Research Design in Ethnography, Milton Keynes, Open University Press.

Beiner, L and Taylor, T M (2002). The continuing importance of emotion in tobacco control media campaigns: a response to Hastings and MacFadyen, *Tobacco Control*, 11, 75-77.

Bloor M, Frankland J, Thomas M, Robson K (2000). Focus groups in social research, London, Sage Publications Ltd.

Bonner L (2003). Using theory-based evaluation to build evidence-based health and social care policy and practice, *Critical Public Health*, 13(1): 77 – 92.

Clapp JD, Lange JE, Russell C, and Shillington A, 2003, A failed Norms Social Marketing Campaign, *Journal of Studies on Alcohol*, 64(2): 409-414.

Dewhirst, T and Sparks, R (2003). Intertextuality, tobacco sponsorship of sports and adolescent male smoking culture: a selective review of tobacco industry documents, *Journal of Sport and Social Issues*, 27(4), 372-398.

Ernster, V, Kaufman, N, Nichter, M, Sarnet, J & Yoon, SY (2000). Women and tobacco: moving from policy to action, *Bulletin of the World Health Organisation*, 78, 7.

Evans J and Tsatsaroni A, 1996, Linking the cognitive and the affective in educational research: cognitivist, psychoanalytic and poststructuralist models, *British Educational Research Journal*, 22, No. 3, June 1996, pp.347 – 358.

Ewles L. and Simmet I, 1992, Promoting Health: A Practical Guide, London, Scutari Press.

Gonzales, R, Glik, D, Davoudi, M, Ang, A (2004). Media literacy and public health, *American Behavioural Scientist*, 48(2), 189-201.

Hastings G and MacFayden L (2002). The limitations of fear messages, *Tobacco Control*, 11, 73-75.

Hayes N, 1994, Foundations of Psychology, London, Routledge.

Jason LA, Pokorny SB, and Schoeny ME, 2003, Evaluating the effects of enforcements and fines on youth smoking, *Critical Public Health*, 13(1): 33 – 45.

Kotler P, Zaltman G (1971). Social marketing: an approach to planned social change, *Journal of Marketing*, 35, 3-12.

Kevin Maguire and Dr Kevin Love

Maguire K, 1997, "A qualitative study of attitudes and perceptions of Environmental Health towards people with mental illness and learning difficulties", *International Journal of Environmental Health Research*, 7(3), 203-214.

Musham, C and Trettin, L (2002). Bringing health services to the poor through social marketing: ethical issues, *Journal of Health Care for the Poor and Underserved*, 13(3), 280-287.

National Archive (1945). Coughs and Sneezes. Available online at www.nationalarchives.gov.uk/theartofwar/prop/home-front/INF3-0407J.htm [Accessed 18/07/06].

National Health Service (NHS). (2005a). Too Good to be True, available at: *www.givingupsmoking.co.uk/CNI/Current_Campaign/advertisements/tv_ads/*, [accessed: 10/10/05].

National Health Service (NHS) (2005b). Fingerlegs, available at: www.givingupsmoking.co.uk/CNI/Current_Campaign/advertisements/tv_ads/, [accessed: 10/10/05].

Paton M (1997). Utilization-Focussed Evaluation, London, Sage Publications Ltd.

Pawson R and Tilley N (1997). Realistic Evaluation, London, Sage Publications Ltd.

Royal Society (1993). Risk: analysis, perception and management/report of a Royal Society study group. London, Royal Society.

Stevens R (1983). Freud and Psychoanalysis, Milton Keynes, The Open University Press.

Spear S (2005). Learning from the Behemoths, *Environmental Health Practitioner*, 113(10), 10-12.

Van der Knaap P (2004). Theory-based evaluation and learning: possibilities and challenges, *Evaluation*, 10(1): 16 – 34.

Wiseman J P (1974). The research web, *Urban Life and Culture*, 3, 317-328.

