REPORT TO FSA SCOTLAND ON PROJECT SO1014.

PREVALENCE OF FAECAL SHEDDING ON SCOTTISH BEEF CATTLE FARMS OF VEROCYTOTOXIGENIC *ESCHERICHIA COLI* SEROGROUPS: O26, O103, O111 AND O145.

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2. LAYPERSONS SUMMARY

The aim is to find out how frequent the most important strains of verocytotoxigenic *Escherichia coli* (VTEC) are in Scottish cattle closest to sale or slaughter. The aim is consistent with recommendations of both the Microbiological Safety of Food Funders Group (MSFFG) and World Health Organisation (WHO) who stated "Knowledge of the distribution of non-O157 *VTEC* in animals, including cattle, is limited on a global scale."

The research is to:

- Provide the proportion of Scottish finishing cattle shedding *E. coli* of types O26, O103, O111, O145
- Provide the proportion of Scottish beef farms with shedding present in finishing animals
- Lead to a better understanding of the association between non-O157 shedding and farm characteristics
- Bacterial strains collected in a coherent manner will be available for subsequent characterisation and typing.

APPROACH

Before testing cattle across Scotland it was important to find out how reliable a test based on immunomagnetic separation (IMS) was for finding the VTEC strains. (IMS is an existing method where tiny magnets are placed into cultures of the sample to attach to and separate the VTEC bacteria). There were differences in how sensitive the IMS test was for the recovery of VTEC, but no sign of differences in isolating different strains and no sign that 3 laboratory staff were obtaining different results. The specificity of the IMS test was high and that meant that very few errors would be made in wrongly identifying strains. The lower sensitivity we think is caused by the lack of a simple method to identify the non-O157 VTEC bacteria when they grow on laboratory plates. The results suggested that the reliable detection limits for non-O157 VTEC are at least 10 times higher than for *E. coli* O157.

The IMS method is a valuable tool in the study of non-O157 VTEC. In total 6,086 dung samples were collected from 338 farms across Scotland. We sampled all regions, by Animal Health District, and evenly across seasons so as to avoid unnecessary bias and to our knowledge this work was larger than any previously carried out. It establishes a benchmark as the first true prevalence figure for these non-O157 VTEC strains in UK.

FINDINGS

The *E. coli* of types O26 and O157 were most often verocytotoxin positive (49% and 99% respectively) and these VTEC occurred on 10% and 14.7% of farms. These farms were scattered throughout Scotland and perhaps each of 3,500 Scottish farms have at least one animal carrying VTEC of the O26 or O157 types. Though, *E. coli* of types O103 and O145 occurred on many farms very few of these bacteria were verocytotoxin positive. Therefore, we are 95% confident that verocytotoxin positive strains of these types only occur on 0.3 to 1.6% of all farms (i.e. perhaps fewer than 250 farms in the whole of Scotland). We found no strains of *E. coli* O111. In examining why these strains occurred we found no sign of differences in numbers of non-O157 VTEC positive farms across the regions of Scotland but there are clear seasonal patterns with fewest positives found in the spring and most in summer and autumn. This is opposite to the results for *E. coli* O157 where more are found when the animals are inside buildings rather than during the summer. We found no sign of variation by Animal Health Division (AHD) or differences in occurrence of O26 *E. coli* that were due to the types of cattle management; whether the cattle are housed or at grass; the types of housing; the number of cattle on a farm; or whether the cattle are fed silage, hay or concentrates. Importantly, the occurrence of *E. coli* O157 strains at farm level is lower than that previously found in Scotland and the results show a real decrease in carriage of *E. coli* O157 by cattle. This change has occurred in recent years but the cause of the decline is unclear. The change is unlikely to be the result of any direct intervention as there are no known means for control of the organism in cattle.

The number of individual animals that have VTEC of types O26, O103 or O145 in their dung is low, being generally less than 2% of animals. However, there is a lot of between farm variation with some farms having most of the animals positive. This variation may simply be chance occurrence and further detailed analysis is necessary. The analysis will allow us to tell if the results are due to chance, to some farms having higher rates at which the bacteria spread, or if some farms having more introductions of the bacteria.

In Continental Europe, the most common non-O157 VTEC that cause human disease are *E. coli* types O111, O26, O103 and O145. These bacteria have been reported in 11, 11, 7 and 5 countries respectively. In Scotland this study has shown VTEC strains of types O103, O111 or O145 are uncommon or absent in cattle. In contrast, the type O26 VTEC are considerably more common and at farm level the 10% prevalence is close to the 14.7% found for *E. coli* O157. This is an important finding as O26 *E. coli* are the most common non-O157 VTEC found in human disease in Spain and is the first or second most common type in human non-O157 infection in Germany, Belgium, Denmark, Finland, Canada, US and Japan. In the UK, O26 human infections are unusual but four

Scottish clinical cases occurred during a six-week period in June and July 2003. An FSA funded study (S11001) that collaborated with this project used a laboratory method to compare 33 human isolates of *E. coli* type O26 with 152 of the isolates obtained from cattle. The 33 human isolates commonly carried the same genes as our Scottish cattle isolates and though we found no strains from the two hosts to be exactly the same the human and cattle isolates were related. Thus cattle may be a significant source for the type O26 strains and human infections could develop in Scotland to be as significant a problem as those caused by *E. coli* O157.

SUMMARY

In summary we have provided a benchmark for the occurrence of non-O157 VTEC in cattle faeces in UK and found evidence that *E. coli* positive for the verocytotoxin gene and of type O26 are common and widely dispersed on Scottish farms. There has been rapid emergence of new strains of type O26 in Germany so further work should be carried out to monitor the strains in Scotland. Isolates from humans and animals should be examined and studies carried out to find out the sources of human infection. A number of recommendations (p. 33) are suggested by the authors of this report.

AIMS

3.1 STUDY PROPOSAL

Verocytotoxin (VT) producing *Escherichia coli* (VTEC), and particularly strains of serotype O157:H7, have emerged as significant food poisoning pathogens that can cause a severe and potentially fatal illness in humans (Ammon, 1997). Possible outcomes of infection include haemorrhagic colitis (HC), and haemolytic uraemic syndrome (HUS) which is the main cause of acute renal failure in children. All VTEC strains produce potent phage-encoded cytotoxins called verocytotoxins (producing either vtx1 or vtx2 or both). Another virulence-associated determinant that may be expressed by VTEC is intimin, a surface protein, responsible for the intimate attachment of VTEC to intestinal epithelial cells resulting in attaching and effacing (AE) lesions (Roe and Gally, 2000). Intimin is encoded by the chromosomal gene *eae* that is part of a pathogenicity island termed the locus for enterocyte effacement (LEE).

VTEC strains that cause human infections belong to a large number of O and H serotypes. Most outbreaks and sporadic cases of HC and HUS in the UK are attributed to strains belonging to the serotype O157:H7 (Smith, *et. al*., 2001). However, the detection of non-O157 VTEC infections is limited to a few laboratories because no specific isolation media is available for these non-O157 VTEC serotypes. Thus, there is no routine laboratory testing of human cases in UK for non-O157 strains and their significance for human health is largely unknown (Smith, *et. al.,* 2001).

As evidence of the pathogenic potential for humans of non-O157 VTEC, serogroup O26 is considered the second most common cause of HUS in UK (Smith *et. al*., 2001). *E. coli* serotypes O26 and O111 are important causes of human VTEC infection in Australia, and serogroups O26, O103, O111, O145 are regarded as especially likely to cause severe human infections (Boerlin, *et. al*., 1999; Schmidt, *et. al*., 2001). Despite the problems of detection the non-O157 VTEC serotypes: O26:H–, O26:H11, O91:H–, O103:H2, O111:H–, O113:H21, O118:H16, O128:H2, O145:H–, O145:H28 and O146:H21, have all been associated with illness in humans (Blanco, *et. al*., 2001). Severe diarrhoea, including HC and HUS, has been statistically associated with strains carrying the *eae* gene for intimin and producing vtx2 (Boerlin, *et. al*., 1999) and these are particularly found in serotypes: O26:H11, O103:H2 and O111:NM (WHO, 1998). In contrast, the Study of Infectious Intestinal Disease in England showed serotypes of non-O157 VTEC that are negative for the *eae* gene have been rarely implicated in severe human illness and were more frequently found among asymptomatic carriers, in uncomplicated cases of diarrhoea, or among adult patients (FSA, 2000).

Internationally, Scotland has a relatively high rate of *E. coli* O157 related disease in humans and a considerable amount of work has been conducted to investigate the epidemiology of *E. coli* O157 infection. An unexpectedly high proportion of cases are associated with environmental factors, such as farm animal contact or gardening and living on, or visiting farms were significant risk factors in acquiring the infection (Locking, 2000). It is known that domestic ruminants, especially cattle and sheep, are major reservoirs of VTEC and these bacteria, including *E. coli* O157 strains, are part of the normal intestinal flora. A SAC study of 925 farms has established the prevalence of *E. coli* O157 by immunomagnetic separation (IMS) at 8% of cattle and 25% of farms. However, for non-O157 VTEC, no cattle shedding prevalence data is available in UK and internationally there is very little quantifiable data since there is no standard isolation technique. In Spain it has been reported that 25 VTEC serotypes may be directly cultured from faecal samples from 84% of 32 farms and from 20% of adult cattle. The majority of strains were *eae* negative (Blanco, *et. al*., 1996). In a number of studies there has been examination for VTEC but no subsequent examination for *eae*. The study of Beutin, *et. al*. (1993) identified 21% of cattle as carriers of VTEC and recovered 41 O:H serotypes from 720 normal animals. Montenegro, *et. al*. (1990) used DNA hybridisation to identify VTEC in 11% of animals and Fagan, *et. al*. (1999) used a multiplex PCR that identified *vtx1* in 17% of bovine faeces and *vtx2* in 7%.

The introduction of immunomagnetic separation (IMS) was a significant advance in the detection of *E. coli* O157 (Wright, *et. al*., 1994). IMS beads are now available for the five major serogroups (O157, O26, O103, O111 and O145) causing human infections (Boerlin, *et. al*., 1999; Schmidt, *et. al*., 2001). The technique has considerable advantage over the molecular methods offering greater sensitivity and specificity. The method is preferable to direct culture of individual bacteria or DNA hybridization and most appropriate for large-scale prevalence studies of non-O157 VTEC with subsequent examination of isolates for *vtx* and *eae* gene carriage.

The epidemiology of *E. coli* O157 infections is now partly understood and the importance of direct or indirect contact with the animal reservoirs of infection has been identified. It is considered possible that non-O157 serotypes may emerge as more frequent human pathogens from this animal reservoir and they may be common in Scottish cattle. It is therefore proposed that random sampling of cattle will be undertaken by SAC with collaboration of the IPRAVE studies and the IMS techniques will enable accurate prevalence estimations for non-O157 VTEC.

The aim is to establish the prevalence of the most important serotypes in Scottish cattle closest to slaughter. The aim is consistent with recommendations of both the Microbiological Safety of Food Funders Group (MSFFG) and World Health Organisation

(WHO) who stated "Knowledge of the distribution of non-O157 *VTEC* in animals, including cattle, is limited on a global scale." WHO/CSR/APH/98.8.

The research is to:

- Provide the proportion of Scottish finishing cattle shedding serogroups O26, O103, O111, O145
- Provide the proportion of Scottish beef farms with shedding present in finishing animals
- Lead to a better understanding of the association between non-O157 shedding and farm characteristics
- Bacterial strains collected in a coherent manner will be available for subsequent molecular characterisation and typing.

3.2 STUDY DESIGN AND OBJECTIVES

The proposal is dependent upon collaboration with The Wellcome Trust funded IPRAVE project "Epidemiology and Evolution of Enterobacteriaceae Infections in Humans and Domestic Animals" with fieldwork based at SAC Inverness. The IPRAVE study examines faecal pat samples collected from beef cattle farms in Scotland for the presence of *E. coli* O157:H7. The IPRAVE testing procedure uses bacterial enrichment of 1 g faeces samples in buffered peptone water (BPW) with subsequent immunomagnetic separation (IMS) testing for *E. coli* O157 at SAC Inverness. Faecal samples from the IPRAVE studies and the anonymous background farm data are available from SAC Inverness and the sample collection and initial processing is not included as a cost for FSA (Scotland).

The proposal is to use sensitive sampling methods and Objective 1 will be to establish the validity of the test methods. Our results from the IPRAVE study have shown that the limit of detection of *E. coli* O157 by IMS is in the order of 10 c.f.u. g^{-1} of faeces (unpublished data) but the sensitivity of IMS is determined by both the numbers of bacteria present and the distribution of the target organisms in the faecal samples.

Objective 1 (IMS validation). Determination of the sensitivity and specificity of the IMS techniques for detection of the non-O157 VTEC strains. Faecal material will be collected on a subset of farms. The proposed validation studies will use 1 g spiked faecal samples, enrichment in buffered peptone water (BPW) and O26, O103, O111 and O145 specific IMS. These studies will be confirmed by additional studies subcontracted to the University of Aberdeen. The effect of altering incubation temperature for the BPW to 42°C will be studied and the analyses will include examination of the effect of operator. The faecal samples will be spiked with mixed target serotypes so that the operators are effectively blinded to the intended result. A report on the IMS techniques' sensitivity,

specificity and minimum detection level of target organism will be prepared and a meeting held in February 2002 to confirm the method's acceptability for the farm prevalence study (Objective 2).

Objective 2 (Farm prevalence study). The IPRAVE studies will be directed at farms included in the earlier SAC prevalence work to determine what changes in O157 prevalence have occurred. The previous studies revealed no regional differences in farm prevalence. The farms will be a random sampling of 300 chosen from those included in the original prevalence work and that have indicated a willingness to participate in further studies.

It was our opinion that the FSA (Scotland) study should sample the same proportion of animals as developed for the *E. coli* O157 prevalence study where the estimates were based upon an 80% likelihood of identifying a farm as truly positive, assuming the prevalence of shedding was 2%. The within herd sample sizes for this are estimated at 20 samples per farm for a 300-farm study. This will actually achieve a 90% likelihood for identifying farms as positive for non-O157 VTEC strains, if the on farm prevalence is similar to the actual figure of 8% animals positive for *E. coli* O157. There is sufficient leeway in this estimation to achieve the 80% likelihood of identifying a farm as truly positive should the within herd prevalence actually be 2% of animals, which is lower than that of *E. coli* O157. At the sample level using IMS based isolation methods we expect specificity to be extremely high and to have negligible impact on the estimate of the shedding prevalence.

Thus the study is designed to detect non-O157 VTEC on a farm with 90% likelihood of identifying a farm as truly positive if 8% of finishing cattle are shedding, and with 80% likelihood if 2% of finishing cattle are shedding. If the true on farm prevalence of non-O157 VTEC strains is 2% the prevalence estimates (at 95% confidence) are 0.47%–3.7% and if the true prevalence is 8% then the estimates are 4.7%–10.7%. The estimated prevalences are 2.1% and 7.7% respectively.

Statistical analysis of the results will provide estimates of the proportion of Scottish farms on which cattle are shedding non-O157 VTEC, and the proportion of finishing cattle in Scotland that are shedding non-O157 VTEC. Associations between farm characteristics and management practices, and shedding patterns will be examined using classical statistical techniques and, where necessary, logistic regression and generalised linear mixed modelling.

Objective 3 (Strain collection). From each individual faecal sample a representative isolate of all positive serotypes will be stored at –80°C and this storage of strains will

allow subsequent full characterisation and identification of virulence genes. The isolates will be uniquely identified by bar-coding and this plus the background data recorded from the study will be entered into the database that is a shared resource of IPRAVE and accessible to all collaborators. It is estimated that Objective 3 will include virulence characterisation of 600 bacterial isolates.

3.3 TIMETABLE

IPRAVE studies for primary validation of objective 1 September 2001 – end February 2002.

Subsequent independent validation will be by Aberdeen University complete by end March 2002.

FSA (Scotland) proposal (3rd Jan 2002 – 31st December 2003) with final report at end June 2004.

4. MATERIALS AND METHODS

4.1 LABORATORY METHODS

4.1.1 Bacterial isolation and culture

Within 48 h of collection, 1 g of faeces from each sample was suspended in 20 mL buffered peptone water (BPW), and incubated at 37°C (±1°C) for 6 h. Following incubation, 1 mL of BPW broth was added to 20 µL serogroup specific IMS beads (serogroups O26, O103, O111, O145; LAB M, Bury, Lancashire) in a screw capped microcentrifuge tube. The tube contents were mixed on a blood tube rotator for 30 min then placed in IMS magnet racks for five min. Beads were washed three times in 1 mL 0.01M phosphate buffered saline with 0.05% v/v Tween® (PBS-T). Following the final wash, supernatant was removed and beads were resuspended in 50 µl PBS-T. These suspensions of serogroup O26, O103, O111 and O145 beads were plated onto separate Chromocult TBX plates (Merck, Poole, Dorset) and incubated at 37°C (± 1°C) overnight. From each Chromocult TBX plate, up to ten morphologically distinct colonies were tested against serogroup specific antisera (Statens Serum Institut, Copenhagen, Denmark) using a slide agglutination test. Presumptive positive isolates were stored on Prolab beads (Prolab Diagnostics, South Wirral, Cheshire, UK) at -80°C (± 4°C). Known positive control broths for each serogroup were also included on each IMS test day.

4.1.2 Tube agglutination

For the prevalence study tube agglutination screening was introduced to exclude non-specific slide-agglutinating strains from further study. Frozen bacterial isolates were revived on MacConkey agar plates and a portion of a well isolated colony was re-checked by slide agglutination with the other portion inoculated into 3 mL nutrient broth (Oxoid, Basingstoke, UK). After incubation at 37°C (± 1°C) for 2 h the broth culture was diluted to the equivalent of a MacFarland standard 4 suspension using sterile 0.85% saline. 1 mL of this was placed in a screw capped microcentrifuge tube and heated at 100°C (± 1°C) for 1 h. After this time the tubes were removed from the heat and left to stand undisturbed for a further 1 h (Ørskov and Ørskov, 1984). Dilutions of monospecific *E. coli* antiserum (Statens Serum Institut, Copenhagen, Denmark) were prepared depending on the titre value. Then 25 µL of 0.85% saline was added to all wells of 96 well U-bottomed microtitre plates (Bibby Sterilin, Staffs. UK) and 25 µL of diluted serum were added to all wells in Row A. This was titrated down the wells by double dilution to Row G, discarding the final 25 µL and leaving Row H as a saline control. The boiled and cooled antigen suspension was added to each row from H to A, the plate covered tightly to prevent evaporation and incubated at 50°C (± 1°C) overnight. The reactions were read using an inverted mirror;

typical O agglutination is seen as a precipitate covering the bottom of the well. A negative reaction is seen as a discrete button on the bottom of the well. The titre value was recorded as the last positive well. Isolates that were negative were excluded from further serotyping provided they gave no positive PCR results.

4.1.3 PCR detection of *vtx1, vtx2*, *eae* and *ehl*

A multiplex PCR was used to detect genes encoding verocytotoxins 1 and 2 (*vtx1, vtx2*), intimin (*eae*) and enterohaemolysin (*ehl*). (Paton and Paton, 1998). Briefly, a single bacterial colony was emulsified in 0.85% saline and heated to 100°C for 15 min. The PCR reaction mix was prepared as described except the primers to detect the O antigen encoding portions of the *E. coli* genome were excluded. 2 μL of target was added to the reaction mix, cycling conditions and subsequent electrophoresis were as described. Results were recorded as presence or absence of bands of the expected size (*vtx1*:180bp, *vtx2*:255bp, *eae*:384bp and *ehl*:534bp). A photographic record was taken for each gel.

4.1.4 Serogrouping and biochemical confirmation of identity

Each isolate that gave a positive result on titration and/or PCR was confirmed as *E. coli* and serotyped in the Laboratory of Enteric Pathogens (LEP), Health Protection Agency (HPA), Colindale, UK by the project staff. The biochemistry of each isolate was checked using microtitre plates with the following substrates: lactose peptone water (PW), mannitol PW, cellobiose PW, sorbitol PW, ONPG, urea, Simmon's citrate, lysine, ornithine, malonate, tryptone and a slope of GIA agar was inoculated (Table 1).

At LEP the serogrouping was carried out by titration of a suspension of each strain with specific unabsorbed O antiserum according to the serotyping scheme developed by Kauffmann (1947) that categorises *E. coli* based on the structure of the somatic "O" and flagellar "H" antigens. Isolates showing a diagnostic titre were then titrated with specific absorbed serum and if the result was a diagnostic titre the strain was assigned that serogroup. Strains that failed to titrate were retested by mixing 1:1 with a panel of 186 unabsorbed sera in microtitre plates. Any serum showing agglutination were re-tested as described above, if again there was no diagnostic titre a fresh broth was prepared and autoclaved at 121°C for 30 min before repeating the procedure. (NB. The submission and workload of clinical human isolates at LEP precluded the H serotyping of study isolates and there remain 429 *E. coli* isolates for which we do not have the full O & H serotype designations. It has been proposed to FSA that because of the backlog and the unavailability of another site for H typing in UK that the 221 serogroup O26 isolates

should be typed by PCR restriction fragment length polymorphism of the flagellin encoding *fliC* gene (Zhang, *et. al*., 2000)).

4.2 IMMUNOMAGNETIC SEPARATION (IMS) EXPERIMENTS

4.2.1 Experiment 1 to estimate approximate sensitivity and specificity of IMS procedure for *E. coli* O26, O103, O111 and O145

This experiment was to provide approximate estimates of the IMS test sensitivity and specificity for the target organism in the presence of non-target bacteria.

A single faecal pat from an adult cow was collected and divided into 12 samples. Three samples were inoculated with 1 mL maximum recovery diluent (MRD) containing a target *E. coli* serogroup (e.g. *E. coli* O26) at a concentration of 10 c.f.u. mL^{-1}, another three samples at a concentration of 100 c.f.u. mL^{-1}, and a further three samples at a concentration of 1000 c.f.u. mL^{-1}. The final three samples were inoculated with 1 mL MRD containing no *E. coli*. Samples were then randomised and inoculated with one of the other *E. coli* serogroups (e.g. *E. coli* O103), and the process repeated until samples had been inoculated with all four serogroups. Samples therefore comprised different combinations of the different *E. coli* serogroups at different concentrations.

E. coli were recovered from samples with immunomagnetic separation (IMS) beads specific to either *E. coli* O26, O103, O111 or O145 using the method described (4.1.1). IMS beads were plated on TBX agar and the serogroup of up to 10 positive colonies from a representative range of morphologies screened by slide agglutination. The inoculated strains were all nalidixic acid resistant and recovered isolates were plated to agar containing nalidixic acid to ensure that they were consistent with those added to the samples. This trial was repeated over three weeks using a fresh faecal pat on each occasion. Two different strains for each *E. coli* serogroup were used.

4.2.2. Experiment 2 to estimate sensitivity of IMS for *E. coli* O26

The accuracy of the test procedure sensitivity was evaluated for *E. coli* O26 using a more sophisticated experiment where improvements to the experimental design included:

- Six bovine faecal pats, from different farms, tested as being negative for endogenous *E. coli* O26 were used - these are more likely to be representative of field samples and considered less likely to contain endogenous *E. coli* O26 than in experiment 1 (4.2.1).
- Six bacterial strains of *E. coli* O26 were used, five of which (C683.1, C1991, C1414.1, C1528.4 & 56C280/2) are known to have been isolated from cattle; the origin of the sixth strain (UA3552) is not known. The strains are more representative of the field

samples as they were deliberately chosen to avoid those initially isolated by the IMS technique. The consistency of inoculated strains and recovered isolates was evaluated by testing for the presence of the *vtx1*, *vtx2*, *eae* and *ehl* genes using PCR (4.1.3) rather than by screening for nalidixic acid resistance.

- Experiment 2 was designed so that faecal pat and strain variation could be statistically examined.
- The number of colony forming units of inoculated *E. coli* ranged between $1.6x10^1$ to $8.4x10^5$ c.f.u. per sample. This increased range provided more accurate estimates of sensitivity and error.

4.2.3. Experiment 3 to examine variation in IMS recovery from different faecal pats

A third experiment was designed to examine the hypothesis that the recovery of inoculated *E. coli* and sensitivity of the IMS test procedure varies between faecal pats. The recovery of inoculated *E. coli* O26 from 12 faecal pats from housed animals and 12 faecal pats from grazing animals was investigated. All faecal pats were obtained from different farms and had been tested as negative for endogenous *E. coli* O26. Nine 1 g samples from each faecal pat were inoculated with 100 c.f.u. of the nalidixic acid resistant *E. coli* O26 strain UA3552 and tested by IMS as previously described.

4.2.4. Experiment 4 to optimise enrichment methods for detecting non-O157 strains

Twelve strains of serogroups O26 and O111 were screened conductimetrically on a Malthus Growth Analyser. Strains were grown separately in the 4 enrichment broths (Buffered peptone water (BPW); BPW + vancomycin (8 mg L^{-1}); modified tryptone soya + novobiocin (20 mg L^{-1}); *E. coli* broth + novobiocin (20 mg L^{-1}) and at 37°C and 42°C for the standard IMS enrichment period of 6 h. Volumes (0.1 mL) were transferred into a specific *E. coli* conductance medium at 37°C and the growth curves compared.

Decimal dilutions (-3 : -8) of cocktails of representative strains (nalidixic acid resistant mutants) of O26 and O111 were spiked into bovine faeces (containing no target VTEC) and enriched in the 2 optimum enrichment broths (BPW at 37°C and BPW-V at 42°C) for 6 h prior to testing in quintuplicate by IMS. Beads were plated onto MacConkey agar with nalidixic acid and incubated at 37°C for 18-24 h. An exponential model was fitted to the data using the maximum-likelihood estimate method (Haas, *et. al*., 1999).

4.3 SCOTTISH NON-O157 VTEC PREVALENCE STUDY - FARM SELECTION AND SAMPLING

4.3.1 Sampling plan

A five-stage sampling plan was used to sample faecal pats for the Scottish national prevalence study. The stages of the sampling plan were: State Veterinary Service Animal Health Division; farm cluster; individual farm; animal group within farm and faecal pat samples within group. An illustration of the sampling stages is given in Table 2.

4.3.2 Sampling frame

SEERAD originally provided SAC Inverness with a randomly selected list of 3,111 cattle farms, to undertake a study to determine the prevalence of *E. coli* O157 shedding by cattle during the period 1998–2000. This list represented 20% of the holdings with cattle in Scotland. Of these cattle holdings, 953 farms were sampled as part of the SEERAD-funded SAC prevalence study and owners or managers of 925 farms consented to a revisit for the IPRAVE and FSA prevalence studies. Farms were selected for the IPRAVE and FSA prevalence studies from the list of 925. For two State Veterinary Service (SVS) Animal Health Divisions (Highlands, Islands) the list of farms visited with consent to revisit was exhausted and for these two Animal Health Divisions some farms that had not been sampled in the SEERAD study were also selected from the original list of 3,111. The IPRAVE O157 prevalence study sampled from 481 holdings and the FSA non-O157 prevalence study from a subset of 338 holdings.

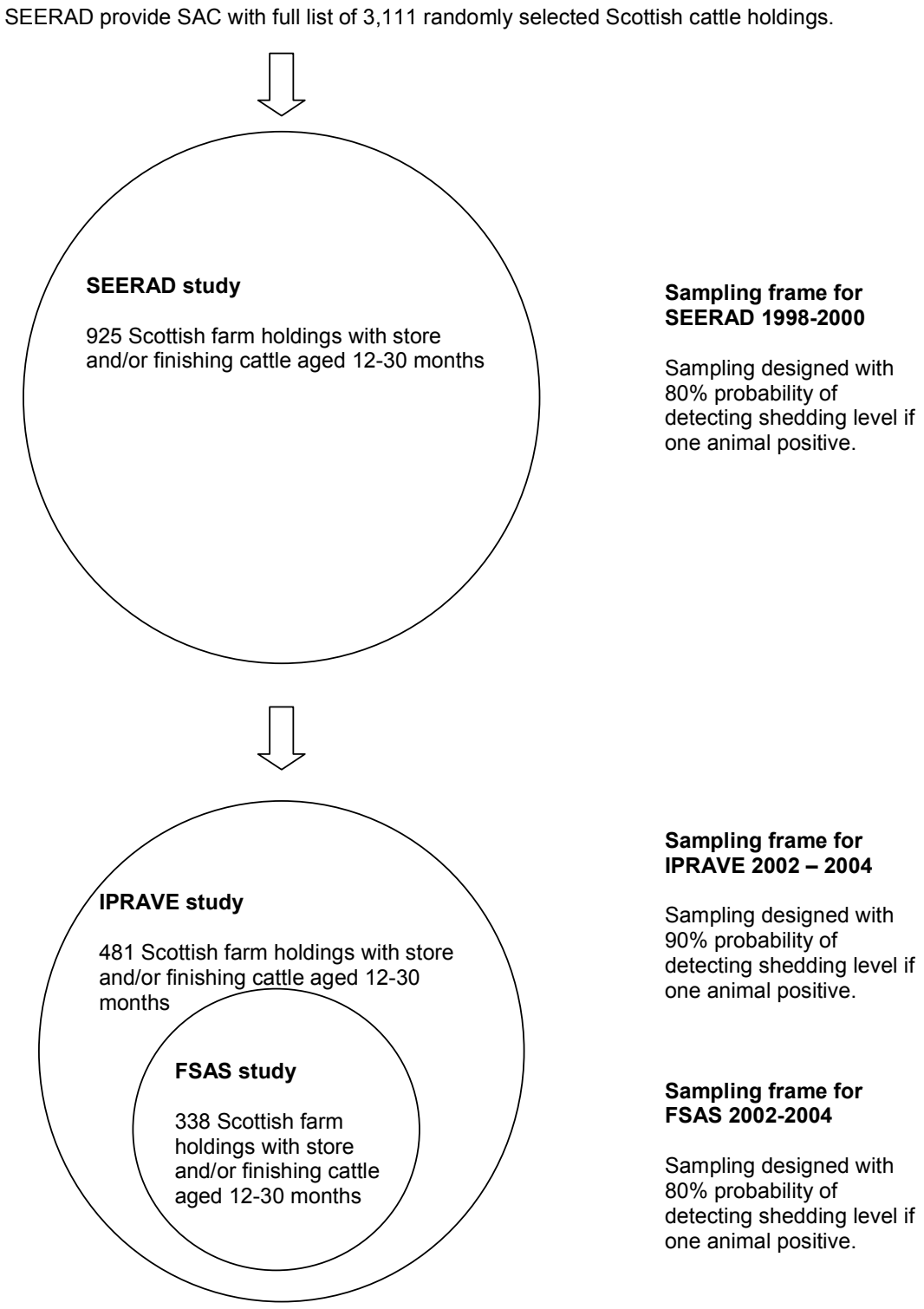
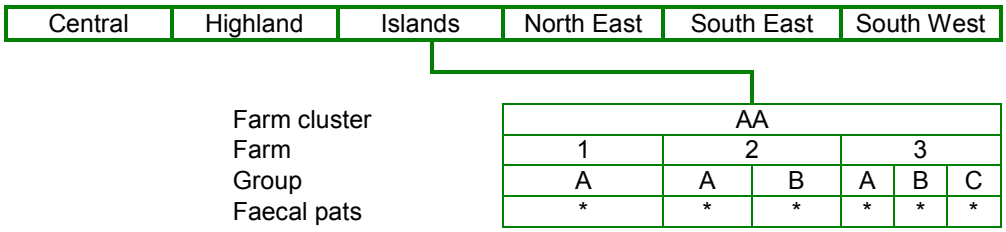


Figure 1. Sampling frames for O157 and non-O157 VTEC prevalence studies

4.3.3 Sample selection

Within each Animal Health Division the farms were sampled in clusters of three to make the visits manageable. Farms in the same cluster were sampled on the same or contiguous days. To select farms in each cluster the primary farm was selected randomly. Then a list was generated of the six farms nearest the primary farm, and within the same Animal Health Division, and farms on the list were ranked in ascending order according to distance from the primary farm. Following consent to visit the primary farm, the sampling cluster of three farms was completed by sequentially approaching farms, according to rank, on the list of farms nearest the primary farm.

At each farm visit, all the groups of cattle closest to sale or slaughter were identified. The number of cattle within each group was determined and for each group the number of faecal pats to be sampled within the enclosure was taken from a predetermined sampling schedule. In each enclosure, faecal pats were selected randomly and for the FSA study the predetermined sampling schedule actually ensured sufficient numbers to give an 80% chance of sampling at least one positive pat if there was one or more shedding animals within a group. This sampling was more rigorous than the proposed sampling intended to give an 80% chance of sampling at least one positive pat if there was a 2% shedding level. A questionnaire regarding farm management factors including: type, numbers, sources and purpose of livestock; housing and feeding of cattle; management practices (including the type and size of farm) and housing was also completed.



* sampling of faecal pats actually carried out with 80% probability of detecting at least one positive pat if there was one or more shedding animals within a group.

Figure 2. Sample selection for non-O157 VTEC prevalence study

4.4 STATISTICAL ANALYSIS

4.4.1 Descriptive analyses

Statistical analysis of the prevalence data was conducted using the SURVEYMEANS procedure in SAS v8.2 (SAS Institute Inc, Cary, North Carolina, USA).

The primary sampling unit (PSU) for the farm level and pat level analysis was the **farm cluster** and records were weighted for the analyses. For the analysis of farm level prevalence, records were weighted by the number of cattle farms with store and finishing cattle aged more than 1 year in the respective Animal Health Division divided by the number of farms in the respective farm cluster. Thus positive isolations from an Animal Health Division with fewer farms carry less weight than positive isolations from an Animal Health Division with more farms. This weighting is to eliminate undue bias in the calculation of a national prevalence figure.

4.4.2 Preliminary evaluation of risk factors for the recovery of *E. coli* O26 from faecal pats from store/finishing cattle and farm management variables

Associations between the recovery of *E. coli* O26 and farm management variables were tested using grouped data with the approach of Generalised Estimating Equations (Zeger and Liang, 1986; Liang and Zeger, 1986) using a logit link function and assuming a binomial error distribution. Multiple faecal pats within each farm were modelled as repeat measures with an exchangeable correlation structure. Associations were evaluated using the score statistic (Rotnitzky and Jewell, 1990) and adjusted Wald Statistic (Shah *et al*., 1977). Odds ratios and approximate 95% confidence intervals were estimated for each explanatory variable.

The farm management explanatory variables that were individually examined included type of farm, size of farm, animal housing, Animal Health District and type of feeding, together with season of sampling. Unifactorial analyses associated with a probability of $\leq$ 0.250 were then included in a preliminary multifactorial model and variables with a probability of $\leq$ 0.150 were retained in a final multifactorial analysis. The fit of the final multifactorial model was evaluated by plotting Pearson residuals against predicted values and estimating the Wald-Wolfowitz run statistic (Chang, 2000).

5 RESULTS

5.1 IMMUNOMAGNETIC SEPARATION (IMS) VALIDATION EXPERIMENTS

The sensitivity and specificity of the IMS procedures used to recover *E. coli* O111, O145, O26 and O103 was evaluated in four experiments. Experiment 1 calculated approximate estimates of sensitivity and specificity for the test procedure; experiment 2 confirmed the accuracy of the approximate sensitivity for *E. coli* O26 using a more sophisticated experimental design and experiment 3 further explored the variation in the recovery of inoculated *E. coli* between faecal pats. A fourth experiment conducted at University of Aberdeen examined the optimum enrichment methods for the subsequent IMS technique.

5.1.1 Experiment 1 approximate estimates of sensitivity and specificity for *E. coli* serogroups O26, O103, O111 and O145

The summary results of experiment 1 (4.2.1) to calculate approximate estimates of sensitivity and specificity for the test procedure for each serogroup are presented in Table 3. Ninety five per cent confidence intervals are approximate and take into account clustering. The sensitivity estimates differed across the serogroups but are small compared to the confidence intervals and sensitivity is very dependent upon inoculated bacterial concentration. At $1x10^3$ c.f.u. g^{-1} faeces the sensitivities varied from 52$\pm$21% for the serogroup O145 strain to 73$\pm$17% for *E. coli* serogroup O103. The specificity of the IMS procedures were high varying from 88$\pm$11% to 100%.

5.1.2 Experiment 2 to estimate sensitivity of IMS for *E. coli* O26

The estimates presented in Table 3 are approximate and the accuracy of the IMS test sensitivity for *E. coli* O26 was evaluated with a more sophisticated experimental design (4.2.2). A dose-response curve, with approximate 95% confidence limits, was fitted to the data by assuming that the proportion of samples from which *E. coli* O26 was recovered follows a linear function of the logit transformation. Sensitivities for the procedure for between 10^2 and 10^5 c.f.u., inoculated *E. coli* serogroup O26 strains, are from 33% (95%CI: 23 - 45%) to 86% (95%CI: 77 - 92%) (Table 4). The estimates of the sensitivity for the test procedure are very similar to those from experiment 1.

5.1.3. Experiment 3 to examine variation in IMS recovery from different faecal pats

The recovery of inoculated *E. coli* O26 from 12 faecal pats from housed animals and 12 faecal pats from grazing animals was investigated in experiment 3 (4.2.3). Inoculated *E. coli* was recovered from between 0 and 8 of the samples from each faecal pat and the differences in recovery between faecal pats are statistically very highly significant

(Fishers Exact Probability Test, p<0.001). Inoculated *E. coli* was recovered from 25% and 45% of the samples from housed and grazing animals respectively and this difference is statistically significant ($F_{1,17}$=4.43, p=0.05) but there is also very highly significant variation in recovery between faecal pats of the same origin i.e. housed or grazing (χ_1=67.4, p<0.001).

5.1.4. Experiment 4 to optimise enrichment methods for detecting non-O157 strains

The results of experiment 4 clearly showed that numbers of O26 and O111 reached during the 6 h enrichment (prior to the start of conductance measurements) were significantly greater in the BPW based broths compared to the other enrichment media. The values in Fig. 3 for the detection of a cocktail of strains indicate BPW-V enriched at 42°C is on average more sensitive than BPW at 37°C but this difference is not statistically significant. Both methods are less sensitive than BPW-V at 42°C for *E. coli* O157 reported by Omisakin *et. al.* (2003).

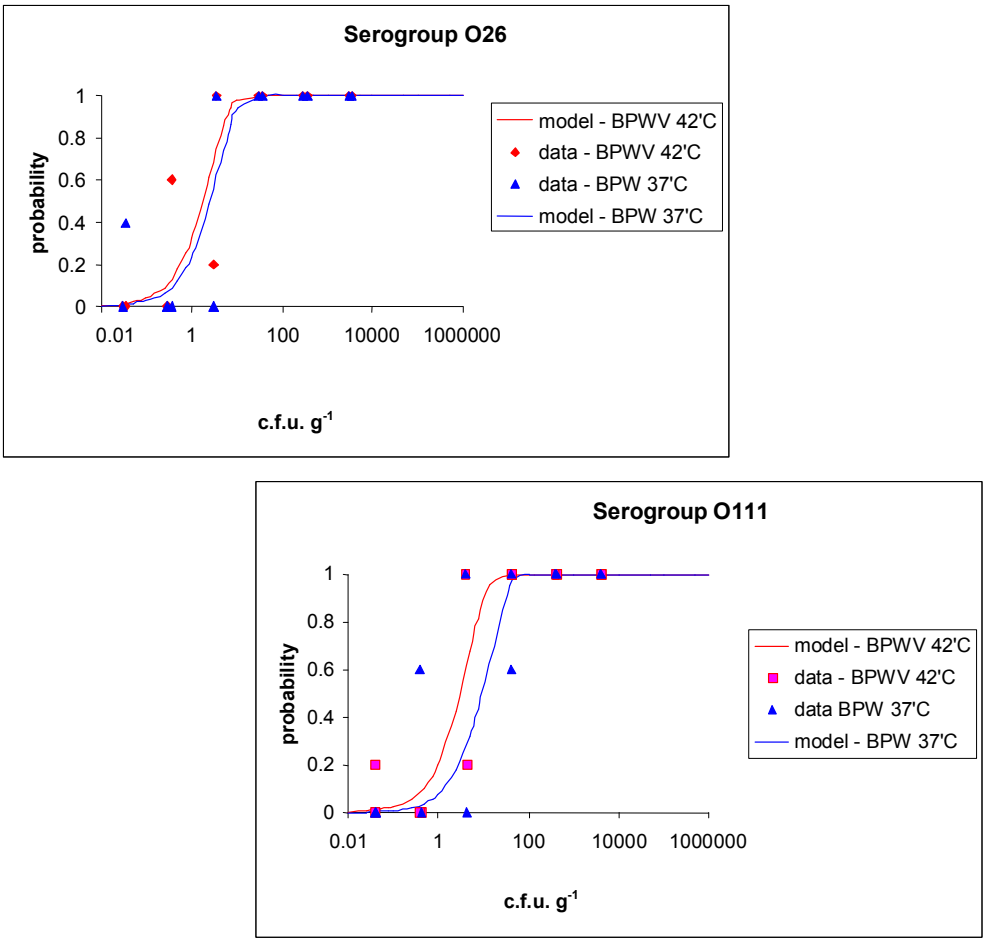


Figure 3. Exponential fit of the IMS method for VTEC O26 and O111 strain detection as applied to cattle faecal samples.

5.2 SCOTTISH PREVALENCE OF SEROGROUP O26, O103, O111 OR O145 SHEDDING BY CATTLE CLOSEST TO SALE OR SLAUGHTER

5.2.1 Farm sampling

338 farms were sampled between 30.04.2002 and 26.01.2004. A breakdown of the farms sampled by season and State Veterinary Service Animal Health Division is given in Table 5. Farms sampled per Division were from 51 to 59 and from 79 to 90 farms per season. From these farms a total of 6,086 faecal pat samples were collected with between 912 and 1,142 samples from each Division and between 1,291 and 1,748 per season (Table 6).

5.2.2 Bacterial characterisations

A total of 638 isolates were recovered by culture with subsequent testing by slide agglutination, tube agglutination and PCR at SAC Inverness. From the 638 isolates there were 552 isolates that gave a significant serogroup tube agglutination titre or positive PCR result that were taken to LEP in HPA Colindale. Two serogroup O103 isolates sent to LEP were from the same faecal pat and proved to be identical and the prevalence results are therefore based upon the data from 551 recovered isolates.

All isolates were confirmed as *E. coli* but some atypical biochemical results were seen. *E. coli* with atypical biochemistry results were also tested for their ability to produce 2-ketogluconate from gluconate and for growth in potassium cyanide (KCN). In all cases these were negative. Fifty-three serogroup O26 isolates from 17 farms were anaerogenic, of these 18 were *vtx1*, *vtx2* and the remainder *vtx1* positive. Twenty-seven O103 isolates from 8 farms were able to ferment cellobiose, a characteristic usually associated with *E. hermanii* but as these isolates decarboxylated lysine and did not grow in KCN they were classified as *E. coli*. Fifteen serogroup O26 isolates from the same farm and 2 from 2 different farms were urease positive. Further testing confirmed their identity as *E. coli*.

The 551 isolates were identified as *E. coli* and using the LEP serotyping scheme 460 (82.8% of putative seropositive isolates) were reported as the target serogroup with 249 of the isolates confirmed as serogroup O26, 168 isolates as O103 and 43 as O145 respectively. There was no isolation of serogroup O111 strains. Two isolates have been designated O rough where there was no lipopolysaccharide expression and 56 as O? where they did not fit a pattern recognised by the current *E. coli* typing system. Thirty-three non-target serogroup isolates were recovered belonging to 14 individual serogroups shown in Table 7.

5.2.3 Scottish prevalence of serogroups O26, O103, O111 or O145 shedding by cattle closest to sale or slaughter

The proportion of the 338 farms positive for each serogroup can be calculated ignoring whether the identified strains possessed any virulence determinants and using the weightings to eliminate undue bias in the calculation of prevalence figures. **The weighted farm level prevalence for *E. coli* serogroup O26 was 19.8% (68/338), 20.3% (75/338) for serogroup O103 and 7.1% (26/338) for serogroup O145 strains. Serogroup O111 was not isolated from any farm (Table 8). In comparison, the IPRAVE prevalence study showed 52 farms (15.3%) positive for serogroup O157 strains from the same subset of 338 study farms.** The location of farms with cattle shedding *E. coli* of serogroups O26, O103 or O145 are shown in Figure 4.

5.2.4 Variation in farm level prevalence of shedding by season and by Animal Health Division

The weighted prevalence of shedding for *E. coli* serogroups O26, O103 or O145, broken down by season is given in Table 9 and it is apparent that for all three serogroups faecal shedding is lowest during the spring (March to May) and peaks during the summer (June to August) and autumn (September to November). Differences between the prevalence of shedding across seasons were statistically significant at the farm level for *E. coli* serogroups O26 ($p<0.0005$) and O103 ($p=0.0041$) but not O145.

Differences between the weighted farm level prevalences of shedding across Animal Health Divisions are shown in Table 10. The weighted regional prevalences were statistically significant at the farm level for *E. coli* serogroup O145 ($p=0.04$) and there was an absence of this serogroup in the north east Animal Health Division. However, it is notable that the 95% confidence intervals are wide and too much emphasis should not be placed on this result. No significant regional differences were found for the farm level prevalence of serogroups O26 or O103.

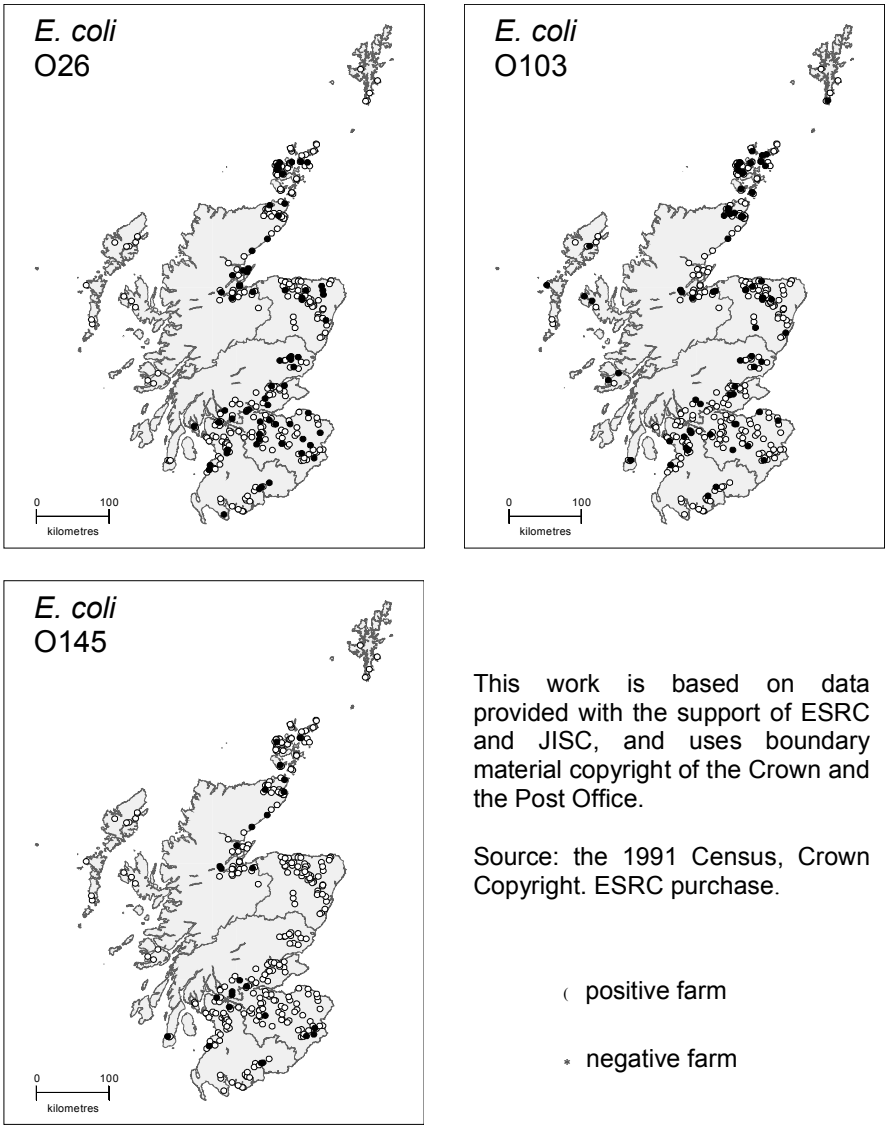


Figure 4. Farms positive for *E. coli* serogroups O26, O103 or O145 in faecal pats collected from Scottish cattle closest to sale or slaughter

5.2.5 Preliminary evaluation of risk factors for the recovery of *E. coli* O26 from faecal pats from store/finishing cattle and farm management variables

No association was found between the recovery of *E. coli* O26 and purchase of cattle; whether animals were housed or grazing; housing type; numbers of cattle on the farm or availability of different feedstuffs. Three explanatory variables (season, AHD, and farm type i.e. dairy, suckler or specialist finisher) were associated with a probability of ≤ 0.250 in the unifactorial analyses. The preliminary multifactorial analysis identified two variables with a probability of ≤ 0.150 (season and AHD). These two variables were

retained in a final multifactorial analysis and modelled using multifactorial Generalised Estimating Equations (4.4.2) and fixed explanatory variables of season (winter, spring, summer, autumn) and Animal Health Division (central, south east, south west, highland, islands & north east) and the random explanatory variable of farm. There is no evidence of an interaction between season and Animal Health Division (χ^2_{15}=13.14, P>0.250). **The final model has identified an association between the recovery of *E. coli* O26 and season with a four times higher likelihood of recovering *E. coli* O26 associated with faecal pats collected in autumn and summer than during the spring** (Table 11) (Season χ^2_3= 9.78, P=0.021; $F_{3,335}$= 3.24, P=0.022). No association with Animal Health Division was found (Region χ^2_1= 8.84, P<0.150; $F_{1,333}$= 1.75, P<0.150).