Evaluation of extraction methods for the isolation of dust mites, bacterial, and fungal PCR-quality DNA from indoor environmental dust samples: A new scope for indoors research

Dr Edu B Suárez-Martínez¹, Miss Maryliz González-Santos², Dr Federico Montealegre³

- ¹ Department of Biology, University of Puerto Rico in Ponce, Ponce, PR
- ² Department of Chemistry, Pontifical Catholic University of Puerto Rico, Ponce, PR
- ³ Department Microbiology, Ponce School of Medicine, Ponce, PR

Correspondence: Edu B Suarez-Martinez, University of Puerto Rico in Ponce, HC 08 Box 243, Ponce, Puerto Rico 00731. Telephone: (787)844-8181 ext.2365, Email: edu6@yahoo.com

Abstract

Dust mites, bacteria, and fungi are ubiquitous organisms found in household dust and have been related to the development of three types of human diseases: hypersensitivity responses (allergic reactions), infections, and toxicosis of the respiratory system. Sometimes, the presence of these organisms cannot be detected by typical methods, which prevents an effective and accurate identification of these pathological sources.

An effective isolation of PCR-quality DNA from indoorsenvironmental samples will allow employing the PCR technology as an indoor detection technique for these biological sources. Several DNA extraction methods have been employed with soil or sediment samples, but their DNA products usually contain organic contaminants, polysaccharides, proteins, and tannins that inhibit the PCR amplification.

We compared for the first time, seven DNA extraction methods to identify the most efficient protocol for obtaining dust mites, bacteria and fungi PCR-quality DNA from indoor settings. The SoilMaster™ DNA Extraction Kit showed a highly sensitive, reproducible and fast (less than an hour) method for obtaining PCR-quality DNA from dust mites, bacteria, and fungi when compared with the other methods studied. An added modification (sample homogenization) to this kit positively influenced the dust mites' mitochondrial DNA extraction by increasing its ratio over proteins. By using the DNA extraction and PCR approach in indoors-dust samples, non-cultivable indoor biological contaminant organisms can also be identified, contributing to an accurate diagnostic test.

Key Words: Bacteria, DNA extraction; dust mites, environmental health; fungi; indoor-dust.

Introduction

Several extraction methods for the isolation of DNA from various sources (e.g. cells, tissue, and plants) have been extensively described in past literature (Sharma, et al., 2003; Laird, et al., 1991; Tkach and Pawlowski, 1999). Among the most commonly employed methods are the CTAB (cethyltrimethylammonium)(Doyle and Doyle, 1987) and its modifications (Huang, et al., 2000), and the Phenol/Chloroform extraction using several lysis buffers (SDS, Triton X100). However, when these methods are employed on soil or sediment samples, the DNA product usually contains organic contaminants

such as humic and fulvic acids (Tebbe and Vahjen, 1993). These components can act as inhibitors in the DNA amplification by polymerase chain reaction (PCR), and false negative results may be obtained (Morera, 2001). In addition, these extraction methods are not efficient in obtaining PCR-quality DNA from samples that are inherently rich in polysaccharides, proteins, and tannins (e.g. bacteria, arthropods and fungi) (Krause, et al., 2001). To achieve PCR-quality DNA from these sources, further purification protocols must be performed (Braid, et al., 2003). These procedures result in extensive processing time and a high cost per sample. Some methods have overcome the limitations inherent in these protocols and are capable of extracting PCR-quality DNA of bacterial and fungal origin from soil and sediment samples (Yeates, et al., 1998; Burgmann, et al., 2001; Krsek and Wellington, 1999), as well as fungal DNA from indoor air settings (Haugland, et al., 2002). However, in the current literature, there is no description of an extraction method that can be used on indoor-collected dust samples for obtaining PCR-quality DNA from bacteria and/or domestic mites. These organisms (bacteria, fungi, and domestic mites) are consistently found world wide in households dust and have been related to the development of allergic diseases such as asthma, rhinitis, and atopic dermatitis, infections, and toxicosis at the respiratory system (Arlian and Platts-Mills, 2001; Nelson, 2001; Menetrez, et al., 2001; Meis, 2002). Certain species of these agents are also known to sensitize some individuals to other diseases (Foster, et al., 2003).