5.2.6 Frequency of virulence determinants in *E. coli* serogroups O26, O103, O111 and O145

The non-O157 verocytotoxigenic *E. coli* isolates showed considerable diversity possessing a variety of combinations of the four virulence determinants (*vtx1*, *vtx2*, *eae* or *ehl*). In total, serogroup O26 *E. coli* isolates were found with 10 different combinations of the four investigated virulence determinants. Serogroup O103 and O145 isolates each had 5 different combinations. Tables 12 to 14 show, for each serogroup, the number of individual strains and the frequency of individual virulence determinants in each serogroup. In contrast to the diversity of the non-O157 isolates the vast majority (99%) of O157 isolates were *vtx2*, *eae* and *ehl* positive or *vtx1*, *vtx2*, *eae* and *ehl* positive (Table 15).

Many serogroup O26 *E. coli* were potentially verocytotoxigenic with 122 (49.0%) *vtx1* positive and of these 31 (12.4%) were both *vtx1* and *vtx2* positive. No serogroup O26 *E. coli* isolates were found to carry *vtx2* gene alone. Of the 249 serogroup O26 isolates there were 209 (83.9%) isolates with *eae*, 129 (51.8%) carried *ehl* and 97 (39.0%) possessed both *eae* and *vtx* genes (Table 12). Isolates carrying the *eae* gene were over 12 times more likely to carry *ehl* than those isolates without *eae* (RR=12.15. 95%CI: 3.1 to 47.1%).

Carriage of *vtx* genes was very uncommon among the 168 isolates of *E. coli* O103. Only two (1.2%) isolates possessed the genes encoding *vtx*, one with vtx*1* only and the other with both *vtx1* and *vtx2* (Table 13). Carriage of *eae* and *ehl* was more common with 64 (38.1%) isolates and 62 (36.9%) isolates carrying *eae* and *ehl* respectively and most frequently both. Genes for *vtx* were also very uncommon among the 43 isolates of *E. coli* O145 (Table 14). One isolate carried the *vtx1* gene and one carried *vtx2* only. Carriage of *eae* and *ehl* was common with, respectively, 36 (83.7%) isolates and 28 (65.1%) isolates carrying *eae* and *ehl*.

The weighted faecal farm level prevalences for the different strains of each serogroup O26, O103, O145 and O157 have been calculated and are shown in Tables 16 to 19. **The weighted farm level prevalence for verocytotoxigenic *E. coli* serogroup O26 was 10.1% (52/338), 0.7% (2/338) for serogroup O103 and 0.7% (2/338) for verocytotoxigenic serogroup O145 strains. In comparison, 51 farms (14.7%) were positive for verocytotoxigenic serogroup O157 strains from the same subset of 338 study farms.**

5.2.7 Within farm frequency of *E. coli* serogroups O26, O103, O111 or O145

The within farm frequency of serogroups O26, O103 and O145 are illustrated in Figures 5 a, b and c. It is apparent that these serogroups do not occur on the majority of farms but the frequency for each serogroup on a small number of farms is high with more than 50% of samples positive. Verocytotoxigenic *E. coli* of serogroups O103 and O145 are rare but an examination of the frequency of verocytotoxigenic serogroup O26 strains suggests clustering on a subset of farms but this is more pronounced for *eae* positive serogroup O26 strains than for *vtx* positive strains (Figures 5 d and e). We found no evidence for a linkage between the isolation of *E. coli* O157 and isolation of any of the other non-O157 *E. coli* serogroups with the relative risk ratios for serogroup O26 being 0.83, for O103 being 0.96 and 1.08 for serogroup O145.

5.2.8 Rhamnose fermentation patterns for *E. coli* serogroup O26 strains

All of the serogroup O26 isolates were examined for their ability to ferment rhamnose. The results (Table 20) demonstrate that among serogroup O26 isolates the strains that have *vtx* genes are less likely to ferment rhamnose than those that do not carry *vtx* genes. 115 of 122 *vtx* positive isolates were unable to ferment rhamnose in 24 h but 7 isolates did ferment rhamnose in that time. Of the 127 *vtx* negative isolates 92 strains fermented rhamnose and the remaining 35 were negative. One isolate gave an inconclusive result and has been scored as positive for the purpose of this report. Seventeen isolates of non-O26 serogroups all fermented rhamnose.

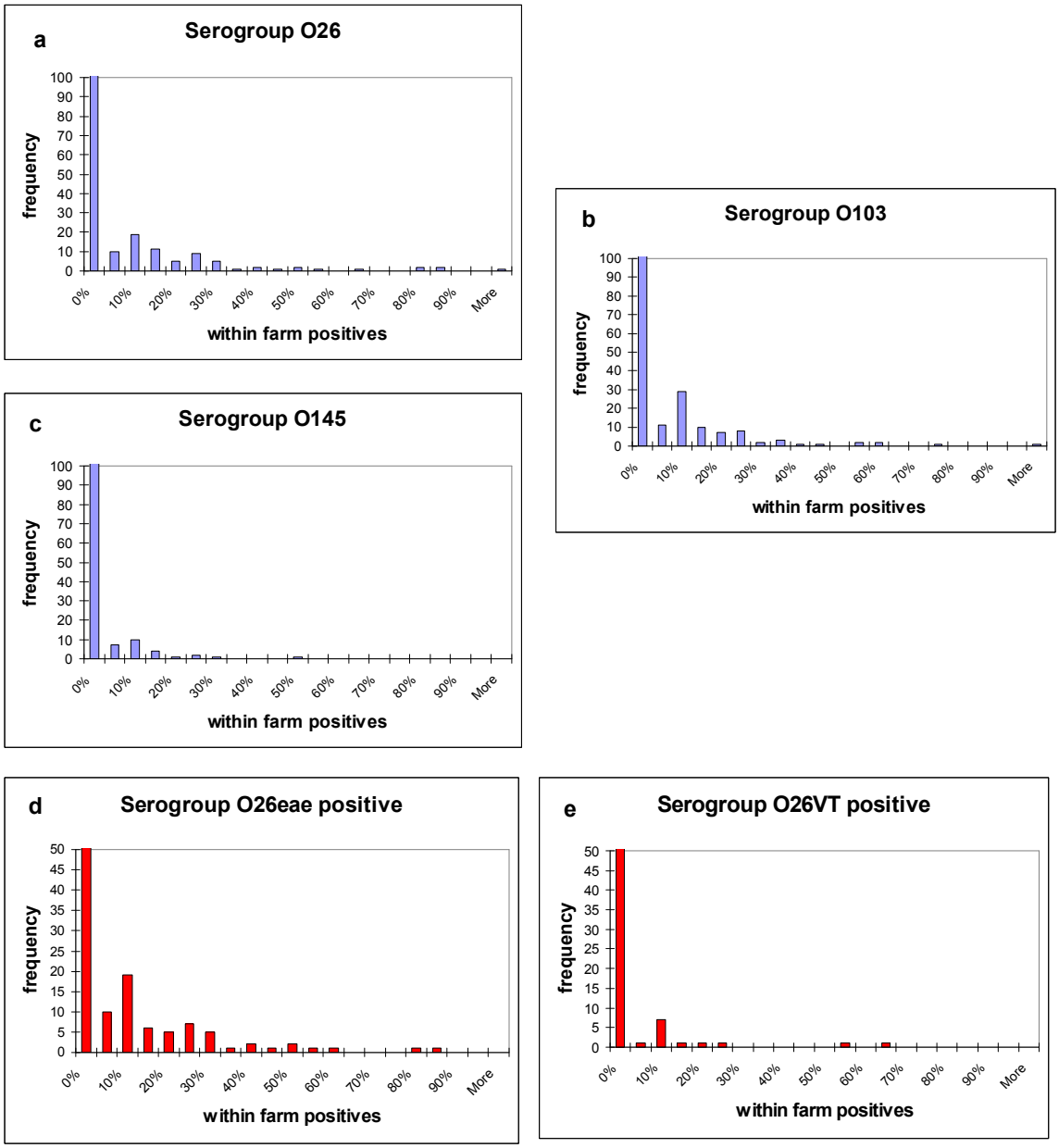


Figure 5. Within farm frequency of *E. coli* serogroups O26, O103, O111 and O145

6 DISCUSSION

The primary objective of the project was to establish the prevalence of VTEC serogroups O26, O103, O111 or O145 in faecal pat samples from Scottish cattle closest to sale or slaughter. However, prior to the field study it was necessary to examine the sensitivity and specificity of the proposed IMS procedures. Experiment 1 gave approximate estimates of sensitivity and specificity for IMS detection of *E. coli* O26, O103, O111 and O145 and experiment 2 subsequently gave an accurate sensitivity result for *E. coli* O26 IMS and confirmed the original results. For each serogroup the test sensitivity was dependent upon the concentration of target organisms and only approached 100% beyond 1,000 c.f.u. g^{-1} faeces. When the concentration of target organisms was 100 c.f.u. g^{-1} faeces or lower then the test sensitivity was poor. Variation in recovery was observed for both faecal pats and strains, although differences in recovery of the latter were not statistically significant. There was no evidence of a difference in recovery between laboratory operators. The specificity of the IMS procedures was high and the subsequent serogroup identification by slide and tube agglutination and later exclusion of 91 from the 551 recovered isolates allowed the isolation procedure to approach 100% specificity for all four serogroups. The lower sensitivity we largely attribute to factors such as inhibition or overgrowth by competing microflora and a major constraint was that after the IMS procedure there was no serogroup specific indicator medium such as sorbitol MacConkey as used for *E. coli* O157 detection.

We made a detailed examination of IMS sensitivity and no similar study is available from the literature. This is apparent even for *E. coli* O157 where IMS has become widely adopted as the international standard. Previous reports have probably over estimated IMS sensitivity and this is apparent in Experiment 4, which was designed to confirm the optimum isolation temperature and best pre-enrichment broth. In studies that use a limited number of faecal samples (Chapman *et. al*., 1997) or examine recovery of a cocktail of strains from single samples (Omisakin *et. al*., 2003) it is possible for the IMS procedure to achieve apparently high levels of sensitivity for the detection of low bacterial numbers. Whilst it is undoubtedly true that IMS for serogroup O157 significantly enhances sensitivity over direct plating, the data of Omisakin *et. al*. (2003) suggest that the reliable limit of detection for *E. coli* O157 by IMS may be in the order of 100 c.f.u. g^{-1} faeces and it is notable that Chapman *et. al*. (1997) give a detection limit of 1,000 c.f.u. g^{-1} faeces for some individual *E. coli* O157 strains. Our results are in accord with these results. We considered the reliable minimal bacterial detection limits for non-O157 VTEC to be at least 10 fold higher than for *E. coli* O157.

There is no previous suggestion that IMS recovery varies between faecal pats. However, we found very highly significant ($p<0.001$) differences between faecal pats for recovery of *E. coli* O26 and were concerned that these differences may not be caused by competing

microflora but through death of inoculated strains. However, no association (χ_1=0.93, p>0.10) (data not shown) was found between the efficiency of recovery of inoculated *E. coli* O26 and the faecal extracts' ability to inhibit the test strain growth. *E. coli* recovery by IMS was compared with the faecal pat compositions, comprising pH and water content, and gives a rank correlation coefficient (r_s) of 0.387 (p=0.062).

Experiment 4 showed that enrichment in buffered peptone water was the most suitable enrichment (Foster, 2003; Drysdale, 2004) and though our results indicated incubation at 42°C offered slight advantage this was not feasible as the IPRAVE field work protocols used 37°C as the incubation temperature. The use of 1 g faecal samples was considered justified since this sample size, or smaller, has been widely used in major prevalence and investigative studies of *E. coli* O157 (Besser *et. al.*, 1997; Chapman *et. al.,* 1997; Mechie *et. al*., 1997; Rahn *et. al.*, 1997; Pritchard *et. al.*, 2000; Wray *et. al*., 2000). This sample size was also important to allow comparison with the results of the IPRAVE project and retrospectively with the SEERAD funded study on the prevalence of *E. coli* O157 shedding in Scottish cattle. Though the examination of 10 g faecal samples may have increased the sensitivity of the IMS method this is a complex issue and paradoxically, increasing the amount of faeces tested does not necessarily increase test sensitivity as both methods use the same volume of broth culture for testing by IMS.

Using the IMS procedure the prevalence study was successfully carried out in the planned timetable and 6,086 faecal pats were sampled on 338 farms across Scotland. The sampling strategy avoided major regional and seasonal bias and to our knowledge provides the first true prevalence figures in UK. Other studies have examined collections of *E. coli* isolates or have used PCR or DNA probe to screen small numbers or groups of cattle for verocytotoxin positive *E. coli* irrespective of serogroup and the majority of these publications do not provide a true national prevalence figure. In Spain (Blanco, *et al*., 1996) only 2 animals carried serotypes O26 or O157 from an examination of 328 faecal samples by direct culture. Similarly, though Beutin, *et al*. (1993) identified 21% of cattle as carriers of VTEC and recovered 41 O:H serotypes there were no O26, O103 or O145 strains. Montenegro, *et al*. (1990) used DNA hybridisation to identify VTEC in 11% of 259 animals but again identified no serogroup O26, O103 or O145 strains and only 2 *E. coli* O157 isolates. Jenkins, *et. al.*, (2002) found *vtx* genes in 21% of bovine faecal samples from Scotland but recovered no serogroup O26 or O157 strains amongst the 31 serotypes subsequently identified.

In examining the weighted farm level prevalence for each serogroup it should be noted that the figures for non-O157 VTEC are minimum prevalence estimates as the sensitivity of the IMS procedure is lower than anticipated and lower than for *E. coli* O157. The results showed 19.8% and 15.3% of farms respectively positive for serogroup O26

serogroup and O157 strains. However, we identified major differences between the target serogroups in the likelihood of isolates being verocytotoxin positive, with serogroup O26 and O157 isolates having significant numbers of verocytotoxin positive strains (49% and 99% respectively). **Therefore, the weighted farm level prevalences of VTEC serogroups O26 and O157 were respectively 10% and 14.7% and these farms are distributed throughout Scotland. This can be interpreted to mean that over 3,500 Scottish farms have at least one animal carrying VTEC of serogroups O26 or O157**.

The weighted farm level prevalence for all *E. coli* serogroup O103 isolates was 20.3% and 7.1% for serogroup O145 strains but very few isolates are *vtx* positive**. Therefore the 95% confidence intervals show that verocytotoxin positive strains of these serogroups only occur on 0.3 to 1.6% of all farms (i.e. fewer than 250 farms in the whole of Scotland). Additionally, no strains of serogroup O111 were recovered.** The absence of *E. coli* O111 shedding was striking since this serogroup has been found elsewhere in cattle (Dorn, *et. al*., 1993; Sandhu *et. al*., 1996; Wieler, *et. al*., 1996; Blanco, *et. al*., 1996b; Holland, *et. al*., 1999). Historically, there are also reports of O111 recovery in Scotland or North of England (Sherwood, *et. al*., 1985). However, the current absence of serogroup O111 from Scottish samples is in agreement with our previous work, that included testing for *vtx* by DNA hybridisation, and which also identified a low shedding prevalence of serogroup O145 VTEC (Jenkins *et. al*., 2002; Jenkins *et. al*., 2003; Pearce *et. al*., 2004).

There was no convincing evidence for regional differences in distribution. **However, for all three non-O157 serogroups there were clear seasonal differences with farm level prevalence lowest in the spring and highest in summer and autumn.** This contrasts to the results for *E. coli* O157 where the prevalence appears to be higher in the housing period than the summer (Synge and Paiba, 2000; Ogden *et. al*., 2004). **The multifactorial analyses focussed upon *E. coli* O26 as the most frequent non-O157 VTEC and examination was made for farm factors that contribute to the variation in the recovery of *E. coli* O26 at both pat and farm level.** No differences can be attributed to Animal Health Division or differences in the types of cattle management; whether the sample group are housed or at grass; the types of housing; the number of cattle on a farm; or whether the cattle are fed silage, hay or concentrates. Again, significant seasonal variation was found with the highest number of serogroup O26 isolations made in summer and autumn. Thus the weighted farm level prevalences and the multifactorial analyses of serogroup O26 indicated a seasonal variation for both serogroup O26 and O103 strains. However, care needs to be taken in interpretation, as it is possible this is an artefact arising from use of the IMS test. The IMS validations showed a greater sensitivity for recovery of inoculated *E. coli* O26 from faeces collected from grazing animals and this may bias the results. Intriguingly, the multifactorial analysis

showed no difference for whether the sample group is housed or at grass and this potential contradiction with seasonality requires explanation. The simplest and most logical being that higher numbers of the target bacterium are present in faecal samples taken in the winter thus raising the sensitivity of the IMS test for these samples.

The number of individual animals shedding VTEC of serogroups O26, O103 or O145 was low, being generally <2% of animals. However, there was considerable between farm variation in animal level prevalence shown in Figures 5 a, b and c. Geue *et. al*. (2002) also showed considerable variation in prevalence across four farm groups and as has been described for *E. coli* O157 (Matthews *et. al*., 2003) our results showed a minority of farms with very high proportions of animal shedding. This between herd variation may simply be chance occurrence and further detailed modelling is necessary to explore the transmission dynamics of the different serogroups and of the individual strains. This will allow an examination of whether the results can be attributed to chance, to some farms having higher transmission rates, to more introductions of the organism or to some animals being significantly greater risks for spread.

A variety of methods have been used for the detection of non-O157 VTEC strains. However, our previous work has shown that for the detection of VTEC O26 strains the IMS technique is 2.5 times more sensitive than replica colony plating and DNA hybridisation using *vtx* probes (Jenkins *et. al*., 2003). IMS is also substantially more sensitive for detection of *E. coli* O157 (Pearce *et. al*., 2004). We thus believe that the IMS technique is a valuable tool in the epidemiological study of VTEC and that our prevalence data is more accurate than previously obtained. The scale of the work is larger than any described in the literature and establishes a benchmark for shedding of VTEC by cattle in UK. **It is notable that the IPRAVE study revealed the farm level *E. coli* O157 prevalence figure of 14.7% (95% CI: 10.4 to 19.0) as significantly lower than that previously described in Scotland (Synge and Paiba, 2000). These results show a real decrease in carriage of *E. coli* O157 by cattle.** This change has occurred in recent years but the cause of the decline is unclear and may be attributable to a number of different factors. The change is unlikely to be the result of any direct intervention since there are no established means for control of the organism in the bovine host.

In Continental Europe, the most common non-O157 VTEC serogroups causing human disease are *E. coli* O111, O26, O103 and O145 (WHO, 1998); which have been reported in 11, 11, 7 and 5 countries respectively (Eklund *et. al*., 2001; Caprioli and Tozzi, 1998). In Scotland we have shown VTEC strains of serogroups O103, O111 or O145 are of low prevalence or absent from cattle. In contrast, the prevalence of serogroup O26 VTEC is considerably higher and at farm level the 10% prevalence approaches the 14.7% found

for *E. coli* O157. This is an important finding as serogroup O26 is the most common non-O157 VTEC found in human disease in Spain (Blanco *et. al*., 2004). The authors also refer to it as the first or second most common type in human non-O157 infection in Germany, Belgium, Denmark, Finland, Canada, US and Japan (Blanco *et. al*., 2004). In the UK, serogroup O26 infections are uncommon but have been recognised (Smith, *et. al*., 2001; Willshaw *et. al*., 2001; Evans *et. al*., 2002) and strains with *vtx1*, *eae* and *ehl* have been identified as particularly likely to cause severe human infections (Willshaw *et. al*., 2001; Blanco, *et. al*., 2004). Additionally, enhanced surveillance in Scotland has led to the Scottish *E. coli* Reference Laboratory identifying four Scottish clinical cases of *E. coli* O26 in people during a six-week period in June and July 2003 (*Lesley Allison personal communication*).

Zhang *et. al*. (2000) describe *E. coli* O26 as a heterogeneous group that may carry the *vtx1* gene or the *vtx2* gene, *vtx1* and *vtx2* genes or neither of these genes. It is of note that though we isolated no serogroup O26 strain possessing *vtx2* alone we did recover isolates with both *vtx1* and *vtx2* genes and these may pose an increased threat to human health. It has been reported that *vtx2* positive isolates have emerged in Germany in recent years (Zhang *et. al*., 2000b) and are particularly associated with severe human disease. The mechanisms behind such a shift in *vtx1* to *vtx2* genotype are obscure but could be the result of infection of endemic *E. coli* O26 strains with *vtx2* converting bacteriophage (Zhang et. al., 2000b). These authors have suggested that such isolates have recently emerged and belong to a single clonal type characterised by an identical *fliC* gene for H11.

An FSA funded study (S11001) used pulsed-field gel electrophoresis (PFGE) to compare human isolates of serogroup O26 with 152 of the isolates recovered from cattle in this project (SO1014). The most common virulence profile from the 33 human serogroup O26 isolates was *vtx1* with *eae* and *ehl* genes (*vtx1*+, *eae*+, *ehl*+) and this is the most common virulence profile in our Scottish cattle isolates representing almost 30% of all serogroup O26 isolates. Though the work identified no identical PFGE profiles from the two hosts there was overlap of the virulence profiles and the relationships, determined by PFGE, showed human and cattle isolates to cluster together. **Thus cattle represent a significant reservoir for serogroup O26 strains and human infections might develop in Scotland to be as significant a problem as those caused by *E. coli* O157**.

Other than VTEC strains we identified considerable numbers of isolates from the non-O157 serogroups that were *eae* positive or *eae* and *ehl* positive. The very strong association that was found between carriage of *eae* and *ehl* genes has previously been described (Boerlin *et. al*., 1999; Blanco *et. al*., 2004). Whilst both *eae* and *ehl* encode important virulence determinants the isolates from cattle that are *eae* gene positive but

vtx negative are generally regarded as atypical enteropathogenic *E. coli* (EPEC) and may be less likely than VTEC to cause human disease (Ramachandran, *et. al*., 2003).

Thus we have provided a benchmark for shedding of VTEC by cattle in UK and evidence that VTEC of serogroup O26 are common and widely dispersed in Scottish cattle. It is important that because of the rapid emergence of new strains in Germany that further work should be carried out to monitor the strains of non-O157 VTEC, particularly of serogroup O26. Isolates from diverse sources should be examined and epidemiological follow-ups to clinical cases conducted so as to clarify their reservoirs of infection and to identify their routes of transmission.

7. RECOMMENDATIONS

1. It is recommended that further effort be given to establishing the validity of an indicator medium for serogroup O26 VTEC strains. The study identified biochemically atypical clones and confirmed that rhamnose non-fermenting *E. coli* O26 isolates were predominately verocytotoxin positive strains. This finding is consistent with previous results (Wieler, *et. al*., 1995; Hiramatsu *et. al*., 2002) and we have initiated further epidemiological studies utilising a rhamnose indicator medium.

2. The variation in the sensitivity of the IMS procedure between faecal pats indicates that further work investigating the effects of faecal pat composition on the recovery of inoculated *E. coli* may be appropriate.

3. Further detailed modelling is necessary to explore the transmission dynamics of the different serogroups and individual strains. This work may explain if some farms having higher proportions of positive animals can be attributed to higher transmission or immigration rates of the organism or if some animals are significantly greater risks for spread. (The work will be published in peer-reviewed journals and whilst it is expected that there will be no change in emphasis to the findings presented in this report it should be noted that the detailed analyses may lead to minor alterations in statistical significance).

4. There has been a significant fall in *E. coli* O157 carriage by cattle in Scotland and it is recommended that at an interval of 2 to 3 years further prevalence work should be conducted to confirm the change. (NB. This result arises directly from the collaboration with the Wellcome Trust IPRAVE study that underpinned this work).

5. The absence of serogroup O111 strains from Scottish cattle is remarkable and further monitoring is recommended.

6. Possibly because of difficulties in routinely identifying non-O157 VTEC there has been no actual epidemiological linkage between human cases of serogroup O26 disease and animal sources of infection. This is in contrast to *E. coli* O157 infection and an epidemiological follow-up to clinical cases is recommended with further typing of isolates.

7. There is a need for studies into the distribution and the number of *E. coli* O26 in faecal samples. The numbers of endogenous *E. coli* of non-O157 serogroups from positive field samples is unknown though Widiasih *et. al*., 2003 have suggested that serogroup O157 VTEC are excreted for longer periods and in higher numbers than serogroup O26 VTEC strains. This work requires validation as it may offer a logical explanation for the greater numbers of *E. coli* O157 human infections. Our current work is showing that calves may shed *E. coli* O26 strains in faeces for many weeks and at high bacterial numbers.

8. Cattle may represent a significant reservoir for serogroup O26 strains and human infections might develop to be as significant a problem as those caused by *E. coli* O157 in Scotland. The evolutionary history of VTEC serogroup O26 from across Europe should be examined and the virulence potential of strains studied by further phenotypic and genotypic characterisation.

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9. REFERENCES

1 Ammon, A. (1997). Surveillance of enterohaemorrhagic *E. coli* (EHEC) infections and haemolytic uraemic syndrome (HUS) in Europe. Eurosurveillance. 2: 91-96.

2 Anon. (1993). Cowan and Steel's manual for the identification of medical bacteria. Cambridge University Press, Cambridge (UK).

3 Besser, T.E., Hancock, D.D., Pritchett, L.C., McRae, E.M., Rice, D.H. and Tarr. P.I. (1997). Duration of detection of fecal excretion of *Escherichia coli* O157H7 in cattle. Journal of Infectious Diseases. 175: 726-729.

4 Beutin, L., Geier, D., Steinrück, H., Zimmermann, S. and Scheutz, F. (1993). Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. Journal of Clinical Microbiology 31: 2483-2488.

5 Beutin, L., Zimmermann, S. and Gleier, K. (1998). Human infections with Shiga toxin-producing *Escherichia coli* other than serogroup O157 in Germany. Emerging Infectious Diseases. 4: 635-639.

6 Blanco, M., Blanco, J.E., Blanco, J., Gonzalez, E.A., Mora, A., Prado, C., Fernandez, L., Rio, M., Ramos, J., and Alonso, M.P. (1996). Prevalence and characteristics of *Escherichia coli* serotype O157:H7 and other verotoxin-producing *E. coli* in healthy cattle. Epidemiology & Infection. 117: 251-257.

7 Blanco, M., Blanco, J.E., Blanco, J., Gonzalez, E.A., Alonso, M.P., Maas, H. and Jansen, W.H. (1996b). Prevalence and characteristics of human and bovine Vero toxigenic Escherichia coli strains isolated in Galicia (North-western Spain). European Journal of Epidemiology. 12: 13-19.

8 Blanco, J., Blanco, M., Blanco, J.E., Mora, A., Alonso, M.P., Gonzalez, E.A., and Bernardez, M.I. (2001). O:H serotypes of human verocytotoxigenic *E. coli* (VTEC). http://secuslugo.lugo.usc.es/ecoli/SEROTIPOSHUM.htm

9 Blanco, J. E., Blanco, M., Alonso, M. P., Mora, A., Dahbi, G., Coira, M. A., Blanco, J. (2004). Serotypes, Virulence Genes, and Intimin Types of Shiga Toxin (Verotoxin)-Producing *Escherichia coli* Isolates from Human Patients: Prevalence in Lugo, Spain, from 1992 through 1999. Journal of Clinical Microbiology. 42: 311-319.

10 Boerlin, P., McEwen, S.A., Boerlin-Petzold, F., Wilson, J.B., Johnson, R.P., and Gyles, C.L. (1999). Association between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. Journal of Clinical Microbiology 37: 497-503.

11 Caprioli, A., and Tozzi, A. E. (1998). Epidemiology of Shiga toxin-producing *Escherichia coli* infections in continental Europe, p. 38-48. *In* J. B. Kaper, and A. D. O'Brien (ed.), *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. American Society for Microbiology, Washington, D.C.

12 Chang, Y. (2000). Residuals analysis of the generalized linear models for longitudinal data. *Statistics in Medicine* 19:1277-1293

13 Chapman, P. A., Cerdan Malo, A.T., Siddons, C.A. and Harkin. M. A. (1997). Use of commercial enzyme immunoassays and immunomagnetic separation systems for detecting *Escherichia coli* O157 in bovine fecal samples. Applied and Environmental Microbiology. 63: 2549-2553.

14 Chapman, P. A., Siddons, C. A., Cerdan Malo, A. T. and Harkin, M. A. (1997). A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiology & Infection. 119: 245-250.

15 Dorn, C.R., Francis, D.H., Angrick, E.J., Willgohs, J.A., Wilson, R.A., Collins, J.E., Jenke, B.H. and Shawd, S.J. (1993). Characteristics of Vero cytotoxin producing *Escherichia coli* associated with intestinal colonization and diarrhea in calves. Veterinary Microbiology. 36: 149-159.

16 Drysdale, M., MacRae, M., Strachan, N.J.C., Reid, T.M.S. and Ogden, I.D. (2004). The detection of non-O157 *E. coli* in food by immunomagnetic separation. Journal of Applied Microbiology. 97: 220-224.

17 Eklund, M., Scheutz, F. and Siitonen, A. (2001). Clinical isolates of non-O157 Shiga toxin-producing *Escherichia coli*: serotypes, virulence characteristics and molecular profiles of strains of the same serotype. Journal of Clinical Microbiology. 39: 2829-2834

18 Evans, J., Wilson, A., Willshaw, G.A., Cheasty, T., Tompkins, D.S., Wheeler, J.G. and Smith. H.R. (2002). Vero cytotoxin-producing *Escherichia coli* in a study of infectious intestinal disease in England. Clinical Microbiological Infectious Disease. 8: 183-186.

19 Fagan, P.K., Hornitzky, M.A., Bettelheim, K.A., and Djordjevic, S.P. (1999). Detection of Shiga like toxin (*stx1* and *stx2*), intimin (*eaeA*) and enterohemorrhagic *Escherichia coli* (EHEC) hemolysin (EHEC *hlyA*) genes in animal feces by multiplex PCR. Applied and Environmental Microbiology. 65: 868-872.

20 Foster, G., Hopkins, G.F., Gunn, G.J., Ternent, H., Thomson-Carter, F. and others. (2003). A comparison of two pre-enrichment media prior to immunomagnetic separation for the isolation of *E. coli* O157 from bovine faeces. Journal of Applied Microbiology. 95: 155-159.

21 FSA. 2000. “UK publicly funded research relating to verocytotoxin-producing *Escherichia coli* (VTEC). Report of the Microbiological Safety of Food Funders Group (MSFFG). http://www.foodstandards.gov.uk/research/vtec.htm

22 Geue, L., Segura-Alvarez, M., Conraths, F.J., Kuczius, T., Bockemuhl, J., Karch, H. and Gallien, P. (2002). A long-term study on the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) on four German cattle farms. Epidemiology and Infection. 129: 173-185.

23 Gross, R.J. and Rowe, B. (1985). Serotyping of *Escherichia coli*. The Virulence of *Escherichia coli*. 14: 345-360

24 Haas, C.N., Rose, J.B., and Gerba, C.P. (1999). Quantitative Microbial Risk Assessment. John Wiley. New York. USA.

25 Hiramatsu, R., Matsumoto, M., Miwa, Y., Suzuki, Y., Saito, M. and Miyazaki, Y. (2002). Characterization of Shiga toxin-producing *Escherichia coli* O26 strains and establishment of selective isolation media for these strains. Journal of Clinical Microbiology. 40: 922-925

26 Holland, R.E., Wilson, R.A., Holland, M.S., Yuzbasiyan-Gurkan, V., Mullaney, T.P. and White, D.G. (1999). Characterization of eae^{+} *Escherichia coli* isolated from healthy and diarrheic calves. Veterinary Microbiology. 66: 251-263.

27 Jenkins, C., Chart, H., Cheasty, T., Willshaw, G., Pearce, M. and others. (2002). Verocytotoxin-producing *Escherichia coli* (VTEC) other than serogroup O157 from Scottish cattle. Veterinary Record. 151: 58-60.

28 Jenkins, C., Pearce, M.C., Smith, A.W., Knight, H.I., Shaw, D.J. and others. (2003). Detection of *Escherichia coli* serogroups O26, O103, O111 and O145 from bovine faeces using immunomagnetic separation and PCR/DNA probe techniques. Letters in Applied Microbiology. 37: 207-212.

29 Jenkins, C., Willshaw, G.A., Evans, J., Cheasty, T., Chart, H., Shaw, D.J, Dougan, G., Frankel, G. and Smith, H.R. (2003b). Subtyping of virulence genes in verocytotoxin-producing *Escherichia coli* (VTEC) other than serogroup O157 associated with disease in the United Kingdom. Journal of Medical Microbiology. 52: 941-7.

30 Kauffmann, A. F. (1947). Zur serologie der Coli-Gruppe. Acta Pathologica Microbiologica Scandinavica 21: 20-45.

31 Kobayashi, H., Miura, A., Hayashi, H., Ogawa, T., Endo, T., Hata, E., Eguchi, M., and Yamamoto, K. (2003). Prevalence and characteristics of eae-positive *Escherichia coli* from healthy cattle in Japan. Applied and Environmental Microbiology. 69: 5690-5692.

32 Liang, K.Y. and Zeger, S.L. (1986). Longitudinal data-analysis using generalized linear models. Biometrika 73:13-22.

33 Locking, M. (2000). A case-control study of sporadic cases of *Escherichia coli* O157 infection in Scotland. SCIEH weekly report. ISSN. 1357-4493. 34: 9-10.

34 Matthews, L., Gunn, G., Synge, B., McKendrick, I., and Woolhouse, M.E.J. (2003). Super-spreaders or super-farms: the transmission dynamics of E. coli O157 on Scottish farms. Proceedings of VTEC 2003, Edinburgh. p.208.

35 Mechie, S.C., Chapman, P.A. and Siddons, C.A. (1997). A fifteen month study of *Escherichia coli* O157:H7 in a dairy herd. Epidemiology & Infection. 118: 17-25.

36 Montenegro, M.A., Bulte, M., Trumpf, T., Aleksic, S., Reuter, G., Bulling, E., and Helmuth, R. (1990). Detection and characterization of fecal verotoxin-producing *Escherichia coli* from healthy cattle. Journal of Clinical Microbiology. 28: 1417-1421.

37 Ogden, I.D., MacRae, M., and Strachan, N.J.C. (2004). Is the prevalence and shedding of *E. coli O157* in beef cattle in Scotland seasonal? FEMS Microbiology Letters. 233: 297-300.

38 Omisakin, F., MacRae, M., Ogden, I. D. and Strachan. N. J. C. (2003). Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. Applied and Environmental Microbiology. 69: 2444-2447.

39 Ørskov, F. and Ørskov, I. (1984). Serotyping of *Escherichia coli*. Methods in Microbiology. 14: 43-112

40 Paton, A.W. and Paton, J.C. (1998). Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, eae, enterohemorrhagic E. coli hylA, rfb_{O111}, and rfb_{O157}. Journal of Clinical Microbiology. 36: 598-602.

41 Pearce, M.C., Jenkins, C., Vali, L., Smith, A.W., Knight, H.I. and others. (2004). Temporal shedding patterns and virulence factors of *Escherichia coli* serogroups O26, O103, O111, O145 and O157 in a cohort of beef calves and their dams. Applied and Environmental Microbiology. 70: 1708-1716.

42 Pritchard, G. C., Willshaw, G. A., Bailey, J. R., Carson, T. and Cheasty, T. (2000). Verocytotoxin-producing *Escherichia coli* O157 on a farm open to the public. Veterinary Record. 147: 259-264.

43 Rahn, K., Renwick, S. A., Johnson, R. P., Wilson, J. B., Clarke, R. C., Alves, D., McEwen, S., Lior, H. and Spika, J. (1997). Persistence of *Escherichia coli* O157:H7 in dairy cattle and the dairy farm environment. Epidemiology & Infection. 119: 251-259.

44 Ramachandran, V., Brett, K., Hornitsky, M.A., Dowton, M., Bettelheim, K.A., Walker, M.J., and Djordjevic, S.P. (2003). Distribution of intimin subtypes among *Escherichia coli* isolates from ruminant and human sources. Journal of Clinical Microbiology. 41: 5022-5032.

45 Roe, A. J. and Gally, D. L. (2000). Enteropathogenic and enterohaemorrhagic *Escherichia coli* and diarrhoea. Current Opinion in Infectious diseases. 13. 511-517.

46 Rotnitzky, A. and Jewell, N.P. (1990). Hypothesis testing of regression parameters in semiparametric generalized linear-models for cluster correlated data. Biometrika 77: 485-497

47 Sanderson, M. W., Gay, J. M., Hancock, D.D., Gay, C. C., Fox, L. K. and Besser, T. E. (1995). Sensitivity of bacteriologic culture for detection of *Escherichia coli* O157:H7 in bovine feces. Journal of Clinical Microbiology. 33: 2616-2619.

48 Sandhu, K.S., Clarke, R.C., McFadden, K., Brouwer, A., Louie, M., Wilson, J., Lior, H., and Gyles, C.L. (1996). Prevalence of the eaeA gene in verotoxigenic *Escherichia coli* strains from dairy cattle in Southwest Ontario. Epidemiology and Infection. 116: 1-7.

49 Schmidt, H., Bielaszewska, M. and Karch, H. (2001). Characterization and typing of non-O157 Shiga toxin producing *Escherichia coli* by molecular methods. Eds. G. Duffy, P. Garvey, J. Coia, Y. Wasteson and D.A. McDowell. Conference Proceedings on 'Epidemiology of Verocytotoxigenic *E. coli*' organised by an EU Concerted Action (CT98-3935) in Malahide, Dublin, Ireland 8-10th February 2001. ISBN 1 84170 147 5. p.124-129.

50 Shah B.V., Holt, M.M. and Folsom, R.E. (1977). Inference about regression models from sample survey data. *Bulletin of the International Statistical Association* 47: 43-57.

51 Sherwood, D., Snodgrass, D.R. and O'Brien, A.D. (1985). Shiga-like toxin production from *Escherichia coli* associated with calf diarrhoea. Veterinary Record. 116: 217-218.

52 Smith, H. R., Willshaw, G. A., Cheasty, T. and O'Brien. S.J. (2001). Verocytotoxin producing *Escherichia coli* in England and Wales. Eds. G. Duffy, P. Garvey, J. Coia, Y. Wasteson and D.A. McDowell. Conference Proceedings on 'Epidemiology of Verocytotoxigenic *E. coli*' organised by an EU Concerted Action (CT98-3935) in Malahide, Dublin, Ireland 8-10th February 2001. ISBN 1 84170 147 5. p.28-34.

53 Synge, B., and G. Paiba. (2000). Verocytotoxin- producing *E. coli* O157. Veterinary Record. 147: 27.

54 Wieler, L.H., Bauerfeind, R., Weiss, R., Pirro, F. and Baljer, G. (1995). Association of enterohemolysin and non-fermentation of rhamnose and sucrose with Shiga-like toxin genes in *Escherichia coli* from calves. Zentralblatt fur Bakteriologie. 282: 265-274.

55 Wieler, L.H., Vieler, E., Erpenstein, C., Schlapp, T., Steinruck, H., Bauerfeind, R., Byomi, A. and Baljer, G. (1996). Shiga toxin producing *Escherichia coli* from bovines: association of adhesion with carriage of *eae* and other genes. Journal of Clinical Microbiology. 34: 2980-2984.

56 WHO. Zoonotic Non-O157 Shiga Toxin-Producing *Escherichia Coli* (STEC). Report of a WHO Scientific Working Group Meeting. Berlin, Germany 23-26 June 1998. http://www.who.int/emc

57 Widiasih, D.A., Ido, N., Omoe, K., Sugii, S. and Shinagawa, K. (2003). Duration and magnitude of faecal shedding of Shiga toxin-producing *Escherichia coli* from naturally infected cattle. Epidemiology and Infection. 132: 67-75.

58 Willshaw, G.A., Cheasty, T., Smith, H.R., O'Brien, S.J. and Adak, G. (2001). Verocytotoxin-producing *Escherichia coli* (VTEC) O157 and other VTEC from human infections in England and Wales 1995-1998. Journal of Medical Microbiology. 50: 135-142.

59 Wray, C., McLaren, I.M., Randall, L.P. and Pearson, G.R. (2000). Natural and experimental infection of normal cattle with *Escherichia coli* O157. Veterinary Record. 147: 65-68.

60 Wright, D.J., Chapman, P.A., and Siddons, C.A. (1994). Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. Epidemiology & Infection. 113: 31-39.

61 Zeger, S.L. and Liang, K.Y. (1986). Longitudinal data-analysis for discrete and continuous outcomes. Biometrics. 42: 121-130.

62 Zhang, W.L., Bielaszewska, M., Bockemuhl, J., Schmidt, H., Scheutz, F. and Karch, H. (2000). Molecular analysis of H antigens reveals that human diarrheagenic *Escherichia coli* O26 strains that carry the *eae* gene belong to the H11 clonal complex. Journal of Clinical Microbiology. 38: 2989-2993.

63 Zhang, W.L., Bielaszewska, M., Liesegang, A., Tschape, H., Schmidt, H., Bitzan, M. and Karch, H. (2000b). Molecular characteristics and epidemiological significance of Shiga toxin-producing *Escherichia coli* O26 strains. Journal of Clinical Microbiology. 38: 2134-2140.

10. TABLES

Table 1. Biochemical identification of bacterial isolates

Substrate	Typical *E. coli*
Lactose	Acid production
D-Mannitol	Acid production
ONPG	O-nitrophenol production
Simmon's Citrate	No reaction
Urea	No reaction
Lysine	Decarboxylation
Ornithine	Variable
Control	No reaction
Cellobiose	No reaction
Sorbitol	Variable
Peptone	Indole production
Malonate/TDA	No reactions
Glucose Iron Agar {	Acid + gas production No H_2S production

Table 2. Sampling strategy for non-O157 VTEC prevalence study

Stage	Description	Sampling type
First	SVS Animal Health Division	Strata
Second	Farm cluster	Cluster
Third	Individual farm	Strata
Fourth	Animal group within farm	Strata
Fifth	Faecal pat samples in group	Strata

Table 3. Approximate sensitivity and specificity of IMS test procedure

Concentration (c.f.u. inoculated *E. coli* g^{-1} faeces)	Serogroup			
	Sensitivity% (approx. 95% CI)			
	E. coli O103	*E. coli* O111	*E. coli* O26	*E. coli 0145*
$1x10^1$	49 (43-55)	40 (13-67)	20 (0-40)	12 (0-30)
$1x10^2$	51 (8-94)	51 (8-94)	31 (13-49)	39 (21-57)
$1x10^3$	73 (39-100)	56 (13-99)	59 (16-100)	52 (11-93)
Specificity%	88 (66-100)	99 (97-100)	100 (100)	96 (88-100)

Table 4. Estimated sensitivity of IMS test for *E. coli* O26

Concentration (c.f.u. inoculated *E. coli* g^{-1} faeces)	Sensitivity%	95% CI
$1x10^2$	33	23 – 45
$1x10^3$	53	44 – 62
$1x10^4$	73	64 – 81
$1x10^5$	86	77 – 92

Table 5. Number of farms sampled, by SVS Animal Health Division and season

	SVS Animal Health Divisions						
Season	Central	South east	South west	Highland	North east	Islands	Total
Spring	9	14	15	12	15	14	79
Summer	12	15	13	15	12	15	82
Autumn	15	14	15	13	15	15	87
Winter	15	15	15	15	15	15	90
Total	51	58	58	55	57	59	338

Table 6. Number of faecal pat samples, by SVS Animal Health Division and season

	SVS Animal Health Divisions						
Season	Central	South east	South west	Highland	North east	Islands	Total
Spring	145	257	269	208	225	187	1291
Summer	222	289	228	280	265	299	1583
Autumn	245	298	251	218	245	207	1464
Winter	300	298	218	300	362	270	1748
Total	912	1142	966	1006	1097	963	6086

Table 7. Summary of identified serogroups from non-O157 VTEC prevalence study

Serogroup	Number of isolates
O26	249
O103	168
O145	43
O2	1
O8	1
O11	5
O36	4
O43	1
O75	3
O77	1
O110	1
O117	5
O126	1
O150	2
O162	2
O168	5
E54071/88	1
O rough	2
O unidentifiable	56

Table 8. Weighted farm level prevalence of *E. coli* O26, O103, O111 and O145 shedding by Scottish cattle closest to sale or slaughter

Serogroup	No. Farms Positive	Weighted Prevalence%	S.E.%	95% CI
O26	68	19.8	2.6	14.6 - 25.0
O103	75	20.3	2.6	15.3 - 25.4
O111	0	0.0	n.a.	n.a.
O145	26	7.1	1.6	4.0 - 10.2
O157	52	15.3	2.3	10.8 – 19.8

Table 9. Weighted farm level prevalence of *E. coli* O26, O103 or O145 shedding by Scottish cattle closest to sale or slaughter by season

Serogroup	Season	Weighted Prevalence%	S.E.%	95% CI
O26	spring	7.4	3.2	0.8 - 14.0
	summer	32.7	6.5	19.3 - 46.1
	autumn	23.2	4.5	13.9 - 32.5
	winter	15.8	5.8	3.8 - 27.7
O103	spring	8.7	4.1	0.1 - 17.3
	summer	27.3	5.8	15.3 - 39.3
	autumn	24.3	5.7	12.6 - 36.0
	winter	20.5	4.7	10.7 - 30.3
O145	spring	4.4	2.7	0.0 - 9.9
	summer	11.2	4.1	2.7 - 19.7
	autumn	8.1	3.6	0.6 - 15.6
	winter	4.8	2.2	0.3 - 9.3

Table 10. Weighted farm level prevalence of *E. coli* O26, O103, O111 or O145 shedding by Scottish cattle closest to sale or slaughter by Animal Health Division

Serogroup	AHD	Weighted Prevalence%	S.E.%	95% CI
O26	Central	27.5	7.7	11.2 - 43.8
	South east	23.3	6.0	10.8 - 35.8
	South west	18.3	5.1	7.6 - 29.0
	Highland	23.6	6.5	10.0 - 37.3
	North east	14.0	6.4	0.6 - 27.5
	Islands	13.3	5.1	2.7 - 24.0
O103	Central	23.5	5.6	11.8 - 35.3
	South east	13.3	3.8	5.5 - 21.2
	South west	16.7	6.2	3.8 - 29.6
	Highland	30.1	6.1	17.1 - 43.1
	North east	17.5	6.4	4.0 - 31.1
	Islands	30.0	6.8	15.8 - 44.2
O145	Central	9.8	4.8	0.0 - 19.9
	South east	6.7	3.9	0.0 - 14.8
	South west	8.3	4.1	0.0 - 16.9
	Highland	16.7	5.6	5.0 - 28.4
	North east	0	n.a.	n.a.
	Islands	5.0	2.7	0.0 - 10.7

Table 11. Multifactorial analysis of risk factors for the recovery of *E. coli* O26 from faecal pats

Season	Samples			Farms			Odds ratios
	Positive	Number tested	%	Positive	Number tested	%*	
Spring	19	1315	1	6	79	8	Reference Class
Summer	86	1583	5	27	82	33	4.0 (1.4 - 11.9)
Autumn	86	1464	6	20	87	23	4.1 (1.3 - 12.9)
Winter	58	1748	3	15	90	17	2.4 (0.8 - 7.5)
AHD	Samples			Farms			Odds ratios
	Positive	Number tested	%	Positive	Number tested	%*	
Central	64	912	7	14	51	27	3.7 (1.4 - 10.2)
South east	68	1166	6	14	58	24	3.2 (1.2 - 9.0)
South west	39	966	4	11	58	19	2.2 (0.8 - 6.3)
Highland	38	1006	4	13	55	24	2.0 (0.8 - 5.0)
North east	21	1097	2	8	57	14	1.0 (0.3 - 3.0)
Islands	19	963	2	8	59	14	Reference Class

* the % figure is not weighted and therefore is not identical to the weighted prevalence % given in Tables 9 and 10.