In this project, we compared for the first time, seven DNA extraction procedures (the Epicentre SoilMaster™ DNA Extraction Kit protocol, a modification to the Epicentre SoilMaster™ DNA Extraction Kit protocol, QIAmp DNA Mini Kit, CTAB, and three modifications of Phenol/Chloroform protocol) to obtain PCR-quality DNA of dust mites, bacteria, and fungi from indoor environmental samples. We intended to identify the most efficient, simple, and least time consuming protocol. The generation of PCR-quality DNA from indoor environmental samples will allow the use of PCR technology as an innovative detection tool of these organisms independently of their cultivable status.

Experimental procedures

Indoors sample collection

Using a hand held vacuum cleaner (Medivac, England), dust was collected from 10 mattresses at a rate of 2 m²/min during three minutes. The vacuum cleaner's nozzle was equipped with a $300\mu m$ stainless steel mesh

Dr Edu B Suárez-Martínez, Miss Maryliz González-Santos, Dr Federico Montealegre

and a 50µm diameter filter. Approximately 1g of dust was collected from 10 different locations and each sample was placed in sterile plastic bags. The 10 individual samples were mixed in a sterile plastic container in order to obtain a pooled dust sample. Eightyfour samples (12 per method, 100mg each and selected randomly) were weighed in 2ml Eppendorf sterile tubes and stored at -20°C until DNA extraction.

DNA Extraction

Total DNA from indoor dust samples (100mg each) was extracted using seven DNA extraction protocols and 12 repetitions per method:

- Epicentre SoilMaster™ DNA Extraction Kit (EpiN): Protocol was followed as described in Meis and Chen (2002).
- 2. Epicentre SoilMaster™ DNA Extraction Kit with modification (EpiH): At step 2 (according to the manufacturer's manual), after the addition of the Soil DNA Extraction Buffer, a 30 seconds homogenization procedure was included and then the protocol was continued, by following the remaining steps as described by the manufacturer.
- 3. QIAGEN® (QIAmp DNA Mini Kit): DNA extraction was performed following the manufacturer's instruction manual.
- CTAB (cethyltrimethylammonium bromide): A volume of 500 μL of 1% CTAB, 50mM Tris-HCl, pH 8.0, 10mM EDTA, 0.7 M NaCl, 0.1% 2-mercaptoethanol, was added to the sample and incubated at 60°C for one hour. Chloroform/isoamyl alcohol (700 μL) was added; samples were mixed gently and

thoroughly and spun at 14,000 rpm for 10 minutes. The upper aqueous phase was transferred to a new tube and the process was repeated. Precipitation was done with 1X volume of isopropanol, followed by 2X ethanol and 1/10 3M Sodium Acetate (pH 5.2). The samples were stored at -80°C overnight followed by a 15 minutes maximum speed centrifugation at 4°C. Supernatant was removed.

- Phenol/Chloroform: Samples were treated with 200 µL of three different lysis buffers (B1, B2 and B3), homogenized for 30 seconds, and spun briefly. A total of 200µg of Proteinase K were added to the samples, followed by three hours incubation at 64°C. Saturated phenol (500µl) was added and mixed by inversion. A maximum speed centrifugation for 15 minutes at room temperature was performed. The upper aqueous phase was transferred to a new tube and chloroform (500 μ l) was added, followed by 15 minutes of centrifugation (RT). The upper phase was retained in a new tube and treated with 2.5X volume ethanol and 1/10 volume of 3M sodium acetate. The samples were stored at -80°C overnight followed by a 15 minutes maximum speed centrifugation at 4°C. Supernatant was removed. Components of the three lyses buffers were:
 - a. Buffer 1 (B1): 10mM Tris-HCl, 320mM sucrose, 5mM MgCl2, 1% Triton X100
 - b. Buffer 2 (B2): 100mM Tris-HCl, 100mM EDTA, 0.25 % SDS
 - Buffer 3 (B3): 50mM Tris, pH 8; 100mM EDTA, 100mM NaCl, 1% SDS