Table 12. Number of *E. coli* serogroup O26 strains, and frequency by virulence determinant

	Virulence determinants				Number of strains
	*vtx*1	*vtx*2	*Eae*	*ehl*	
	-	-	-	-	15
	+	-	-	-	16
	-	-	+	-	57
	+	+	-	-	7
	+	-	+	-	20
	+	-	-	+	2
	-	-	+	+	55
	+	+	+	-	5
	+	-	+	+	53
	+	+	+	+	19
Number of determinants	122	31	209	129	**249**
%	49.0%	12.4%	83.9%	51.8%	**100%**

Table 13. Number of *E. coli* serogroup O103 strains, and frequency by virulence determinant

	Virulence determinants				Number of strains
	*vtx*1	*vtx*2	*Eae*	*Ehl*	
	-	-	-	-	103
	+	-	-	-	1
	-	-	+	-	2
	-	-	+	+	61
	+	+	+	+	1
Number of determinants	2	1	64	62	**168**
%	1.2%	0.6%	38.1%	36.9%	100%

Table 14. Number of *E. coli* serogroup O145 strains, and frequency by virulence determinant

	Virulence determinants				Number of strains
	*vtx*1	*vtx*2	*Eae*	*Ehl*	
	-	-	-	-	7
	-	-	+	-	8
	-	-	+	+	26
	+	-	+	+	1
	-	+	+	+	1
Number of determinants	1	1	36	28	**43**
%	2.3%	2.3%	83.7%	65.1%	100%

Table 15. Number of *E. coli* serogroup O157 strains, and frequency by virulence determinant

	Virulence determinants			Number of strains
	*vtx*1	*vtx*2	*eae*	
	-	-	-	0
	-	-	+	2
	+	-	+	2
	-	+	+	235
	+	+	+	18
Number of determinants	20	253	257	**257**
%	7.8%	98.4%	100%	100%

Table 16. Weighted farm level prevalence estimates of shedding of *E. coli* serogroup O26 strains, by virulence determinant

Virulence determinants				Number	Prev.%	S.E.%	95% CI
*vtx*1	*vtx*2	*Eae*	*ehl*				
-	-	-	-	8	2.7	1.1	0.5 – 4.9
+	-	-	-	8	2.1	0.8	0.6 – 3.6
-	-	+	-	17	5.9	1.6	2.7 – 9.0
+	+	-	-	3	1.0	0.6	0.0 – 2.2
+	-	+	-	7	1.6	0.6	0.4 – 2.8
+	-	-	+	2	0.6	0.4	0.0 – 1.4
-	-	+	+	18	5.2	1.2	2.8 – 7.6
+	+	+	-	4	1.2	0.8	0.0 – 2.7
+	-	+	+	19	4.5	1.1	2.3 – 6.7
+	+	+	+	9	2.8	1.0	0.7 – 4.8
VT positive serogroup O26				**52**	**10.1**	**1.7**	**6.8 – 13.4**
All serogroup O26				**68**	**19.8**	**2.6**	**14.6 – 25.0**

Table 17. Weighted farm level prevalence estimates of shedding of *E. coli* serogroup O103 strains, by virulence determinant.

Virulence determinants				Number	Prev.%	S.E.%	95% CI
*vtx*1	*vtx*2	*Eae*	*ehl*				
-	-	-	-	51	13.4	1.9	9.7 – 17.1
+	-	-	-	1	0.4	0.4	0.0 – 1.3
-	-	+	-	2	0.6	0.4	0.0 – 1.4
-	-	+	+	31	8.3	1.7	5.0 – 11.6
+	+	+	+	1	0.3	0.3	0.0 – 0.7
VT positive serogroup O103				**2**	**0.7**	**0.5**	**0.3 – 1.6**
All serogroup O103				**75**	**20.3**	**2.6**	**15.3 – 25.4**

Table 18. Weighted farm level prevalence estimates of shedding of *E. coli* serogroup O145 strains, by virulence determinant

Virulence determinants				Number	Prev.%	S.E.%	95% CI
*vtx*1	*vtx*2	*Eae*	*ehl*				
-	-	-	-	4	1.0	0.5	0.0 – 2.0
-	-	+	-	4	1.2	0.9	0.0 – 3.0
-	-	+	+	17	4.4	1.3	1.9 – 6.9
+	-	+	+	1	0.3	0.0	0.0 – 1.0
-	+	+	+	1	0.3	0.3	0.0 – 1.0
VT positive serogroup O145				**2**	**0.7**	**0.5**	**0.2 – 1.6**
All serogroup O145				**26**	**7.1**	**1.6**	**4.0 – 10.2**

Table 19. Weighted farm level prevalence estimates of shedding of *E. coli* serogroup O157 strains, by virulence determinant

Virulence determinants			Number	Prev.%	S.E.%	95% CI
*vtx*1	*vtx*2	*eae*				
-	-	-	0	n.a.	n.a.	n.a.
-	-	+	1	0.4	0.4	0.0 – 1.3
+	-	+	1	0.3	0.3	0.0 – 0.7
-	+	+	46	13.6	2.1	9.5 – 17.8
+	+	+	4	1.3	0.7	0.0 – 2.6
VT positive serogroup O157			**51**	**14.7**	**2.2**	**10.4 – 19.0**
All serogroup O157			**52**	**15.3**	**2.3**	**10.8 – 19.8**

Table 20. Summary of rhamnose fermentation in O26 isolates

	Virulence determinants		
	vtx1	*vtx1+2*	*vtx -ve*
Rhamnose fermentation	3	4	92
No rhamnose fermentation	88	27	35

11. PUBLICATIONS AND PRESENTATIONS

11.1 REFEREED PUBLICATIONS

None. However, three papers are in preparation and should be submitted to peer-review.

11.2 POSTER PRESENTATIONS ARISING FROM FSA FUNDED NON-O157 VTEC PROJECT

1. "Prevalence of faecal shedding on Scottish beef cattle farms of verocytotoxigenic *Escherichia coli* serogroups O26, O103, O111 and O145". Poster presented by **Chris Low** at the 5th International Symposium on 'Shiga Toxin (Verocytotoxin) - Producing *Escherichia coli* infections', Edinburgh 8th-11th June 2003.

2. "Immunomagnetic separation detection of Verocytotoxin-producing Escherichia coli serogroups O26, O103, O111 and O145". Poster presented by **Jude Evans** at the 5th International Symposium on 'Shiga Toxin (Verocytotoxin) - Producing *Escherichia coli* infections', Edinburgh 8th-11th June 2003.

3. "A comparison of media for the isolation of *Escherichia coli* O26". Poster presented by **Hazel Knight** at the 5th International Symposium on 'Shiga Toxin (Verocytotoxin) - Producing *Escherichia coli* infections', Edinburgh 8th-11th June 2003.

4. "Isolation methods for detecting non-O157 VTEC from cattle faeces." Poster presented by **Iain Ogden** at the 5th International Symposium on 'Shiga Toxin (Verocytotoxin) - Producing *Escherichia coli* infections', Edinburgh 8th-11th June 2003.

5. "Unravelling the epidemiology of verocytotoxin producing *Escherichia coli* in Scotland". Poster presented by **George Gunn** at the 10th International Symposium for Veterinary Epidemiology and Economics, Viña del Mar Chile 17th-21st November 2003.

6. "Prevalence of faecal shedding of verocytotoxigenic *Escherichia coli* serogroups O26, O103, O111 and O145 on Scottish cattle farms". Paper presented by **Michael Pearce** at the 10th International Symposium for Veterinary Epidemiology and Economics, Viña del Mar Chile 17th-21st November 2003.

7. "Sensititivity for recovery of *Escherichia coli* O26 from cattle faeces". Poster presented by **Malcolm Hall** at the Annual meeting of the Society for Veterinary Epidemiology and Preventive Medicine, Martigny, Switzerland, 24th-26th March 2004.

11.3 OTHER PUBLICATIONS

Refereed publications arising from the IPRAVE funded non-O157 VTEC projects. NB. These were not from FSA Scotland funded work.

1. Jenkins, C., Chart, H., Cheasty, T., Willshaw, G.A., Pearce, M.C., Foster, G., **Gunn, G.J.**, Smith, H.R., Dougan, G., **Synge, B.A.** and Frankel, G. (2002) Verocytotoxin-producing *Escherichia coli* (VTEC) other than serogroup O157 from Scottish cattle. Veterinary Record. 151:58-60

2. Jenkins, C., Pearce, M.C., Chart, H., Cheasty, T., Willshaw, G.A., **Gunn, G.J.**, Dougan, G., Smith, H.R., **Synge, B.A.** and Frankel, G. (2002) An eight-month study of a population of Verocytotoxigenic *Escherichia coli* (VTEC) in a Scottish cattle herd. Journal of Applied Microbiology. 93:944-953.

3. Jenkins, C., Willshaw, G.A., Cheasty, T., Shaw, D.J., Frankel, G., Dougan, G., **Gunn, G.J.**, Smith, H.R., Paton, A.W. and Paton, J.C. (2003). Distribution of the *saa* gene in strains of Shiga toxin-producing *Escherichia coli* of human and bovine origins. Journal of Clinical Microbiology. 41:1775-1778.

4. Jenkins, C., Pearce, M.C., Smith, A.W., Knight, H.I., Shaw, D.J., Cheasty, T., **Foster, G., Gunn, G.J.**, Dougan, G., Smith, H.R. and Frankel, G. (2003) Detection of *Escherichia coli* serogroups O26, O103, O111 and O145 from bovine faeces using immunomagnetic separation and PCR/DNA probe techniques. Letters in Applied Microbiology. 37:207-212.

5. Jenkins, C., Willshaw, G.A., **Evans, J.**, Cheasty, T., Chart, H., Shaw, D.J., Dougan, G., Frankel, G. and Smith, H.R. (2003) Subtyping of virulence genes in Verocytotoxin-producing *Escherichia coli* (VTEC) other than serogroup O157 associated with disease in the United Kingdom. Journal of Medical Microbiology. 52: 941-947.

6. Pearce, M.C., Jenkins, C., Vali, L., Smith, A.W., Knight, H.I., Cheasty, T., Smith, H.R., **Gunn, G.J.**, Woolhouse, M.E.J., Amyes, S.G.B. and Frankel, G. (2004) Temporal shedding patterns and virulence factors of *Escherichia coli* serogroups O26, O103, O111, O145 and O157 in a cohort of beef calves and their dams. Applied and Environmental Microbiology. 70:1708-1716.

12. DATA DICTIONARY FOR TABLE FSAISOL3_27APR04.XLS

Text	Description	Characteristics	Values	Source of Data
isolno	unique isolate identifier, IPRAVE project	alphanumeric length 12	e.g. WX006275S01K	SAC Inverness IPRAVE project
fsano	unique isolate identifier, FSAS project	alphanumeric length 7	e.g. FSA1000	SAC Inverness
hpano	unique isolate identifier, HPA, Colindale	alphanumeric length 12	e.g. E167966	SAC Inverness
fmcd	farm identifier IPRAVE / FSAS project	alphanumeric length 4	e.g. E033	SAC Inverness IPRAVE project
dos	date of sampling	numeric dd/mm/yyyy	e.g. 01/01/2004	SAC Inverness IPRAVE project
sergrp	Provisional serogroup following slide agglutination	alphanumeric length 4	O26, O103, O111, O145	SAC Inverness
sldagg1	result, primary slide agglutination	alphanumeric length 2	0 = negative 1 = positive nt = not tested	SAC Inverness
sldagg2	result, secondary slide agglutination	alphanumeric length 2	0 = negative 1 = positive nt = not tested ? = indeterminate	SAC Inverness
tubagg	result, tube agglutination	alphanumeric length 6	e.g. 0, 1, 40, 160 nt not tested	SAC Inverness
ecoli	result, *E. coli* confirmation	alphanumeric length 2	1 = *E. coli* confirmed	SAC Inverness
ogrp	result, serogrouping, O antigen, HPA, Colindale	alphanumeric length 9	O antigen NB: this value used for prevalence estimates	SAC Inverness HPA, Colindale
htyp	result, serotyping, H antigen HPA, Colindale	alphanumeric length 6	H antigen NB some results pending	SAC Inverness HPA, Colindale
pcr_vtx1	result, verocytotoxin 1 gene (vtx_1), PCR	alphanumeric length 2	0 = negative 1 = positive	SAC Inverness
pcr_vtx2	result, verocytotoxin 2 gene (vtx_2), PCR	alphanumeric length 2	0 = negative 1 = positive	SAC Inverness
eae	result, intimin gene (*eae*), PCR	Alphanumeric length 2	0 = negative 1 = positive	SAC Inverness
hyl	Result, enterohaemolysin gene (*hyl*), PCR	Alphanumeric length 2	0 = negative 1 = positive	SAC Inverness
rhamnose	result, rhamnose fermentation after 4days incubation	Alphanumeric length 2	0 = negative 1 = positive w = weak nt = not tested	SAC Inverness

isolno	fsano	Hpano	fmcd	dos	sergrp	sldagg1	sldagg2	Tubagg	ecoli	ogrp	htyp	pcr_vtx1	pcr_vtx2	eae	hyl	rhamnose
WX006275 S01K	FSA1000	E167966	E033	30/04/2002	O26	1	1	0	1	O?	H39	0	0	0	0	nt
WX006279 S01M	FSA2000	E167990	E033	30/04/2002	O103	1	0	0	1	O?	H8	0	0	0	0	nt
WX006280 S01M	FSA2001	E167991	E033	30/04/2002	O103	1	0	0	1	O168	H8	0	0	0	0	nt
WX006281 S01M	FSA2002	E167992	E033	30/04/2002	O103	1	0	0	1	O168	H8	0	0	0	0	nt
WX006295 S01W	FSA4000	E168009	E034	30/04/2002	O145	1	nt	0	1	O75	H37	0	0	0	0	nt
WX006301 S01W	FSA4001	E168010	E034	30/04/2002	O145	1	nt	1	1	O145	H37	0	0	0	0	nt
WX006302 S01W	FSA4002	E168011	E034	30/04/2002	O145	1	nt	0	1	O75		0	0	0	0	nt
WX006305 S01W	FSA4003	E168012	E034	30/04/2002	O145	1	nt	0	1	O?	H42	0	0	0	0	nt
WX006306 S01W	FSA4004	E168013	E034	30/04/2002	O145	1	nt	0	1	O75		0	0	0	0	nt
WX006317 S01M	FSA2003	E167993	E035	30/04/2002	O103	1	1	3200	1	O103	H-	0	0	1	1	nt
WX006329 S01W	FSA4005	E168014	E035	30/04/2002	O145	1	nt	0	1	O?	H39	0	0	0	0	nt
WX006850 S01M	FSA2004	E167994	E039	13/05/2002	O103	1	0	0	1	O2	H8	0	0	0	0	nt
WX006858 S01M	FSA2005	E167995	E040	13/05/2002	O103	1	0	0	1	O?	H8	0	0	0	0	nt
WX006875 S01M	FSA2006	E167996	E002	13/05/2002	O103	1	0	0	1	O168	H8	0	0	0	0	nt
WX006876 S01M	FSA2007	E167997	E002	13/05/2002	O103	1	0	0	1	O168	H8	0	0	0	0	nt
WX006878 S01K	FSA1001	E167967	E002	13/05/2002	O26	1	1	0	1	O117		0	0	0	0	nt
WX006882 S01M	FSA2008	E167998	E002	13/05/2002	O103	1	0	0	1	O162	H2	0	0	0	0	nt
WX006886 S01M	FSA2009	E167999	E002	13/05/2002	O103	1	0	0	1	O168	H2	0	0	0	0	nt
WX006984 S01M	FSA2010	E168000	E017	20/05/2002	O103	1	0	0	1	O?	H8	0	0	0	0	nt
WX007035 S01W	FSA4006	E168015	E020	20/05/2002	O145	1	nt	0	1	O126	H20	0	0	0	0	nt
WX007098 S01W	FSA4007	E168016	S001	22/05/2002	O145	1	nt	0	1	O77	H18	0	0	0	0	nt
WX007100 S01K	FSA1002	E167968	S001	22/05/2002	O26	1	1	0	1	O?		0	0	0	0	nt
WX007142 S01K	FSA1003	E167969	E009	22/05/2002	O26	1	1	0	1	O?	H39	0	0	0	0	nt

WX007145 S01K	FSA1004	E167970	E009	22/05/2002	O26	1	1	320	1	O26	H11	0	0	1	0	1
WX007148 S01K	FSA1005	E167971	E009	22/05/2002	O26	1	1	0	1	O?		0	0	0	0	nt
WX007193 S01M	FSA2011	E168001	E304	27/05/2002	O103	1	1	1600	1	O103		0	0	0	0	nt
WX007199 S01K	FSA1006	E167972	E304	27/05/2002	O26	1	1	0	1	O117	H40	0	0	0	0	nt
WX007202 S01K	FSA1007	E167973	E304	27/05/2002	O26	1	1	0	1	O117	H7	0	0	0	0	nt
WX007216 S01K	FSA1008	E167974	E303	27/05/2002	O26	1	0	0	1	O?		0	0	0	0	nt
WX007220 S01K	FSA1009	E167975	E303	27/05/2002	O26	1	0	0	1	O?		0	0	0	0	nt
WX007231 S01W	FSA4008	E168017	E303	27/05/2002	O145	1	nt	0	1	O?	H42	0	0	0	0	nt
WX007270 S01W	FSA4009	E168018	E044	27/05/2002	O145	1	nt	1	1	O145	H37	0	0	0	0	nt
WX007275 S01M	FSA2012	E168002	E044	27/05/2002	O103	1	0	0	1	O?	H8	0	0	0	0	nt
WX007283 S01K	FSA1010	E167976	E044	27/05/2002	O26	1	1	0	1	O43	H2	0	0	0	0	nt
WX007366 S01M	FSA2013	H0 4090 0452	E310	04/06/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
WX007426 S01K	FSA1011	E167977	E047	04/06/2002	O26	1	0	160	1	O26	H11	0	0	1	1	1
WX007587 S01W	FSA4010	E168019	E008	10/06/2002	O145	1	nt	0	1	O rough		0	0	0	0	nt
WX007647 S01M	FSA2014	H0 4090 0453	E006	10/06/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
WX007648 S01M	FSA2015	E168005	E006	10/06/2002	O103	1	1	0	1	O162	H8	0	0	0	0	nt
WX007663 S01M	FSA2016	E168006	E006	10/06/2002	O103	1	0	0	1	O150	H8	0	0	0	0	nt
WX007764 S01K	FSA1012	E167978	E018	12/06/2002	O26	1	1	0	1	O117	H16	0	0	0	0	nt
WX007861 S01K	FSA1013	E167979	E314	12/06/2002	O26	1	1	320	1	O26	H11	0	0	1	0	1
WX007862 S01K	FSA1014	E167980	E314	12/06/2002	O26	1	1	320	1	O26		0	0	1	0	1
WX007866 S01M	FSA2017	E168007	E314	12/06/2002	O103	1	0	0	1	O150	H8	0	0	0	0	nt
WX007868 S01W	FSA4011	E168020	E314	12/06/2002	O145	1	nt	0	1	O11	H25	0	0	0	0	nt
WX007869 S01K	FSA1015	E167981	E314	12/06/2002	O26	1	1	640	1	O26	H11	1	1	1	1	0
WX007881	FSA1016	E167982	E315	12/06/2002	O26	1	1	640	1	O26	H-	0	0	1	0	1

S01K																
WX007895	FSA1017	E167983	E315	12/06/2002	O26	1	1	640	1	O26		0	0	1	0	1
S01K																
WX008003	FSA2018	E168008	E056	02/07/2002	O103	1	0	0	1	O?		0	0	0	0	nt
S01M																
WX008267	FSA1018	E167984	E322	15/07/2002	O26	1	0	0	1	O117		0	0	0	0	nt
S01K																
WX008275	FSA4012	E168021	E322	15/07/2002	O145	1	nt	1	1	O145		0	0	0	0	nt
S01W																
WX008276	FSA4013	E168022	E322	15/07/2002	O145	1	nt	1	1	O145		0	0	0	0	nt
S01W																
WX008277	FSA4014	E168023	E322	15/07/2002	O145	1	nt	1	1	O145	H-	0	0	0	0	nt
S01W																
WX008278	FSA4015	E168024	E322	15/07/2002	O145	1	nt	1	1	O145		0	0	0	0	nt
S01W																
WX008306	FSA4016	E168025	E327	15/07/2002	O145	1	nt	0	1	O?		0	0	0	0	nt
S01W																
WX008325	FSA1019	E167985	E065	22/07/2002	O26	1	1	320	1	O26	H11	1	0	1	1	0
S01K																
WX008326	FSA1020	E167986	E065	22/07/2002	O26	1	1	320	1	O26	H11	1	0	1	1	0
S01K																
WX008327	FSA1021	E167987	E065	22/07/2002	O26	1	1	320	1	O26	H11	1	0	1	1	0
S01K																
WX008336	FSA4017	E168026	E066	22/07/2002	O145	1	nt	0	1	O11	H25	0	0	0	0	nt
S01W																
WX008337	FSA4018	E168027	E066	22/07/2002	O145	1	nt	0	1	O11	H25	0	0	0	0	nt
S01W																
WX008338	FSA4019	E168028	E066	22/07/2002	O145	1	nt	0	1	O11	H25	0	0	0	0	nt
S01W																
WX008341	FSA4020	E168029	E066	22/07/2002	O145	1	0	0	1	O11		0	0	0	0	nt
S01W																
WX008349	FSA1022	E167988	E067	22/07/2002	O26	1	1	320	1	O26	H11	1	0	1	1	0
S01K																
WX008355	FSA1023	E167989	E067	22/07/2002	O26	1	1	640	1	O26	H11	1	0	1	1	0
S01K																
WX008372	FSA4021	E168030	E068	22/07/2002	O145	1	0	0	1	O36	H2	0	0	0	0	nt
S01W																
WX008376	FSA4022	E168031	E068	22/07/2002	O145	1	0	0	1	O36	H2	0	0	0	0	nt
S01W																
WX008382	FSA4023	E168032	E068	22/07/2002	O145	1	0	0	1	O36	H2	0	0	0	0	nt
S01W																
WX008388	FSA4024	E168033	E068	22/07/2002	O145	1	0	0	1	O36	H2	0	0	0	0	nt
S01W																
WX008488	FSA1024	E172747	E332	05/08/2002	O26	1	1	320	1	O26		0	0	1	0	1
S01K																
WX008526	FSA1025		E332	05/08/2002	O26	1	1	0				0	0	0	0	
S01K																