Table 1.0Primer sets
sequences and
expected fragment
size employed in
PCR of DNA
from indoorsenvironmental
samples

| Organism Group | Primer Pair | DNA sequence | Product Size (bp) |
|-------------------------------------|--------------|---|----------------------|
| Arthropods | 12SF 12SR | 5'-TACTATGTTAGCACTTA-3' 5'-AAACTAGGATTAGATACCA-3' | ~400 |
| Fungi, Protists, and Green Algae | NS1 NS2 | 5'-GTAGTCATATGCTTGTCTC-3' 5'-GGCTGCTGGCACCAGACTTGC-3' | 555 |
| Bacteria consensus | P4 P5 | 5'-AACGCGAAGAACCTTAC-3' 5'-CGGTGTGTACAAGGCCCGGGAACG-3' | 450 |

Evaluation of extraction methods for the isolation of dust mites, bacterial, and fungal PCR-quality DNA from indoor environmental dust samples:

A New Scope for Indoors Research

The air-dried precipitated DNA from all seven protocols was re-suspended in $25\mu L$ TE (Epicentre SoilMasterTM DNA Extraction Kit). Further purification of $200\mu l$ from the CTAB, B1, B2 and B3 DNA extractions was performed using the QIAmp DNA Mini Kit (QIAGEN®) following the manufacturer's instructions: CTABP, B1P, B2P, and B3P.

Absorbance ratios

Absorbance ratios (A260/A280) were determined by a GeneQuant Spectrophotometer (Pharmacia Biotech.). Two readings per sample were performed, the mean value per tube was calculated (84) and then the mean values for each method (12) were used to construct a comparison table (Table 2.0).

PCR amplification and Gel electroforesis

PCR from the DNA of dust mites, fungi, and bacteria was performed using a Triple Master Kit (Eppendorf Co.). A PCR reaction volume of 20µl: 5.0µl dust DNA, 0.5µl of each 10mM primer (Table 1.0), 3.2µl of 10x High Fidelity Buffer with Mg²+, 0.4µl of dntp's mix (10mM each), 0.3µl of Triple Master Enzyme Mix (1.5U), and 10.1µl of molecular grade distilled water. PCR conditions were set in an Eppendorf Gradient Mastercycler as follows: denaturation for 5 min at 94°C and 30 cycles of amplification using a step program of 20 sec at 94°C, 20 sec at 38.4°C (12S) or 52.0°C (P4/P5 and NS1/NS2), and 5 min at 72°C. This was followed by a final extension of 10 min at 72°C and held at 4°C. PCR products were stored at -20°C until electrophoresis was performed in a 3% agarose gel. PCR products were visualized under UV light by using ethidium bromide.

Results

The quality of the seven DNA extraction methods and purifications were evaluated by comparing their absorbance ratios (A₂₆₀/A₂₈₀) (Table 2.0). The obtained A₂₆₀/A₂₈₀ ranged from 0.5770 (CTAB) to 2.1545 (EpiH). The lysis buffers B1, B2, B3, and CTAB experienced an increase in their ratio after the purification was

performed: B1 from 1.1885 to 1.3695; B2 from 0.9305 to 1.6065; B3 from 1.1675 to 1.5305; and CTAB from 0.5770 to 1.6300 (Table 2.0).

A successful DNA amplification of dust mites, bacteria, and fungi was observed only with the use of the Epicentre SoilMaster DNA Extraction Kit: EpiN and EpiH (Figures 1.0, 2.0 and 3.0). The homogenization step, included as a modification of the Epicentre SoilMaster DNA Extraction Kit, had a positive influence in the DNA yield of dust mites (Figure 3.0) but no changes were observed in the other organisms (Figures 1.0 and 2.0).

Discussion

Frequently, there are remaining proteins present in the DNA solution, when it is isolated. These proteins are tightly bound to DNA and may interfere with the PCR. To determine the concentration and purity of a DNA solution, the absorbance of UV light is measured in a spectrophotometer. Both proteins and DNA absorb UV light, but they have different absorbance curves. The peak of light absorption is at 260nm for DNA and at 280nm for protein. By dividing these two absorbance values, one can calculate the purity of the DNA solution or the absorbance ratio (A260/A280). Accordingly, a pure sample of DNA substantially free of protein will have a high A260/A280 (between 1.9-2.1).

We noticed an increase in the (A₂₅₀/A₂₅₀) ratio when the purification was performed on the DNA obtained from the three phenol/chloroform (B1-B3) and the CTAB extractions. Although this finding suggests higher purity of the DNA after the purification step in these extractions, the Epicentre SoilMaster™ DNA Extraction Kit (EpiN and EpiH) processed samples showed not only higher A₂₅₀/A₂₅₀ when compared with all other methods, but also values closer to optimal range: EpiN: 2.0575 and EpiH: 2.1545. At this stage, the results pointed to the Epicentre SoilMaster™ DNA Extraction Kit as being the

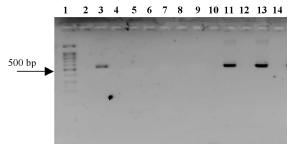
Table 2.0
Average absorbance ratios (A₂₆₀/A₂₈₀) from DNA samples after seven DNA extraction treatments and purification treatments.

| Sample | B1 | В1Р | В2 | B2P | В3 | ВЗР | СТАВ | СТАВР | Q1AGEN | EpiN | ЕріН |
|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| A260/A280 | 1.1885 | 1.3695 | 0.9305 | 1.6065 | 1.1675 | 1.5305 | 0.5770 | 1.6300 | 1.1705 | 2.0575 | 2.1545 |

B1: Buffer 1 of Phenol Chloroform extraction; **B1P**: Buffer 1 of Phenol Chloroform extraction purified; **B2**: Buffer 2 of Phenol Chloroform extraction; **B2P**: Buffer 2 of Phenol Chloroform extraction purified; **B3**: Buffer 3 of Phenol Chloroform extraction purified; **CTAB**: cethyltrimethylammonium bromide protocol for PCR; **CTABP**: cethyltrimethylammonium bromide protocol for PCR purified; **QIAGEN**: QIAmp DNA Mini Kit; **EpiN**: Epicentre SoilMasterTM DNA Extraction Kit; **EpiH**: Epicentre SoilMasterTM DNA Extraction Kit with homogenization step.

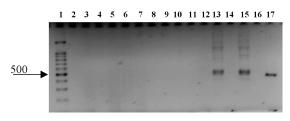
Dr Edu B Suárez-Martínez, Miss Maryliz González-Santos, Dr Federico Montealegre

Figure 1.0
PCR amplification of indoors-dust sample DNA: ~555 bp fragment, amplified with the NS1/NS2
Fungi, algae and protists universal primers.



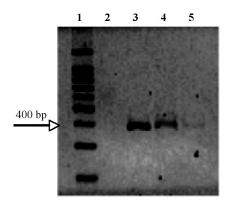
Primers: 3 % agarose gel, ethidium bromide stained: Lane 1: LMW marker; Lane 2: Negative control (water); Lane 3:Positive Control (Penincillum DNA); Lane 4: Empty; Lane 5: B1P; Lane 6: B2P; Lane 7: B3P; Lane 8: Empty; Lane 9: CTABP; Lane 10: QIAGEN; Lane 11: Epicentre SoilMasterTM homogenized (EpiH); Lane 12: Empty; Lane 13: Epicentre SoilMasterTM non-homogenized (EpiN); Lane 14: Empty.

Figure 2.0 PCR amplification of indoors-dust sample DNA: ~450-500 bp fragment, amplified with the P4/P5 Bacteria consensus primers.



Primers: 3 % agarose gel, ethidium bromide stained: Lane 1: LMW marker; Lane 2: Empty; Lane 3:B1; Lane 4: B1P; Lane 5: B2; Lane 6:B2P; Lane 7: B3; Lane 8: B3P; Lane 9: CTAB; Lane 10: CTABP; Lane 11: QIAGEN; Lane 12: Empty; Lane 13: Epicentre SoilMasterTM homogenized (EpiH); Lane 14: Empty; Lane 15: Epicentre SoilMasterTM non-homogenized (EpiN); Lane 16: Negative control (water); Lane 17: Positive Control (E. coli DNA); Lane 18: Empty.

Figure 3.0 PCR amplification with the 12S arthropods universal primers.