WX008527 S01K	FSA1026		E332	05/08/2002	O26	1	0	0			0	0	0	0	
WX008530 S01W	FSA4025	E176210	E332	05/08/2002	O145	1	1	1	1	O145	0	0	1	1	nt
WX008540 S01W	FSA4026	E176211	E333	05/08/2002	O145	1	1	1	1	O145	0	0	1	1	nt
WX008546 S01W	FSA4027	E176212	E333	05/08/2002	O145	1	1	1	1	O145	0	0	1	1	nt
WX008547 S01W	FSA4028	E176213	E333	05/08/2002	O145	1	1	1	1	O145	0	0	1	1	nt
WX008548 S01W	FSA4029	E176214	E333	05/08/2002	O145	1	1	1	1	O145	0	0	1	1	nt
WX008551 S01W	FSA4030	E176215	E333	05/08/2002	O145	1	1	1	1	O145	0	0	1	1	nt
WX008576 S01K	FSA1027	E172748	E328	29/07/2002	O26	1	1	160	1	O26	0	0	1	1	0
WX008592 S01W	FSA4031	E176216	E329	29/07/2002	O145	1	1	1	1	O145	0	0	1	1	nt
WX008593 S01W	FSA4032	E176217	E329	29/07/2002	O145	1	1	1	1	O145	0	0	1	1	nt
WX008614 S01M	FSA2019	H0 4090 0454	E330	29/07/2002	O103	1	0	0	1	O?	0	0	0	0	nt
WX008650 S01K	FSA1028	E172749	E071	12/08/2002	O26	1	1	640	1	O26	1	0	1	1	0
WX008657 S01K	FSA1029	E172750	E071	12/08/2002	O26	1	1	640	1	O26	1	0	1	1	0
WX008661 S01K	FSA1030	E172751	E071	12/08/2002	O26	1	1	640	1	O26	1	0	1	1	0
WX008703 S01M	FSA2020	H0 4090 0455	E072	12/08/2002	O103	1	0	0	1	O?	0	0	0	0	nt
WX008751 S01M	FSA2021		E073	12/08/2002	O103	1	0	0			0	0	0	0	
WX008758 S01W	FSA4033	E176218	E073	12/08/2002	O145	1	1	1	1	O145	0	0	1	1	nt
WX008782 S01M	FSA2022	E176074	E074	19/08/2002	O103	1	1	3200	1	O103	0	0	1	1	nt
WX008794 S01M	FSA2023		E075	19/08/2002	O103	1	0	0			0	0	1	1	
WX008796 S01K	FSA1031	E172752	E075	19/08/2002	O26	1	1	320	1	O26	1	0	1	1	0
WX008798 S01K	FSA1032	E175753	E075	19/08/2002	O26	1	1	640	1	O26	1	0	1	1	w
WX008801 S01K	FSA1033	E172754	E075	19/08/2002	O26	1	1	640	1	O26	1	0	1	1	0
WX008806 S01M	FSA2024	E176075	E075	19/08/2002	O103	1	1	1600	1	O103	0	0	0	0	nt
WX008807	FSA2025		E075	19/08/2002	O103	1	0	0			0	0	0	0	

S01M															
WX008808	FSA1034	E175755	E075	19/08/2002	O26	1	1	640	1	O26	1	0	1	1	0
S01K															
WX008809	FSA1035	E175756	E075	19/08/2002	O26	1	1	640	1	O26	1	1	1	1	0
S01K															
WX008821	FSA1036	E172757	E076	19/08/2002	O26	1	1	40	1	O26	1	1	1	1	0
S01K															
WX008825	FSA1037	E172758	E076	19/08/2002	O26	1	1	640	1	O26	1	1	1	1	0
S01K															
WX008828	FSA1038	E172759	E076	19/08/2002	O26	1	1	640	1	O26	1	1	1	1	0
S01K															
WX008837	FSA1039	E172760	E076	19/08/2002	O26	1	1	640	1	O26	1	1	1	1	0
S01K															
WX008838	FSA2026	E176076	E076	19/08/2002	O103	1	1	1600	1	O103	0	0	0	0	nt
S01M															
WX008857	FSA1040		E334	19/08/2002	O26	1	0	0			0	0	0	0	
S01K															
WX008859	FSA1041	E172761	E334	19/08/2002	O26	1	1	640	1	O26	1	0	0	0	1
S01K															
WX008865	FSA1042	E172762	E334	19/08/2002	O26	1	1	320	1	O26	1	0	1	1	0
S01K															
WX008933	FSA2027	E176077	E336	19/08/2002	O103	1	1	3200	1	O?	0	0	0	0	nt
S01M															
WX008934	FSA2028	E176078	E336	19/08/2002	O103	1	1	1600	1	O103	0	0	0	0	nt
S01M															
WX008937	FSA2029	E176079	E336	19/08/2002	O103	1	1	1600	1	O103	0	0	0	0	nt
S01M															
WX008997	FSA2030		E077	26/08/2002	O103	1	1	0			0	0	0	0	
S01M															
WX008999	FSA2031	E176080	E077	26/08/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
S01M															
WX009001	FSA1043	E172763	E077	26/08/2002	O26	1	1	640	1	O26	0	0	1	1	1
S01K															
WX009002	FSA1044	E172764	E077	26/08/2002	O26	1	1	640	1	O26	0	0	1	1	1
S01K															
WX009009	FSA1045	E172765	E077	26/08/2002	O26	1	1	640	1	O26	0	0	1	1	1
S01K															
WX009022	FSA2032		E078	26/08/2002	O103	1	0	0			0	0	1	1	
S01M															
WX009026	FSA2033		E078	26/08/2002	O103	1	0	0			0	0	1	1	
S01M															
WX009026	FSA4034	E176219	E078	26/08/2002	O145	1	1	1	1	O145	0	0	1	0	nt
S01W															
WX009028	FSA2034		E078	26/08/2002	O103	1	0	0			0	0	0	0	
S01M															
WX009031	FSA2035	E176081	E078	26/08/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
S01M															

WX009037 S01M	FSA2036		E078	26/08/2002	O103	1	0	0			0	0	0	0		
WX009038 S01W	FSA4035		E078	26/08/2002	O145	1	0	0			0	0	0	0		
WX009039 S01M	FSA2037		E078	26/08/2002	O103	1	0	0			0	0	0	0		
WX009098 S01M	FSA2038	E176082	E079	26/08/2002	O103	1	0	800	1	O103	0	0	1	1	nt	
WX009099 S01W	FSA4036		E079	26/08/2002	O145	1	0	0			0	0	0	0		
WX009102 S01W	FSA4037		E079	26/08/2002	O145	1	0	0			0	0	0	0		
WX009122 S01M	FSA2039	E176083	E337	26/08/2002	O103	1	1	3200	1	O103	0	0	0	0	nt	
WX009128 S01M	FSA2040	E176084	E337	26/08/2002	O103	1	1	3200	1	O103	0	0	0	0	nt	
WX009129 S01M	FSA2041	E176085	E337	26/08/2002	O103	1	1	3200	1	O103	0	0	0	0	nt	
WX009143 S01K	FSA1046	E176766	E338	26/08/2002	O26	1	1	320	1	O26	0	0	1	0	1	
WX009165 S01M	FSA2042		E339	26/08/2002	O103	1	1	0			0	0	0	0		
WX009168 S01K	FSA1047	E176244	E339	26/08/2002	O26	1	1	320	1	O26	1	0	1	1	0	
WX009774 S01M	FSA2043	H0 4090 0459	E081	09/09/2002	O103	1	0	0	1	O?	0	0	1	1	nt	
WX009776 S01M	FSA2044		E081	09/09/2002	O103	1	0	0			0	0	0	0		
WX009779 S01K	FSA1048	E176245	E082	09/09/2002	O26	1	1	80	1	O26	0	0	0	0	1	
WX009791 S01K	FSA1049	E176246	E082	09/09/2002	O26	1	1	80	1	O26	0	0	0	0	1	
WX009801 S01W	FSA4038	E176220	E340	09/09/2002	O145	1	1	1	1	O145	0	0	1	0	nt	
WX009806 S01W	FSA4039	E176221	E340	09/09/2002	O145	1	1	1	1	O145	0	0	1	0	nt	
WX009808 S01W	FSA4040	E176222	E340	09/09/2002	O145	1	1	1	1	O145	0	0	1	0	nt	
WX009812 S01K	FSA1050		E340	09/09/2002	O26	1	0	0			0	0	0	0		
WX009812 S01M	FSA2045		E340	09/09/2002	O103	1	0	0			0	0	0	0		
WX009815 S01W	FSA4041	E176223	E340	09/09/2002	O145	1	1	1	1	O145	0	0	1	0	nt	
WX009816 S01W	FSA4042	E176224	E340	09/09/2002	O145	1	1	1	1	O145	0	0	1	0	nt	
WX009829	FSA1051	E176247	E341	09/09/2002	O26	1	1	320	1	O26	0	0	0	0	1	

S01K																
WX009831	FSA1052	E176248	E341	09/09/2002	O26	1	1	320	1	O26		1	1	0	0	1
S01K																
WX009838	FSA1053	E176249	E341	09/09/2002	O26	1	1	320	1	O26		0	0	0	0	1
S01K																
WX009840	FSA1054	E176250	E341	09/09/2002	O26	1	1	320	1	O26	H-	0	0	1	0	1
S01K																
WX009847	FSA1055	E176251	E341	09/09/2002	O26	1	1	320	1	O26		0	0	0	0	1
S01K																
WX009848	FSA1056	E176252	E341	09/09/2002	O26	1	1	320	1	O26	H-	0	0	1	0	1
S01K																
WX009855	FSA1057	E176253	E341	09/09/2002	O26	1	1	320	1	O26		0	0	0	0	1
S01K																
WX009875	FSA1058	E176254	E342	09/09/2002	O26	1	1	320	1	O26		0	0	0	0	1
S01K																
WX009878	FSA1059	E176255	E342	09/09/2002	O26	1	1	320	1	O26		0	0	1	0	1
S01K																
WX009879	FSA4043	E176225	E342	09/09/2002	O145	1	1	1	1	O145		0	0	1	0	nt
S01W																
WX009911	FSA2046	E176086	E083	16/09/2002	O103	1	1	6400	1	O103		0	0	0	0	nt
S01M																
WX009918	FSA2047	E176087	E084	16/09/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
S01M																
WX009919	FSA2048	E176088	E084	16/09/2002	O103	1	1	1600	1	O103		0	0	0	0	nt
S01M																
WX009920	FSA2049	E176089	E084	16/09/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
S01M																
WX009921	FSA2050	E176090	E084	16/09/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
S01M																
WX009923	FSA4044		E085	16/09/2002	O145	1	0	0				0	0	0	0	
S01W																
WX009926	FSA1060	E176256	E085	16/09/2002	O26	1	1	320	1	O26		1	0	1	0	0
S01K																
WX009927	FSA1061		E085	16/09/2002	O26	1	0	0				0	0	0	0	
S01K																
WX009929	FSA1062		E085	16/09/2002	O26	1	1	0				0	0	0	0	
S01K																
WX009931	FSA1063	E176257	E085	16/09/2002	O26	1	1	640	1	O26	H-	0	0	1	1	1
S01K																
WX009936	FSA1064		E085	16/09/2002	O26	1	1	0				0	0	0	0	
S01K																
WX009940	FSA4045		E085	16/09/2002	O145	1	0	0				0	0	0	0	
S01W																
WX009979	FSA2051	E176091	E086	16/09/2002	O103	1	1	3200	1	O103		0	0	1	1	nt
S01M																
WX009987	FSA4046		E086	16/09/2002	O145	1	0	0				0	0	0	0	
S01W																

WX009988 S01M		FSA2052	E086	16/09/2002	O103	1	1	0			0	0	0	0	
WX010211 S01W		FSA4047	E344	17/09/2002	O145	1	0	0			0	0	0	0	
WX010217 S01W		FSA4048	E344	17/09/2002	O145	1	0	0			0	0	0	0	
WX010228 S01M		FSA2053	E345	17/09/2002	O103	1	1	0			0	0	0	0	
WX010468 S01K		FSA1065	E343	23/09/2002	O26	1	1	0			0	0	0	0	
WX010468 S02K		FSA1066	E343	23/09/2002	O26	1	1	0			0	0	0	0	
WX010474 S01M		FSA2054	E343	23/09/2002	O103	1	0	0			0	0	0	0	
WX010503 S01M	FSA2055	E176092	E347	23/09/2002	O103	1	1	3200	1	O103	0	0	1	1	nt
WX010506 S01M	FSA2056	E176093	E347	23/09/2002	O103	1	1	1600	1	O103	0	0	1	1	nt
WX010509 S01W	FSA4049	E176226	E347	23/09/2002	O145	1	1	1	1	O145	0	0	1	1	nt
WX010511 S01W	FSA4050	E176227	E347	23/09/2002	O145	1	0	1	1	O145	0	0	1	1	nt
WX010516 S01M	FSA2057	E176094	E347	23/09/2002	O103	1	1	6400	1	O103	0	0	1	1	nt
WX010519 S01M	FSA2058	E176095	E347	23/09/2002	O103	1	1	1600	1	O103	0	0	0	0	nt
WX010531 S01K	FSA1067	E176258	E348	23/09/2002	O26	1	1	320	1	O26	1	0	1	0	0
WX010534 S01M	FSA2059	E176096	E348	23/09/2002	O103	1	1	3200	1	O?	0	0	0	0	nt
WX010540 S01M		FSA2060	E349	23/09/2002	O103	1	0	0			0	0	0	0	
WX010541 S01M	FSA2061	E176097	E349	23/09/2002	O103	1	1	800	1	O103	0	0	0	0	nt
WX010547 S01M		FSA2062	E350	24/09/2002	O103	1	1	0			0	0	0	0	
WX010567 S01M		FSA2063	E087	30/09/2002	O103	1	1	0			0	0	0	0	
WX010571 S01M		FSA2064	E087	30/09/2002	O103	1	1	0			0	0	0	0	
WX010632 S01W		FSA4051	E088	30/09/2002	O145	1	0	0			0	0	0	0	
WX010643 S01M	FSA2065	E176098	E089	30/09/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
WX010644 S01M	FSA2066	E176099	E089	30/09/2002	O103	1	1	6400	1	O103	0	0	0	0	nt
WX010645	FSA2067	E176100	E089	30/09/2002	O103	1	1	6400	1	O103	0	0	0	0	nt

S01M																
WX010646	FSA2068	E176101	E089	30/09/2002	O103	1	1	6400	1	O103		0	0	0	0	nt
S01M																
WX010647	FSA2069	E176102	E089	30/09/2002	O103	1	1	6400	1	O103		0	0	0	0	nt
S01M																
WX010653	FSA2070	E176103	E089	30/09/2002	O103	1	1	6400	1	O103		0	0	0	0	nt
S01M																
WX010656	FSA2071	E176104	E089	30/09/2002	O103	1	1	6400	1	O103		0	0	0	0	nt
S01M																
WX010658	FSA1068		E089	30/09/2002	O26	1	1	0				0	0	0	0	
S01K																
WX010660	FSA2072	E176105	E089	30/09/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
S01M																
WX010765	FSA1069	E176259	E093	07/10/2002	O26	1	1	320	1	O26		1	1	1	0	1
S01K																
WX010765	FSA2073	E176106	E093	07/10/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
S01M																
WX010765	FSA2074	E176107	E093	07/10/2002	O103	1	1	6400	1	O103		0	0	0	0	nt
S02M																
WX010766	FSA1070	E176260	E093	07/10/2002	O26	1	1	40	1	O26		0	0	1	0	1
S01K																
WX010767	FSA2075	E176108	E093	07/10/2002	O103	1	1	6400	1	O103		0	0	1	1	nt
S01M																
WX010769	FSA2076	E176109	E093	07/10/2002	O103	1	1	6400	1	O103		0	0	1	1	nt
S01M																
WX010770	FSA2077	E176110	E093	07/10/2002	O103	1	1	6400	1	O103		0	0	1	1	nt
S01M																
WX010771	FSA2078	E176111	E093	07/10/2002	O103	1	1	6400	1	O103		0	0	1	1	nt
S01M																
WX010772	FSA1071	E176261	E093	07/10/2002	O26	1	1	640	1	O26		0	0	1	0	1
S01K																
WX010777	FSA1072	E176262	E093	07/10/2002	O26	1	1	160	1	O26	H-	0	0	1	0	1
S01K																
WX010778	FSA1073	E176263	E093	07/10/2002	O26	1	1	640	1	O26	H-	0	0	1	0	1
S01K																
WX010778	FSA2079	E176112	E093	07/10/2002	O103	1	1	6400	1	O103		0	0	1	1	nt
S01M																
WX010779	FSA1074	E176264	E093	07/10/2002	O26	1	1	160	1	O26		0	0	1	0	1
S01K																
WX010779	FSA2080		E093	07/10/2002	O103	1	1	0				0	0	0	0	
S01M																
WX010782	FSA1075	E176265	E093	07/10/2002	O26	1	1	320	1	O26		0	0	1	0	1
S01K																
WX010782	FSA2081	E176113	E093	07/10/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
S01M																
WX010784	FSA1076	E176266	E093	07/10/2002	O26	1	1	320	1	O26		0	0	1	0	1
S01K																

WX010788 S01M	FSA2082	E176114	E093	07/10/2002	O103	1	1	12800	1	O103		0	0	0	0	nt
WX010847 S01K	FSA1077	E176267	E095	07/10/2002	O26	1	1	40	1	O26		0	0	1	1	0
WX010848 S01K	FSA1078	E176268	E095	07/10/2002	O26	1	1	160	1	O26		1	1	1	0	0
WX011035 S01M	FSA2083		E351	14/10/2002	O103	1	1	0				0	0	0	0	
WX011039 S01W	FSA4052	E176228	E352	14/10/2002	O145	1	0	1	1	O?		0	0	0	0	nt
WX011040 S01W	FSA4053		E352	14/10/2002	O145	1	0	0				0	0	0	0	
WX011062 S01M	FSA2084		E353	14/10/2002	O103	1	1	0				0	0	0	0	
WX011068 S01M	FSA2085	E176115	E353	14/10/2002	O103	1	1	400	1	O?		0	0	0	0	nt
WX011069 S01M	FSA2086		E353	14/10/2002	O103	1	1	0				0	0	0	0	
WX011149 S01M	FSA2087		E358	22/10/2002	O103	1	1	0				0	0	0	0	
WX011152 S01M	FSA2088		E358	22/10/2002	O103	1	1	0				0	0	0	0	
WX011153 S01M	FSA2089		E358	22/10/2002	O103	1	1	0				0	0	0	0	
WX011309 S01M	FSA2090	E176116	E108	05/11/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
WX011310 S01M	FSA2091	E176117	E108	05/11/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
WX011318 S01M	FSA2092	E176118	E108	05/11/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
WX011321 S01K	FSA1079		E108	05/11/2002	O26	1	1	0				0	0	0	0	
WX011323 S01K	FSA1080		E108	05/11/2002	O26	1	1	0				0	0	0	0	
WX011339 S01M	FSA2093	E176119	E110	05/11/2002	O103	1	1	1600	1	O103		0	0	1	1	nt
WX011347 S01M	FSA2094	E176120	E110	05/11/2002	O103	1	1	6400	1	O103		0	0	1	1	nt
WX011348 S01M	FSA2095	E176121	E110	05/11/2002	O103	1	1	3200	1	O103		0	0	1	1	nt
WX011349 S01M	FSA2096	E176122	E110	05/11/2002	O103	1	1	3200	1	O103		0	0	0	0	nt
WX011350 S01M	FSA2097	E176123	E110	05/11/2002	O103	1	1	1600	1	O103		0	0	1	1	nt
WX011352 S01M	FSA2098	E176124	E110	05/11/2002	O103	1	1	6400	1	O103		0	0	0	0	nt
WX011362	FSA1081		E111	11/11/2002	O26	1	1	0				0	0	0	0	

S01K															
WX011365	FSA1082		E111	11/11/2002	O26	1	1	0			0	0	0	0	
S01K															
WX011375	FSA1083	E176269	E112	11/11/2002	O26	1	1	640	1	O26	1	1	1	1	1
S01K															
WX011375	FSA2099	E176125	E112	11/11/2002	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M															
WX011377	FSA2100	E176126	E112	11/11/2002	O103	1	1	6400	1	O103	0	0	1	1	nt
S01M															
WX011378	FSA1084	E176270	E112	11/11/2002	O26	1	1	40	1	O26	1	0	1	0	0
S01K															
WX011378	FSA2101	E176127	E112	11/11/2002	O103	1	1	6400	1	O103	0	0	1	1	nt
S01M															
WX011379	FSA1085	E176271	E112	11/11/2002	O26	1	1	640	1	O26	1	1	1	1	1
S01K															
WX011380	FSA1086	E176272	E112	11/11/2002	O26	1	1	640	1	O26	0	0	1	1	1
S01K															
WX011380	FSA2102	E176128	E112	11/11/2002	O103	1	1	6400	1	O103	0	0	1	1	nt
S01M															
WX011385	FSA1087	E176273	E112	11/11/2002	O26	1	1	80	1	O26	1	0	1	0	0
S01K															
WX011387	FSA2103	E176129	E112	11/11/2002	O103	1	1	6400	1	O103	0	0	1	1	nt
S01M															
WX011390	FSA2104	E176130	E112	11/11/2002	O103	1	1	6400	1	O103	0	0	1	1	nt
S01M															
WX011393	FSA1088	E176274	E112	11/11/2002	O26	1	1	640	1	O26	0	0	1	1	1
S01K															
WX011393	FSA2105	E176131	E112	11/11/2002	O103	1	1	6400	1	O103	0	0	1	1	nt
S01M															
WX011539	FSA2106	E176132	E115	18/11/2002	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M															
WX011566	FSA1089	E176275	E363	18/11/2002	O26	1	1	320	1	O26	1	0	0	0	0
S01K															
WX011567	FSA1090	E176276	E363	18/11/2002	O26	1	1	640	1	O26	1	0	0	0	0
S01K															
WX011568	FSA1091	E176277	E363	18/11/2002	O26	1	1	320	1	O26	1	0	1	0	0
S01K															
WX011568	FSA2107		E363	18/11/2002	O103	1	0	0			0	0	0	0	
S01M															
WX011569	FSA1092	E176278	E363	18/11/2002	O26	1	1	320	1	O26	1	0	1	0	0
S01K															
WX011571	FSA1093	E176279	E363	18/11/2002	O26	1	1	320	1	O26	1	0	1	0	0
S01K															
WX011572	FSA1094	E176280	E363	18/11/2002	O26	1	1	320	1	O26	1	0	1	0	0
S01K															
WX011573	FSA1095	E176281	E363	18/11/2002	O26	1	1	320	1	O26	1	0	1	0	0
S01K															