Primers: 3 % agarose gel, ethidium bromide stained: Lane 1 LMW marker; Lane 2: Negative control (water); Lane 3: Positive Control (2ÌI dust mite Dermatophagoides pteronissynus DNA); Lane 4: Epicentre SoilMasterTM homogenized (EpiH); Lane 5: Epicentre SoilMasterTM non-homogenized (EpiN).

more efficient method for extracting PCR-quality DNA from indoor environmental samples. However, the measurement of the A₂₆₀/A₂₈₀ has been argued by some studies, which stated that lower ratios do not always translate to non PCR-quality DNA (Glase, 1985).

To determine the verisimilitude of that statement, we performed PCR testing on all of the DNA that resulted from the extractions methods. Our analysis showed that the obtained A₂₆₀/A₂₈₀ ratio was directly related to the resultant success in the amplification of dust mites, fungal, and bacterial DNA. Only the samples processed with the Epicentre SoilMaster^{\mathbb{M}} DNA Extraction Kit: EpiN (with a ratio of 2.0575) and EpiH (with a ratio of 2.1545) showed positive PCR results when compared with the other tested methods (Figures 1.0, 2.0 and 3.0).

Although the CTAB A260/A280 showed a significant increase when the purification was performed, and it was the method that obtained an absorbance ratio closest to 2 after the Epicentre's (Table 2.0), no amplification was detected for any of the tested organisms (dust mites, fungi, and bacteria). These results contrasted with those of previous studies, which identified the CTAB method as the protocol of choice for fungal DNA extraction from clinical samples (Velegraki, et al., 1999) and indoor air samples (Haugland, et al., 2002). Similarly, bacterial DNA extractions have frequently been obtained from clinical samples and cultures using the phenol/ chloroform extraction and the QIAmp DNA Mini Kit, but these techniques didn't work on our indoor samples. These results may be explained by the presence of PCR inhibitors (Tebbe and Vahjen, 1993; Morera, 2001) in the dust, which were not efficiently removed by the employed purification method. It can also be the result of the limited amount of organisms present at indoor settings when compared to outdoors soil.

The slight augment in the A_{260}/A_{280} ratio between the EpiN and EpiH (Table 2.0) can be attributed to an increase in dust mite DNA (Figure 3.0). This is supported by the fact that no significant differences were observed in the PCR amplification of fungal and bacterial DNA when the homogenization was performed (Figures 1.0 and 2.0). We suspect that the homogenization step (EpiH) contributed to a physical breakage of the dust mites' chitin exoskeleton. This breakage allowed more inside cells to be exposed to the lytic reagents, increasing the availability of DNA and thereby its ratio over proteins.

Evaluation of extraction methods for the isolation of dust mites, bacterial, and fungal PCR-quality DNA from indoor environmental dust samples:

A new scope for indoors research

Conclusions

- The obtained ratios (A₂₆₀/A₂₈₀) concurred with the PCR results. Only the dust mite, fungal, and bacterial DNA with ratio values close to 2 were successfully amplified. Although an increase in the ratio was observed in the purified DNA samples, PCR-quality DNA was not achieved.
- We discovered in our study that except for in the case of the Epicentre, all other protocols (even after purification) failed in the amplification of dust mite, fungal, and bacterial DNA from indoor environmental samples. This failure may be explained by the presence of PCR inhibitors in the dust, which were not efficiently removed by the employed purification method or due to the limited amount of organisms. However, the Epicentre SoilMasterTM DNA extraction Kit seems to overcome this limitation.
- In this study, we identified for the first time an efficient, rapid (less than an hour), and sensitive method for the extraction of PCR-quality DNA from the dust mites, fungi, and bacteria that are found in indoor environmental samples: the Epicentre SoilMaster™ DNA Extraction Kit.
- Furthermore, this is the first report that describes DNA isolation of dust mites from indoor dust samples.
- We were also able to demonstrate an increase in the dust mite DNA yield when our homogenization modification was included to the standard Epicentre SoilMaster™ DNA Extraction Kit protocol.
- The generation of PCR-quality DNA from indoor environmental samples of dust mites, bacteria, and fungi, will allow us to employ the PCR technology as an innovative detection tool.
- This new approach may contribute to the detection and quantification, as well as to the identification of different populations of these ubiquitous organisms that cannot be differentiated by typical methods such as microscopy, culturing, and antigen-antibody tests.