WX011574 S01K	FSA1096	E176282	E363	18/11/2002	O26	1	1	320	1	O26	1	0	0	0	0
WX011575 S01K	FSA1097	E176283	E363	18/11/2002	O26	1	1	640	1	O26	1	0	0	0	0
WX011575 S01M	FSA2108		E363	18/11/2002	O103	1	0	0			0	0	0	0	
WX011576 S01K	FSA1098	E176284	E363	18/11/2002	O26	1	1	320	1	O26	1	0	1	0	0
WX011577 S01K	FSA1099	E176285	E363	18/11/2002	O26	1	1	320	1	O26	1	0	1	0	0
WX011579 S01K	FSA1100	E176286	E363	18/11/2002	O26	1	1	320	1	O26	1	0	1	0	0
WX011580 S01K	FSA1101	E176287	E363	18/11/2002	O26	1	1	320	1	O26	1	0	1	0	0
WX011582 S01K	FSA1102	E176288	E363	18/11/2002	O26	1	1	640	1	O26	1	0	1	1	0
WX011588 S01M	FSA2109		E364	18/11/2002	O103	1	0	0			0	0	0	0	
WX011594 S01M	FSA2110		E364	18/11/2002	O103	1	0	0			0	0	0	0	
WX011596 S01M	FSA2111	E176133	E365	18/11/2002	O103	1	1	3200	1	O?	0	0	0	0	nt
WX011604 S01Q	FSA3000		E365	18/11/2002	O111	1	1	nt			0	0	0	0	
WX011609 S01Q	FSA3001		E365	18/11/2002	O111	1	0	nt			0	0	0	0	
WX011616 S01M	FSA2112		E365	18/11/2002	O103	1	0	0			0	0	0	0	
WX011643 S01M	FSA2113	E176134	E365	18/11/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
WX011645 S01Q	FSA3002		E365	18/11/2002	O111	1	1	nt			0	0	0	0	
WX011652 S01M	FSA2114	E176135	E365	18/11/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
WX011653 S01M	FSA2115	E176136	E365	18/11/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
WX011655 S01M	FSA2116	E176137	E365	18/11/2002	O103	1	1	3200	1	O103	0	0	1	1	nt
WX011711 S01M	FSA2117	E176138	E117	25/11/2002	O103	1	1	200	1	O?	0	0	1	1	nt
WX011841 S01M	FSA2118		E367	25/11/2002	O103	1	1	0			0	0	0	0	
WX011847 S01K	FSA1103	E176289	E368	25/11/2002	O26	1	1	320	1	O26	0	0	1	0	1
WX011876 S01M	FSA2119	E176139	E120	02/12/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
WX011950	FSA1104	E176290	E122	02/12/2002	O26	1	1	640	1	O26	0	0	1	1	1

S01K															
WX011993	FSA1105	E176291	E124	03/12/2002	O26	1	1	320	1	O26	1	0	0	0	0
S01K															
WX012004	FSA1106	E176292	E003	03/12/2002	O26	1	1	320	1	O26	1	0	1	0	0
S01K															
WX012007	FSA1107	E176293	E003	03/12/2002	O26	1	1	640	1	O26	1	0	1	0	0
S01K															
WX012016	FSA1108	E176294	E003	03/12/2002	O26	1	1	640	1	O26	1	0	1	1	0
S01K															
WX012077	FSA2120	E176140	E126	09/12/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
S01M															
WX012082	FSA2121	E176141	E126	09/12/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
S01M															
WX012083	FSA2122	E176142	E126	09/12/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
S01M															
WX012116	FSA1109	E176295	E127	09/12/2002	O26	1	1	320	1	O26	1	0	1	0	0
S01K															
WX012118	FSA1110	E176296	E127	09/12/2002	O26	1	1	320	1	O26	1	0	1	0	0
S01K															
WX012125	FSA1111	E176297	E127	09/12/2002	O26	1	1	320	1	O26	1	0	1	0	0
S01K															
WX012127	FSA1112	E176298	E127	09/12/2002	O26	1	1	320	1	O26	1	0	1	0	0
S01K															
WX012277	FSA2123	E176143	E133	16/12/2002	O103	1	1	3200	1	O103	0	0	0	0	nt
S01M															
WX012345	FSA1113		E133	16/12/2002	O26	1	0	0			0	0	0	0	
S01K															
WX012372	FSA2124		E370	16/12/2002	O103	1	0	0			0	0	0	0	
S01M															
WX012386	FSA2125	E176144	E371	16/12/2002	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M															
WX012425	FSA2126	E176145	E134	06/01/2003	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M															
WX012428	FSA2127	E176146	E134	06/01/2003	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M															
WX012429	FSA2128	E176147	E134	06/01/2003	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M															
WX012437	FSA2129	E176148	E135	06/01/2003	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M															
WX012438	FSA2130	H0 4090	E135	06/01/2003	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M		0460													
WX012439	FSA2131	E176149	E135	06/01/2003	O103	1	1	3200	1	O103	0	0	0	0	nt
S01M															
WX012440	FSA2132	E176150	E135	06/01/2003	O103	1	1	1600	1	O103	0	0	0	0	nt
S01M															
WX012442	FSA2133	E176151	E135	06/01/2003	O103	1	1	1600	1	O103	0	0	0	0	nt
S01M															

WX012443 S01M	FSA2134	E176152	E135	06/01/2003	O103	1	1	3200	1	O103	0	0	0	0	nt
WX012445 S01M	FSA2135	E176153	E135	06/01/2003	O103	1	1	1600	1	O103	0	0	0	0	nt
WX012446 S01M	FSA2136	E176154	E135	06/01/2003	O103	1	1	3200	1	O103	0	0	0	0	nt
WX012544 S01M	FSA2137	E176155	E374	06/01/2003	O103	1	1	3200	1	O103	0	0	0	0	nt
WX012568 S01W	FSA4054	E176229	E137	13/01/2003	O145	1	1	1	1	O145	1	0	1	1	nt
WX012591 S01K	FSA1114	E176299	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012592 S01K	FSA1115	E176300	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012593 S01K	FSA1116	E176301	E138	13/01/2003	O26	1	1	640	1	O26	0	0	1	1	0
WX012594 S01K	FSA1117	E176302	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012595 S01K	FSA1118	E176303	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012596 S01K	FSA1119		E138	13/01/2003	O26	1	0	0			0	0	0	0	
WX012599 S01K	FSA1120	E176304	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012600 S01K	FSA1121	E176305	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012601 S01K	FSA1122	E176306	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012602 S01K	FSA1123	E176307	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012603 S01K	FSA1124	E176308	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012604 S01K	FSA1125	E176309	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012605 S01K	FSA1126	E176310	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012607 S01K	FSA1127	E176311	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012608 S01K	FSA1128	E176312	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012609 S01K	FSA1129	E176313	E138	13/01/2003	O26	1	1	320	1	O26	0	0	1	1	0
WX012621 S01K	FSA1130	E176314	E139	13/01/2003	O26	1	1	1280	1	O26	0	0	1	0	1
WX012622 S01K	FSA1131	E176315	E139	13/01/2003	O26	1	1	320	1	O26	0	0	1	0	1
WX012624	FSA1132	E176316	E139	13/01/2003	O26	1	1	640	1	O26	0	0	1	0	1

S01K																
WX012628	FSA1133	E176317	E139	13/01/2003	O26	1	1	320	1	O26		0	0	1	0	1
S01K																
WX012630	FSA1134	E176318	E139	13/01/2003	O26	1	1	320	1	O26		0	0	1	0	1
S01K																
WX012759	FSA2138	E176156	E145	20/01/2003	O103	1	1	6400	1	O103		0	0	0	0	nt
S01M																
WX012771	FSA2139	E176157	E145	20/01/2003	O103	1	1	6400	1	O103		0	0	0	0	nt
S01M																
WX012772	FSA2140	E176158	E145	20/01/2003	O103	1	1	1600	1	O103		0	0	0	0	nt
S01M																
WX012880	FSA1135		E151	27/01/2003	O26	1	1	0				0	0	0	0	
S01K																
WX012883	FSA1136		E151	27/01/2003	O26	1	0	0				0	0	0	0	
S01K																
WX012884	FSA1137		E151	27/01/2003	O26	1	0	0				0	0	0	0	
S01K																
WX012885	FSA1138		E151	27/01/2003	O26	1	0	0				0	0	0	0	
S01K																
WX012920	FSA2141	E176159	E153	28/01/2003	O103	1	1	1600	1	O103		0	0	1	1	nt
S01M																
WX012921	FSA2142	E176160	E153	28/01/2003	O103	1	1	1600	1	O103		0	0	1	1	nt
S01M																
WX012923	FSA2143	E176161	E153	28/01/2003	O103	1	1	3200	1	O103		0	0	1	1	nt
S01M																
WX012925	FSA4055	E176230	E153	28/01/2003	O145	1	1	1	1	O145		0	0	1	1	nt
S01W																
WX012927	FSA4056	E176231	E153	28/01/2003	O145	1	1	1	1	O145		0	0	1	1	nt
S01W																
WX012940	FSA1139		E154	28/01/2003	O26	1	0	0				0	0	0	0	
S01K																
WX012941	FSA1140	E176319	E154	28/01/2003	O26	1	1	640	1	O26		0	0	1	1	0
S01K																
WX012941	FSA2144	E176162	E154	28/01/2003	O103	1	1	1600	1	O103		0	0	1	1	nt
S01M																
WX012945	FSA2145	E176163	E154	28/01/2003	O103	1	1	1600	1	O103		0	0	0	0	nt
S01M																
WX012945	FSA4057	E176232	E154	28/01/2003	O145	1	1	1	1	O145		0	0	1	1	nt
S01W																
WX012949	FSA1141	E176320	E154	28/01/2003	O26	1	1	640	1	O26	H-	0	0	1	1	0
S01K																
WX012953	FSA1142	E176321	E154	28/01/2003	O26	1	1	640	1	O26	H-	0	0	1	1	0
S01K																
WX012988	FSA1143	E176322	E375	03/02/2003	O26	1	1	320	1	O26		0	0	0	0	1
S01K																
WX013041	FSA1144	E176323	E377	03/02/2003	O26	1	1	640	1	O26		0	0	1	1	1
S01K																

WX013054 S01M	FSA2146	E176164	E377	03/02/2003	O103	1	1	400	1	O?	0	0	1	1	nt
WX013064 S01M	FSA2147	E176165	E155	04/02/2003	O103	1	1	1600	1	O103	0	0	1	1	nt
WX013066 S01M	FSA2148	E176166	E155	04/02/2003	O103	1	1	1600	1	O103	0	0	1	1	nt
WX013117 S01M	FSA2149	E176167	E157	04/02/2003	O103	1	1	1600	1	O103	0	0	1	1	nt
WX013119 S01W	FSA4058	E176233	E157	04/02/2003	O145	1	1	1	1	O145	0	1	1	1	nt
WX013120 S01M	FSA2150	E176168	E157	04/02/2003	O103	1	1	3200	1	O103	0	0	1	1	nt
WX013689 S01K	FSA1145		E387	03/03/2003	O26	1	1	0			0	0	0	0	
WX014102 S01K	FSA1146	E176324	E161	28/03/2003	O26	1	0	160	1	O?	1	1	0	1	1
WX014105 S01K	FSA1147	E176325	E161	28/03/2003	O26	1	0	160	1	O?	0	0	0	0	1
WX014381 S01M	FSA2151	E176169	E172	23/04/2003	O103	1	1	1600	1	O103	0	0	1	1	nt
WX014439 S01K	FSA1148	E176326	E176	28/04/2003	O26	1	1	320	1	O26	0	0	0	0	0
WX014441 S01K	FSA1149	E176327	E176	28/04/2003	O26	1	1	320	1	O26	1	1	1	0	0
WX014479 S01M	FSA2152	E176170	E402	28/04/2003	O103	1	1	3200	1	O103	1	0	0	0	nt
WX014598 S01K	FSA1150	E176328	E180	05/05/2003	O26	1	0	80	1	O26	1	0	1	1	0
WX014605 S01K	FSA1151	E176329	E180	05/05/2003	O26	1	0	80	1	O26	1	0	1	1	0
WX014608 S01K	FSA1152	E176330	E180	05/05/2003	O26	1	1	80	1	O26	1	0	1	1	0
WX014611 S01K	FSA1153	E176331	E180	05/05/2003	O26	1	1	80	1	O26	1	0	1	1	0
WX014613 S01K	FSA1154	E176332	E180	05/05/2003	O26	1	1	80	1	O26	1	0	1	1	0
WX014657 S01M	FSA2153	E176171	E405	06/05/2003	O103	1	0	0	1	E54071/88	1	1	0	0	nt
WX014706 S01M	FSA2154	E176172	E407	06/05/2003	O103	1	1	800	1	O?	0	0	1	1	nt
WX014743 S01M	FSA2155	E176173	E182	12/05/2003	O103	1	1	6400	1	O103	0	0	0	0	nt
WX014744 S01K	FSA1155	E176333	E182	12/05/2003	O26	1	1	80	1	O26	1	0	0	1	0
WX014745 S01M	FSA2156	E176174	E182	12/05/2003	O103	1	1	6400	1	O103	0	0	0	0	nt
WX014746	FSA2157	E176175	E182	12/05/2003	O103	1	1	1600	1	O103	0	0	0	0	nt

S01M															
WX014748	FSA2158	E176176	E182	12/05/2003	O103	1	1	3200	1	O103	0	0	0	0	nt
S01M															
WX014750	FSA2159	E176177	E182	12/05/2003	O103	1	1	800	1	O103	0	0	0	0	nt
S01M															
WX014751	FSA2160	E176178	E182	12/05/2003	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M															
WX014752	FSA2161	E176179	E182	12/05/2003	O103	1	1	1600	1	O103	0	0	0	0	nt
S01M															
WX014753	FSA2162	E176180	E182	12/05/2003	O103	1	1	1600	1	O103	0	0	0	0	nt
S01M															
WX014755	FSA2163	E176181	E182	12/05/2003	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M															
WX014775	FSA2164	H0 4090	E183	12/05/2003	O103	1	1	1600	1	O103	0	0	0	0	nt
S01M		0461													
WX014829	FSA2165	H0 4090	E408	13/05/2003	O103	1	1	0	1	O?	1	1	0	1	nt
S01M		0462													
WX014851	FSA1156	E176334	E409	13/05/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K															
WX014853	FSA1157	E176335	E409	13/05/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K															
WX014855	FSA1158	E176336	E409	13/05/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K															
WX014856	FSA1159	E176337	E409	13/05/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K															
WX014858	FSA1160	E176338	E409	13/05/2003	O26	1	1	320	1	O26	1	0	1	1	0
S01K															
WX014860	FSA1161	E176339	E409	13/05/2003	O26	1	1	320	1	O26	1	0	1	1	0
S01K															
WX014862	FSA1162	E176340	E409	13/05/2003	O26	1	1	320	1	O26	1	0	1	1	0
S01K															
WX014879	FSA4059	E176234	E409	13/05/2003	O145	1	1	1	1	O145	0	0	1	1	nt
S01W															
WX014898	FSA4060	E176235	E410	13/05/2003	O145	1	1	1	1	O145	0	0	1	1	nt
S01W															
WX014903	FSA4061	E176236	E410	13/05/2003	O145	1	1	1	1	O145	0	0	1	1	nt
S01W															
WX014906	FSA4062	E176237	E410	13/05/2003	O145	1	1	1	1	O145	0	0	1	1	nt
S01W															
WX015064	FSA2166	E176182	E184	19/05/2003	O103	1	1	6400	1	O103	0	0	0	0	nt
S01M															
WX015094	FSA1163	E176341	E185	19/05/2003	O26	1	1	320	1	O26	1	0	1	1	0
S01K															
WX015103	FSA1164	E176342	E185	19/05/2003	O26	1	1	320	1	O26	1	0	1	1	0
S01K															
WX015105	FSA1165	E176343	E185	19/05/2003	O26	1	1	320	1	O26	1	0	1	1	0
S01K															

WX015451 S01W	FSA4063		E416	27/05/2003	O145	1	0	0			0	0	0	0	
WX015794 S01K	FSA1166		E202	16/06/2003	O26	1	1	0			0	0	0	0	
WX015803 S01K	FSA1167		E202	16/06/2003	O26	1	1	0			0	0	0	0	
WX015808 S01M	FSA2167		E202	16/06/2003	O103	1	0	0			0	0	0	0	
WX015856 S01W	FSA4064	E176238	E203	16/06/2003	O145	1	1	1	1	O?	0	0	0	0	nt
WX015864 S01M	FSA2168		E204	16/06/2003	O103	1	1	0			0	0	0	0	
WX015865 S01M	FSA2169	E176183	E204	16/06/2003	O103	1	1	6400	1	O103	0	0	1	0	nt
WX015905 S01M	FSA2170	E176184	E205	17/06/2003	O103	1	1	3200	1	O103	0	0	1	1	nt
WX015906 S01M	FSA2171	E176185	E205	17/06/2003	O103	1	1	3200	1	O103	0	0	1	1	nt
WX015924 S01M	FSA2172	E176186	E206	17/06/2003	O103	1	1	6400	1	O103	0	0	1	1	nt
WX016114 S01M	FSA2173	E176187	E417	23/06/2003	O103	1	1	400	1	O?	0	0	0	0	nt
WX016119 S01M	FSA2174	E176188	E417	23/06/2003	O103	1	1	0	1	O103	1	1	1	1	nt
WX016129 S01M	FSA2175	E176189	E417	23/06/2003	O103	1	1	200	1	O?	0	0	0	0	nt
WX016194 S01M	FSA2176	H0 4090 0463	E214	30/06/2003	O103	1	1	0	1	O?	0	0	1	1	nt
WX016197 S01M	FSA2177	E176190	E214	30/06/2003	O103	1	1	400	1	O103	0	0	1	1	nt
WX016289 S01K	FSA1168	E176344	E421	30/06/2003	O26	1	1	80	1	O26	0	0	1	1	0
WX016290 S01K	FSA1169	E176345	E421	30/06/2003	O26	1	1	80	1	O26	0	0	1	1	0
WX016292 S01K	FSA1170	E176346	E421	30/06/2003	O26	1	1	80	1	O26	0	0	1	1	0
WX016293 S01M	FSA2178	E176191	E421	30/06/2003	O103	1	1	0	1	O?	0	0	0	0	nt
WX016294 S01K	FSA1171	E176347	E421	30/06/2003	O26	1	1	40	1	O26	0	0	1	1	0
WX016295 S01K	FSA1172	E176348	E421	30/06/2003	O26	1	1	40	1	O26	0	0	1	1	0
WX016296 S01K	FSA1173	E176349	E421	30/06/2003	O26	1	1	40	1	O26	0	0	1	1	0
WX016297 S01K	FSA1174	E176350	E421	30/06/2003	O26	1	1	40	1	O26	0	0	1	1	0
WX016299	FSA1175	E176351	E421	30/06/2003	O26	1	1	40	1	O26	0	0	1	1	0

S01K															
WX016300	FSA1176	E176352	E421	30/06/2003	O26	1	1	80	1	O26	0	0	1	1	0
S01K															
WX016301	FSA1177	E176353	E421	30/06/2003	O26	1	1	320	1	O26	0	0	1	1	0
S01K															
WX016303	FSA1178	H0 4090 0394	E421	30/06/2003	O26	1	1	0	1	O26	0	0	1	1	0
S01K															
WX016549	FSA1179		E221	21/07/2003	O26	1	1	0			0	0	0	0	
S01K															
WX016550	FSA2179	E172192	E221	21/07/2003	O103	1	1	1600	1	O103	0	0	1	1	nt
S01M															
WX016552	FSA1180		E221	21/07/2003	O26	1	1	0			0	0	0	0	
S01K															
WX016691	FSA1182	E176355	E224	28/07/2003	O26	1	1	320	1	O26	1	0	0	0	0
S01K															
WX016692	FSA1183	E176356	E224	28/07/2003	O26	1	1	320	1	O26	1	0	0	0	0
S01K															
WX016693	FSA1184	E176357	E224	28/07/2003	O26	1	1	320	1	O26	1	0	1	1	0
S01K															
WX016694	FSA1185	E176358	E224	28/07/2003	O26	1	1	320	1	O26	1	0	1	1	0
S01K															
WX016695	FSA1186	E176359	E224	28/07/2003	O26	1	1	320	1	O26	1	0	1	1	0
S01K															
WX016696	FSA1187	E176360	E224	28/07/2003	O26	1	1	160	1	O26	1	0	0	0	0
S01K															
WX016696	FSA2180	E176193	E224	28/07/2003	O103	1	1	1600	1	O103	0	0	1	1	nt
S01M															
WX016697	FSA1188	E176361	E224	28/07/2003	O26	1	1	320	1	O26	1	0	1	1	0
S01K															
WX016698	FSA1189	E176362	E224	28/07/2003	O26	1	1	320	1	O26	1	0	0	0	0
S01K															
WX016699	FSA1190		E224	28/07/2003	O26	1	1	0			0	0	0	0	
S01K															
WX016700	FSA1191		E224	28/07/2003	O26	1	?	0			0	0	0	0	
S01K															
WX016701	FSA1192	E176363	E224	28/07/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K															
WX016702	FSA1193	E176364	E224	28/07/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K															
WX016703	FSA1194		E224	28/07/2003	O26	1	?	0			0	0	0	0	
S01K															
WX016704	FSA1195	E176365	E224	28/07/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K															
WX016705	FSA1196		E224	28/07/2003	O26	1	1	0			0	0	0	0	
S01K															
WX016706	FSA1197	E176366	E224	28/07/2003	O26	1	1	320	1	O26	1	0	1	0	0
S01K															

WX016706 S01M	FSA2181	E176194	E224	28/07/2003	O103	1	1	1600	1	O103	0	0	1	1	nt
WX016707 S01K	FSA1198	E176367	E224	28/07/2003	O26	1	1	320	1	O26	1	0	1	1	0
WX016732 S01K	FSA1199	E176368	E225	28/07/2003	O26	1	1	40	1	O26	0	0	1	1	1
WX016736 S01K	FSA1200	E176369	E225	28/07/2003	O26	1	1	320	1	O26	0	0	1	1	1
WX016766 S01K	FSA1201	E176370	E226	28/07/2003	O26	1	1	40	1	O26	0	0	0	0	1
WX016771 S01K	FSA1202		E226	28/07/2003	O26	1	1	0			0	0	0	0	
WX016975 S01K	FSA1203	E176371	E429	04/08/2003	O26	1	1	320	1	O26	0	0	1	1	1
WX016979 S01K	FSA1204	E176372	E429	04/08/2003	O26	1	1	160	1	O26	0	0	1	1	1
WX016980 S01K	FSA1205	E176373	E429	04/08/2003	O26	1	1	320	1	O26	0	0	1	1	1
WX016981 S01K	FSA1206	E176374	E429	04/08/2003	O26	1	1	320	1	O26	0	0	1	1	1
WX016986 S01K	FSA1207	E176375	E429	04/08/2003	O26	1	1	80	1	O26	0	0	1	1	1
WX016987 S01K	FSA1208	E176376	E429	04/08/2003	O26	1	1	160	1	O26	0	0	1	1	1
WX016987 S01W	FSA4065	E176239	E429	04/08/2003	O145	1	1	1	1	O145	0	0	1	1	nt
WX016988 S01K	FSA1209	E176377	E429	04/08/2003	O26	1	1	160	1	O26	0	0	1	1	1
WX017035 S01K	FSA1210	E176378	E431	04/08/2003	O26	1	1	320	1	O26	0	0	1	0	1
WX017035 S01M	FSA2182	E176195	E431	04/08/2003	O103	1	1	1600	1	O103	0	0	0	0	nt
WX017042 S01K	FSA1211	E176379	E431	04/08/2003	O26	1	1	320	1	O26	0	0	1	0	1
WX017043 S01M	FSA2183	E176196	E431	04/08/2003	O103	1	1	3200	1	O103	0	0	0	0	nt
WX017303 S01M	FSA2184	E176197	E227	10/08/2003	O103	1	1	3200	1	O103	0	0	1	0	nt
WX017326 S01M	FSA2185	E176198	E228	11/08/2003	O103	1	1	1600	1	O103	0	0	1	1	nt
WX017359 S01K	FSA1212	E176380	E228	11/08/2003	O26	1	1	160	1	O26	1	0	1	1	0
WX017382 S01M	FSA2186	E176199	E229	11/08/2003	O103	1	1	800	1	O103	0	0	0	0	nt
WX017383 S01K	FSA1213	E176381	E229	11/08/2003	O26	1	1	160	1	O26	1	0	1	1	0
WX017383	FSA2187	E176200	E229	11/08/2003	O103	1	1	1600	1	O103	0	0	0	0	nt

S01M																
WX017384	FSA1214	E176382	E229	11/08/2003	O26	1	1	320	1	O26		1	0	0	0	0
S01K																
WX017385	FSA2188	E176201	E229	11/08/2003	O103	1	1	1600	1	O103		0	0	0	0	nt
S01M																
WX017404	FSA2189	E176202	E229	11/08/2003	O103	1	1	1600	1	O103		0	0	0	0	nt
S01M																
WX017419	FSA2190		E229	11/08/2003	O103	1	1	0				0	0	0	0	
S01M																
WX017422	FSA2191	E176203	E229	11/08/2003	O103	1	1	3200	1	O103		0	0	1	1	nt
S01M																
WX017426	FSA2192	E176204	E229	11/08/2003	O103	1	1	1600	1	O103		0	0	0	0	nt
S01M																
WX017427	FSA4066	E176240	E229	11/08/2003	O145	1	1	1	1	O145		0	0	1	1	nt
S01W																
WX017431	FSA2193	E176205	E229	11/08/2003	O103	1	1	3200	1	O103		0	0	0	0	nt
S01M																
WX017534	FSA2194	E176206	E232	11/08/2003	O103	1	1	3200	1	O103		0	0	0	0	nt
S01M																
WX017535	FSA2195	H0 4090	E232	11/08/2003	O103	1	1	0	1	O103		0	0	1	1	nt
S01M		0464														
WX017536	FSA2196	E176207	E232	11/08/2003	O103	1	1	3200	1	O103		0	0	0	0	nt
S01M																
WX017539	FSA2197	H0 4090	E232	11/08/2003	O103	1	1	0	1	O103		0	0	1	1	nt
S01M		0465														
WX017542	FSA2198	H0 4090	E232	11/08/2003	O103	1	1	0	1	O103		0	0	1	1	nt
S01M		0466														
WX017543	FSA2199	H0 4090	E232	11/08/2003	O103	1	1	0	1	O103		0	0	1	1	nt
S01M		0467														
WX017622	FSA1215	E176383	E234	18/08/2003	O26	1	1	160	1	O26		1	0	0	0	0
S01K																
WX017686	FSA1216	E176384	E432	18/08/2003	O26	1	1	160	1	O26		0	0	1	0	1
S01K																
WX017689	FSA1217	E176385	E432	18/08/2003	O26	1	1	160	1	O26		0	0	1	0	1
S01K																
WX017690	FSA1218		E432	18/08/2003	O26	1	0	0				0	0	0	0	
S01K																
WX017694	FSA1219	E176386	E432	18/08/2003	O26	1	1	160	1	O26	H-	0	0	1	0	1
S01K																
WX017695	FSA1220	E176387	E432	18/08/2003	O26	1	1	320	1	O26		0	0	1	0	1
S01K																
WX017698	FSA1221	E176388	E432	18/08/2003	O26	1	1	160	1	O26		0	0	1	0	1
S01K																
WX017700	FSA1222	E176389	E432	18/08/2003	O26	1	1	320	1	O26	H-	0	0	1	0	1
S01K																
WX017702	FSA1223	E176390	E432	18/08/2003	O26	1	1	320	1	O26		0	0	1	0	1
S01K																

WX017703 S01K	FSA1224	E176391	E432	18/08/2003	O26	1	1	320	1	O26	0	0	1	0	1
WX017721 S01M	FSA2200	E176208	E433	18/08/2003	O103	1	1	3200	1	O103	0	0	0	0	nt
WX017726 S01M	FSA2201	E176209	E433	18/08/2003	O103	1	1	nt	1	O?	0	0	0	0	nt
WX017769 S01W	FSA4067		E434	18/08/2003	O145	1	0	0			0	0	0	0	
WX017770 S01W	FSA4068		E434	18/08/2003	O145	1	0	0			0	0	0	0	
WX017804 S01W	FSA4069	E176241	E236	25/08/2003	O145	1	1	1	1	O145	0	0	1	1	nt
WX017807 S01K	FSA1225	E176392	E236	25/08/2003	O26	1	1	320	1	O26	0	0	1	1	1
WX017842 S01K	FSA1226	E176393	E237	25/08/2003	O26	1	1	640	1	O26	1	0	1	1	0
WX017869 S01W	FSA4070	E176242	E238	25/08/2003	O145	1	1	1	1	O145	0	0	1	1	nt
WX017872 S01K	FSA1227	E176394	E238	25/08/2003	O26	1	0	160	1	O110	0	0	0	0	1
WX017872 S01M	FSA2202	H0 4090 0468	E238	25/08/2003	O103	1	1	200	1	O103	0	0	0	0	nt
WX017873 S01K	FSA1228	H0 4090 0395	E238	25/08/2003	O26	1	1	0	1	O26	1	1	1	1	0
WX017876 S01K	FSA1229	E176395	E238	25/08/2003	O26	1	1	160	1	O26	1	0	1	1	0
WX017878 S01K	FSA1230	E176396	E238	25/08/2003	O26	1	1	160	1	O26	0	0	1	0	1
WX017881 S01K	FSA1231	E176397	E238	25/08/2003	O26	1	1	160	1	O26	0	0	1	0	1
WX017941 S01W	FSA4071		E239	01/09/2003	O145	1	0	0			0	0	0	0	
WX017991 S01M	FSA2203	H0 4090 0469	E435	01/09/2003	O103	1	1	200	1	O103	0	0	0	0	nt
WX017992 S01M	FSA2204	H0 4090 0470	E435	01/09/2003	O103	1	1	200	1	O103	0	0	1	1	nt
WX017993 S01M	FSA2205	H0 4090 0471	E435	01/09/2003	O103	1	0	0	1	O103	0	0	1	1	nt
WX017997 S01M	FSA2206	H0 4090 0472	E435	01/09/2003	O103	1	0	0	1	O103	0	0	1	1	nt
WX017999 S01M	FSA2207	H0 4090 0473	E435	01/09/2003	O103	1	0	0	1	O103	0	0	1	1	nt
WX018002 S01K	FSA1232	E176398	E435	01/09/2003	O26	1	1	160	1	O26	0	0	1	0	1
WX018003 S01M	FSA2208	H0 4090 0481	E435	01/09/2003	O103	1	1	0	1	O103	0	0	1	1	nt
WX018086	FSA2209	H0 4090	E242	08/09/2003	O103	1	1	1600	1	O?	0	0	0	0	nt

S01M		0482														
WX018099	FSA1233	E176399	E242	08/09/2003	O26	1	1	160	1	O26		1	0	0	0	0
S01K																
WX018111	FSA1234	E176400	E242	08/09/2003	O26	1	1	160	1	O26		1	0	0	0	0
S01K																
WX018112	FSA1235	E176401	E242	08/09/2003	O26	1	1	160	1	O26		1	0	0	0	0
S01K																
WX018114	FSA2210	H0 4090	E243	08/09/2003	O103	1	1	200	1	O103		0	0	0	0	nt
S01M		0483														
WX018153	FSA4072	E176243	E244	07/09/2003	O145	1	1	1	1	O145		0	0	1	0	nt
S01W																
WX018159	FSA4073	H0 4090	E244	07/09/2003	O145	1	0	0	1	O145		0	0	0	0	nt
S01W		0515														
WX018172	FSA1236	E176402	E437	08/09/2003	O26	1	1	320	1	O26		0	0	1	1	1
S01K																
WX018173	FSA1237	E176403	E437	08/09/2003	O26	1	1	160	1	O26		0	0	1	0	1
S01K																
WX018174	FSA1238	E176404	E437	08/09/2003	O26	1	1	320	1	O26		0	0	1	0	1
S01K																
WX018175	FSA1239	E176405	E437	08/09/2003	O26	1	1	160	1	O26		0	0	1	0	1
S01K																
WX018177	FSA1240	E176406	E437	08/09/2003	O26	1	1	320	1	O26		0	0	1	0	1
S01K																
WX018178	FSA1241	E176407	E437	08/09/2003	O26	1	1	160	1	O26		0	0	1	0	1
S01K																
WX018179	FSA1242	E176408	E437	08/09/2003	O26	1	1	320	1	O26	H-	0	0	1	0	1
S01K																
WX018182	FSA1243	E176409	E437	08/09/2003	O26	1	1	320	1	O26		0	0	1	0	1
S01K																
WX018183	FSA1244	E176410	E437	08/09/2003	O26	1	1	320	1	O26	H-	0	0	1	0	1
S01K																
WX018184	FSA1245	E176411	E437	08/09/2003	O26	1	1	160	1	O26	H-	0	0	1	0	1
S01K																
WX018186	FSA1246	E176412	E437	08/09/2003	O26	1	1	160	1	O26	H-	0	0	1	0	1
S01K																
WX018187	FSA1247	E176413	E437	08/09/2003	O26	1	1	320	1	O26	H-	0	0	1	0	1
S01K																
WX018188	FSA1248	E176414	E437	08/09/2003	O26	1	1	320	1	O26	H-	0	0	1	0	1
S01K																
WX018188	FSA4074	H0 4090	E437	08/09/2003	O145	1	0	0	1	O8		0	0	0	0	nt
S01W		0516														
WX018189	FSA1249	E176415	E437	08/09/2003	O26	1	1	320	1	O26	H-	0	0	1	0	1
S01K																
WX018190	FSA1250	E176416	E437	08/09/2003	O26	1	1	320	1	O26		0	0	1	0	1
S01K																
WX018191	FSA1251	E176417	E437	08/09/2003	O26	1	1	160	1	O26	H-	0	0	1	0	1
S01K																

WX018192 S01K	FSA1252	E176418	E437	08/09/2003	O26	1	1	320	1	O26		0	0	1	0	1
WX018193 S01K	FSA1253	E176419	E437	08/09/2003	O26	1	1	320	1	O26		0	0	1	0	1
WX018194 S01K	FSA1254	E176420	E437	08/09/2003	O26	1	1	160	1	O26		0	0	1	0	1
WX018195 S01K	FSA1255	E176421	E437	08/09/2003	O26	1	1	320	1	O26	H-	0	0	1	0	1
WX018230 S01K	FSA1256	E176422	E438	08/09/2003	O26	1	1	160	1	O26		0	0	1	1	0
WX018243 S01K	FSA1257	E176423	E438	08/09/2003	O26	1	1	320	1	O26		0	0	1	1	1
WX018337 S01M	FSA2211	H0 4090 0484	E246	15/09/2003	O103	1	1	1600	1	O103		0	0	0	0	nt
WX018401 S01M	FSA2212	H0 4090 0485	E250	16/09/2003	O103	1	1	400	1	O103		0	0	0	0	nt
WX018534 S01M	FSA2213	H0 4090 0487	E439	22/09/2003	O103	1	1	800	1	O103		0	0	1	1	nt
WX018542 S01W	FSA4075	H0 4090 0517	E439	22/09/2003	O145	1	0	0	1	O145		0	0	1	1	nt
WX018551 S01M	FSA2214	H0 4090 0488	E440	22/09/2003	O103	1	1	3200	1	O103		0	0	0	0	nt
WX018555 S01M	FSA2215	H0 4090 0489	E440	22/09/2003	O103	1	0	0	1	O?		0	0	0	0	nt
WX018556 S01M	FSA2216	H0 4090 0490	E440	22/09/2003	O103	1	1	1600	1	O103		0	0	0	0	nt
WX018558 S01M	FSA2217	H0 4090 0491	E441	22/09/2003	O103	1	1	1600	1	O103		0	0	1	1	nt
WX018561 S01M	FSA2218	H0 4090 0492	E441	22/09/2003	O103	1	1	200	1	O103		0	0	1	1	nt
WX018857 S01M	FSA2219	H0 4090 0493	E442	06/10/2003	O103	1	0	nt	1	O rough		0	0	0	0	nt
WX018873 S01W	FSA4076	H0 4090 0518	E442	06/10/2003	O145	1	0	0	1	O?		0	0	0	0	nt
WX018891 S01K	FSA1258	H0 4090 0396	E443	06/10/2003	O26	1	1	320	1	O26		1	1	1	1	0
WX018911 S01K	FSA1259	H0 4090 0397	E444	06/10/2003	O26	1	0	0	1	O26		0	0	0	0	1
WX018922 S01M	FSA2220	H0 4090 0494	E444	06/10/2003	O103	1	1	3200	1	O103		0	0	0	0	nt
WX018966 S01W	FSA4077	H0 4090 0519	E261	13/10/2003	O145	1	0	0	1	O?		0	0	0	0	nt
WX018969 S01K	FSA1260	H0 4090 0398	E261	13/10/2003	O26	1	1	0	1	O?		0	0	0	0	1
WX018991 S01M	FSA2221	H0 4090 0495	E262	13/10/2003	O103	1	1	1600	1	O103		0	0	1	1	nt
WX019040	FSA2222	H0 4090	E264	20/10/2003	O103	1	1	3200	1	O103		0	0	1	1	nt

S01M		0496													
WX019042	FSA2223	H0 4090	E264	20/10/2003	O103	1	1	800	1	O103	0	0	1	1	nt
S01M		0497													
WX019055	FSA1261	H0 4090	E265	20/10/2003	O26	1	1	80	1	O26	0	0	1	1	0
S01K		0399													
WX019055	FSA4078	H0 4090	E265	20/10/2003	O145	1	1	1	1	O145	0	0	1	1	nt
S01W		0520													
WX019091	FSA1262	H0 4090	E445	20/10/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K		0400													
WX019092	FSA1263	H0 4090	E445	20/10/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K		0401													
WX019095	FSA1264	H0 4090	E445	20/10/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K		0402													
WX019098	FSA1265	H0 4090	E445	20/10/2003	O26	1	1	160	1	O26	1	0	1	1	0
S01K		0403													
WX019099	FSA1266	H0 4090	E445	20/10/2003	O26	1	1	160	1	O26	0	0	1	1	0
S01K		0404													
WX019119	FSA4079	H0 4090	E446	20/10/2003	O145	1	1	1	1	O145	0	0	1	1	nt
S01W		0521													
WX019156	FSA2224	H0 4090	E447	20/10/2003	O103	1	1	800	1	O103	0	0	0	0	nt
S01M		0498													
WX019193	FSA1267	H0 4090	E267	27/10/2003	O26	1	1	320	1	O26	0	0	0	0	1
S01K		0405													
WX019194	FSA1268	H0 4090	E267	27/10/2003	O26	1	1	320	1	O26	0	0	0	0	1
S01K		0406													
WX019197	FSA1269	H0 4090	E267	27/10/2003	O26	1	1	320	1	O26	0	0	0	0	1
S01K		0407													
WX019199	FSA1270	H0 4090	E267	27/10/2003	O26	1	1	320	1	O26	1	0	0	0	1
S01K		0408													
WX019203	FSA1271	H0 4090	E267	27/10/2003	O26	1	1	320	1	O26	0	0	0	0	1
S01K		0409													
WX019211	FSA2225	H0 4090	E268	27/10/2003	O103	1	1	800	1	O103	0	0	1	1	nt
S01M		0499													
WX019452	FSA2226	H0 4090	E272	10/11/2003	O103	1	0	0	1	O?	0	0	0	0	nt
S01M		0500													
WX019482	FSA1272	H0 4090	E273	10/11/2003	O26	1	1	320	1	O?	1	1	1	1	w
S01K		0412													
WX019484	FSA1273	H0 4090	E273	10/11/2003	O26	1	1	320	1	O26	1	1	1	1	0
S01K		0413													
WX019490	FSA1274	H0 4090	E273	10/11/2003	O26	1	1	320	1	O26	1	1	1	1	0
S01K		0414													
WX019707	FSA1275	H0 4090	E451	01/12/2003	O26	1	1	0	1	O?	0	0	0	0	1
S01K		0415													
WX019709	FSA1276	H0 4090	E451	01/12/2003	O26	1	0	0	1	O?	0	0	0	0	1
S01K		0416													
WX019710	FSA1277	H0 4090	E451	01/12/2003	O26	1	1	0	1	O?	0	0	0	0	1
S01K		0417													

WX019711 S01K	FSA1278	H0 4090 0418	E451	01/12/2003	O26	1	1	0	1	O?	0	0	0	0	1
WX019711 S01M	FSA2227	H0 4090 0501	E451	01/12/2003	O103	1	1	800	1	O103	0	0	1	1	nt
WX019712 S01K	FSA1279	H0 4090 0419	E451	01/12/2003	O26	1	0	0	1	O?	0	0	0	0	1
WX019713 S01K	FSA1280	H0 4090 0420	E451	01/12/2003	O26	1	0	0	1	O?	0	0	0	0	1
WX019714 S01M	FSA2228	H0 4090 0502	E451	01/12/2003	O103	1	1	400	1	O103	0	0	1	1	nt
WX019715 S01K	FSA1281	H0 4090 0421	E451	01/12/2003	O26	1	1	0	1	O?	0	0	0	0	1
WX019732 S01M	FSA2229	H0 4090 0503	E453	01/12/2003	O103	1	1	1600	1	O103	0	0	0	0	nt
WX019787 S01K	FSA1282	H0 4090 0422	E455	02/12/2003	O26	1	1	0	1	O?	0	0	0	0	1
WX019908 S01K	FSA1283	H0 4090 0423	E279	10/12/2003	O26	1	1	160	1	O26	1	1	0	0	0
WX019909 S01K	FSA1284	H0 4090 0424	E279	10/12/2003	O26	1	1	160	1	O26	1	1	1	0	0
WX019910 S01K	FSA1285	H0 4090 0425	E279	10/12/2003	O26	1	1	640	1	O26	1	1	1	1	0
WX019912 S01K	FSA1286	H0 4090 0426	E279	10/12/2003	O26	1	1	160	1	O26	1	1	1	0	0
WX019912 S01M	FSA2230	H0 4090 0504	E279	10/12/2003	O103	1	1	1600	1	O103	0	0	1	1	nt
WX019913 S01K	FSA1287	H0 4090 0427	E279	10/12/2003	O26	1	1	320	1	O26	1	1	1	1	0
WX019914 S01K	FSA1288	H0 4090 0428	E279	10/12/2003	O26	1	1	320	1	O26	1	1	0	0	0
WX019915 S01M	FSA2231	H0 4090 0505	E279	10/12/2003	O103	1	1	1600	1	O103	0	0	0	0	nt
WX019916 S01K	FSA1289	H0 4090 0429	E279	10/12/2003	O26	1	1	320	1	O26	1	1	0	0	0
WX019916 S01W	FSA4080	H0 4090 0522	E279	10/12/2003	O145	1	1	1	1	O145	0	0	1	1	nt
WX019917 S01K	FSA1290	H0 4090 0430	E279	10/12/2003	O26	1	1	160	1	O26	1	1	0	0	0
WX019921 S01K	FSA1291	H0 4090 0431	E279	10/12/2003	O26	1	1	320	1	O26	1	1	0	0	0
WX019966 S01M	FSA2232	H0 4090 0506	E280	10/12/2003	O103	1	1	3200	1	O103	0	0	0	0	nt
WX020066 S01M	FSA2233	H0 4090 0507	E284	15/12/2003	O103	1	1	800	1	O103	0	0	0	0	nt
WX020067 S01M	FSA2234	H0 4090 0508	E284	15/12/2003	O103	1	1	800	1	O103	0	0	0	0	nt
WX020070	FSA2235	H0 4090	E284	15/12/2003	O103	1	1	800	1	O103	0	0	0	0	nt

S01M		0509													
WX020160	FSA2236	H0 4090	E290	05/01/2004	O103	1	1	0	1	O103	0	0	0	0	nt
S01M		0510													
WX020191	FSA1292	H0 4090	E291	05/01/2004	O26	1	nt	0	1	O?	0	0	0	0	1
S01K		0432													
WX020196	FSA1293	H0 4090	E291	05/01/2004	O26	1	nt	0	1	O?	0	0	0	0	1
S01K		0433													
WX020197	FSA1294	H0 4090	E291	05/01/2004	O26	1	nt	0	1	O?	0	0	0	0	1
S01K		0434													
WX020436	FSA1295	H0 4090	E295	12/01/2004	O26	1	nt	0	1	O?	0	0	0	0	1
S01K		0435													
WX020463	FSA1296	H0 4090	E463	12/01/2004	O26	1	nt	0	1	O?	0	0	0	0	1
S01K		0436													
WX020495	FSA1297	H0 4090	E465	12/01/2004	O26	1	nt	0	1	O26	1	0	1	1	0
S01K		0437													
WX020498	FSA1298	H0 4090	E465	12/01/2004	O26	1	nt	160	1	O26	1	0	1	1	0
S01K		0438													
WX020499	FSA1299	H0 4090	E465	12/01/2004	O26	1	nt	320	1	O26	1	0	1	1	0
S01K		0439													
WX020502	FSA1300	H0 4090	E465	12/01/2004	O26	1	nt	160	1	O26	1	0	1	1	0
S01K		0440													
WX020508	FSA1301	H0 4090	E465	12/01/2004	O26	1	nt	160	1	O26	1	0	1	1	0
S01K		0441													
WX020530	FSA2237	H0 4090	E296	19/01/2004	O103	1	1	800	1	O103	0	0	0	0	nt
S01M		0511													
WX020531	FSA2238	H0 4090	E296	19/01/2004	O103	1	1	400	1	O103	0	0	0	0	nt
S01M		0512													
WX020535	FSA2239	H0 4090	E296	19/01/2004	O103	1	1	200	1	O103	0	0	1	1	nt
S01M		0513													
WX020560	FSA1302	H0 4090	E296	19/01/2004	O26	1	nt	320	1	O26	1	0	0	1	0
S01K		0442													
WX020561	FSA1303	H0 4090	E296	19/01/2004	O26	1	nt	320	1	O26	1	0	1	1	0
S01K		0443													
WX020653	FSA2240	H0 4090	E466	19/01/2004	O103	1	1	800	1	O103	0	0	0	0	nt
S01M		0514													
WX020712	FSA4081	H0 4090	E468	19/01/2004	O145	1	0	0	1	O?	0	0	0	0	nt
S01W		0523													
WX020721	FSA4082		E468	19/01/2004	O145	1	0	0			0	0	0	0	
S01W															
WX020758	FSA1304	H0 4090	E299	26/01/2004	O26	1	nt	320	1	O26	1	1	0	0	0
S01K		0444													
WX020761	FSA1305	H0 4090	E299	26/01/2004	O26	1	nt	160	1	O26	1	1	1	1	0
S01K		0445													
WX020762	FSA1306	H0 4090	E299	26/01/2004	O26	1	nt	160	1	O26	1	1	1	1	0
S01K		0446													
WX020763	FSA1307	H0 4090	E299	26/01/2004	O26	1	nt	160	1	O26	1	1	1	1	0
S01K		0447													

WX020764 S01K	FSA1308	H0 4090 0448	E299	26/01/2004	O26	1	nt	640	1	O26	1	1	1	1	0
WX020765 S01K	FSA1309	H0 4090 0449	E299	26/01/2004	O26	1	nt	160	1	O26	1	1	1	1	0
WX020782 S01K	FSA1310	H0 4090 0450	E478	26/01/2004	O26	1	nt	160	1	O26	0	0	1	0	1
WX020788 S01K	FSA1311	H0 4090 0451	E479	26/01/2004	O26	1	nt	160	1	O26	0	0	1	0	1