Acknowledgments

We appreciate the technical assistance provided by Miss Elizabeth Rivera (PSM Mol Biol Core), Miss Magda Rolón, and Miss Jancy Acosta (PSM) and financial support by NIH grants 2G12RR0030500-18, #P20RR16470, #T42/CCT21042, #S06GM08239 and #5-T34-GM07732.

References

Arlian, L G and Platts-Mills T A (2001). The biology of dust mites and the remediation of mite allergens in allergic disease. *Journal of Allergy and Clinical Immunology*, 107(3 Suppl), S406-S413.

Braid M D, Daniels L M and Kitts C L (2003). Removal of PCR inhibitors from soil DNA by chemical flocculation. *Journal of Microbiology Methods*, 52(3), 389-393.

Burgmann H, Pesaro M, Widmer F and Zeyer J (2001). A strategy for optimizing quality and quantity of DNA extracted from soil. *Journal of Microbiology Methods*, 45(1), 7-20.

Doyle J J and Doyle J L (1987). A rapid DNA Isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin*, 19,11-15.

Foster S, Bedford K J, Gould M, Coward W R and Hewitt C R (2003) Respiratory syncytial virus infection and virus-induced inflammation are modified by contaminants of indoor air. *Immunology* 108(1), 107-109.

Glasel J A (1995). Validity of nucleic acid purities monitored by 260nm/280nm absorbance ratios. Biotechniques, 18(1), 62-63.

Haugland R A, Brinkman N and Vesper S J (2002). Evaluation of rapid DNA extraction methods for the quantitative detection of fungi using real-time PCR analysis. *Journal of Microbiology Methods* 50(3), 319-323.

Huang J, Ge X and Sun M (2000). Modified CTAB protocol using a silica matrix for isolation of plant genomic DNA. *Biotechniques*, 28(3), 432-434.

Krause DO, Smith W J and McSweeney C S (2001). Extraction of microbial DNA from rumen contents containing plant tannins. *Biotechniques*, 31(2), 294-298.

Dr Edu B Suárez-Martínez, Miss Maryliz González-Santos, Dr Federico Montealegre

Krsek M and Wellington E M (1999). Comparison of different methods for the isolation and purification of total community DNA from soil. *Journal of Microbiology Methods*, 39(1), 1-16.

Laird P W, Zijderveld A, Linders K, Rudnicki M A, Jaenisch R and Berns A (1991). Simplified mammalian DNA isolation procedure. *Nucleic Acids Research*, 19(15), 4293.

Meis J and Chen F L (2002). Extract PCR-Ready Soil DNA in less than an hour with the new Soil Master DNA Extraction Kit. *EPICENTRE Forum* 9(2), 1-3.

Morera D (2001). Efficient Removal of PCR inhibitors using agarose-embedded DNA preparations. *Nucleic Acids Research* 26, 3309-3310.

Menetrez M Y, Foarde K K and Ensor D S (2001) An analytical method for the measurement of nonviable bioaerosols. *Journal of the Air & Waste Management Association*, 51(10), 1436-1442.

Nelson H S (2001). The importance of allergens in the development of asthma and the persistence of symptoms. *Disease-a-Month*, 47(1), 5-15.

Sharma R, Jacob J S, Damgaard D M and McAllister T A (2003). Extraction of PCR-Quality plant and microbial DNA from total rumen contents. *Biotechniques*, 34(1), 92-97.

Tebbe C and Vahjen W (1993). Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and yeast. *Applied Environmental Microbiology*, 59(8), 2657-2665.

Tkach V and Pawlowski J (1999). A new method of DNA extraction from the ethanol-fixed parasitic worms. Acta Parasitologica, 44(2), 147-148.

Velegraki A, Kambouris M, Kostourou A, Chalevelakis G, and Legakis NJ (1999). Rapid extraction of fungal DNA from clinical samples for PCR amplification. *Medical Mycology*, 37 (1), 69-73.

Yeates C, Gillings M R, Davison A D, Altavilla N and Veal D A (1998). Methods for microbial DNA extraction from soil for amplification. *Biological Procedures Online*, 1, 40-47.