

Verocytotoxigenic *Escherichia coli* O157 in animals on public amenity premises in England and Wales, 1997 to 2007

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At the request of the public health authorities, 31 public amenity premises in England and Wales containing animals of various species were investigated for the presence of verocytotoxigenic *Escherichia coli* (VTEC) O157 between 1997 and 2007, because of putative associations with human cases. VTEC O157 was confirmed in one or more species on 19 (61.3 per cent) of the premises. There were significant associations between the presence of VTEC O157 and the number of species sampled, the size of the enterprise, the presence of young cattle and the presence of adult pigs. *E. coli* O157 was isolated from 305 (17.8 per cent) of 1715 samples taken from all the premises, and verocytotoxin genes were detected by PCR in 184 (98.4 per cent) of 187 representative isolates. On positive premises, the highest mean proportion of positive samples (29.0 per cent) was in cattle, followed by sheep (24.4 per cent), donkeys (14.6 per cent), pigs (14.3 per cent), horses (12.3 per cent) and goats (9.9 per cent). A high proportion of positive samples was obtained from camelid species sampled on three of the premises. The main phage types (PT) were 2 and 21/28, which were those most commonly isolated from human cases during the same period.

A single PT was detected on 14 of the 19 positive premises, with up to six different species having the same PT.

VEROCYTOTOXIGENIC *Escherichia coli* (VTEC) O157 has emerged as an important human pathogen during the past 25 years. It causes a broad range of human illnesses, including diarrhoea, haemorrhagic colitis and severe systemic complications, particularly haemolytic uraemic syndrome (Lynn and others 2005). Most cases are sporadic and unattributable, although case-control studies have indicated that contact with animals is a major risk factor (Locking and others 2001, O'Brien and others 2001). Infection may be acquired on commercial livestock farms through occupational or residential exposure or the consumption of contaminated unpasteurised milk; cases have also been associated with private farm visits by children (Willshaw and others 2003). Of greater significance are the clearly defined outbreaks that have occurred in England (Milne and others 1999, Pritchard and others 2000, 2001) and elsewhere, including the USA and Canada (Lejeune and Davis 2004, Durso and others 2005), which have been linked to visits by the public to farms or other animal amenity premises. In Great Britain, the major VTEC O157 outbreaks have invariably occurred as a result of indirect contacts with animals via contaminated food or water, but the potential for large outbreaks to occur from direct contact with animals on premises visited by the public has been demonstrated in the USA and Canada (Lejeune and Davis 2004).

Open farms and similar premises often provide the only significant opportunity for the general public to have close contact with animals, and they fulfil an important educational role and provide major contributions to the tourism and leisure industries. The potential risk is the exposure of people to zoonotic hazards such as VTEC O157 (Pritchard and others 2000) and *Cryptosporidium parvum* (Pritchard and others 2007). The prevalence of VTEC O157 in farmed livestock, especially cattle, has been extensively researched, including comprehensive abattoir surveys (Milnes and others 2008) and intensive farm studies (Ellis-Iversen and others 2008), but relatively little is known about its prevalence among the wide range of species found on animal amenity premises. Since the mid-1990s, the Veterinary Laboratories Agency (VLA) has assisted public health authorities in England and Wales with the investigation of human VTEC O157 infections linked to animal contact on such premises, and this paper presents the findings from 1997 to 2007.

Materials and methods

The premises investigated were all essentially 'open farms' – a generic category of animal establishments, specifically operating as an attrac-

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tion for visits by the general public; they included petting zoos, children's farms, city farms, wildlife parks and other premises with animals. The investigations were undertaken at the request of the public health authorities when epidemiological inquiries suggested that animal contact was the likely source of human VTEC O157 infection. They usually concerned outbreaks affecting more than one household, but occasionally followed single sporadic cases or family outbreaks. Open farm premises investigated between 1997 and 2007 were included in the study; conventional commercial farms inaccessible to the public were excluded.

Field investigations and data collection

Visits were undertaken by veterinary investigation officers (VIOs) of the VLA as soon as possible after the request to investigate. They were often accompanied by members of the public health authorities, such as environmental health officers or consultants in communicable disease control. In addition to collecting samples, veterinary advice was provided on animal husbandry and hygiene aspects potentially relevant to the risk of human infection. General health and safety precautions, such as the supervision of visitors, advisory notices and hand-washing facilities, were assessed but not analysed further. A standardised questionnaire was used by VIOs to collect epidemiological data, including the type and size of the premises, the animal species (type, number, age and grouping), the number of days open, and the numbers of staff and visitors. The data were entered into a Microsoft Access database and checked for errors, and validated by a visual check linked to a random selection of 10 per cent of the unique farm identifiers and a search of the database for outliers and impossible values.

Sample collection

The primary aim of collecting samples was to detect VTEC O157 for characterisation and comparison with strains from human cases by phage type (PT) and pulsed-field gel electrophoresis (PFGE), as described by Pritchard and others (2000). A standardised sampling method was used to detect at least one positive sample in each epidemiological group with 95 per cent confidence, assuming a prevalence of at least 10 per cent (Cannon and Roe 1982); in some investigations more samples were collected. An epidemiological group was defined as a group of animals, usually of the same species, which were housed together or had other close contacts through management and husbandry practices. Each sample consisted of a minimum of 1 g of fresh-looking faeces from the animal enclosure or environment, or occasionally from the rectum (depending on availability, safety and practicality), either from single animals or pooled from several animals in the same group (Arnold and others 2008). Pooled samples were usually collected from species producing small faecal pellets, such as sheep and rabbits.

Detection and isolation of VTEC O157

The methods for culture and isolation were as described by Pritchard and others (2000) and Paiba and others (2003); pre-enrichment in buffered peptone water was followed by immunomagnetic separation (Chapman and Ackroyd 1997) and the inoculation of beads (Dynabead anti-*E coli* O157; Dynal) on to sorbitol MacConkey's agar supplemented with cefixime and potassium tellurite (CT-SMAC). Non-sorbitol-fermenting colonies were examined by an *E coli* O157 latex agglutination test (DR260G; Oxoid). Biochemically confirmed *E coli* O157 were examined for verocytotoxin (VT) genes 1 and 2 by PCR (Willshaw and others 2001) and phage typed (Khakhria and others 1990). PTs were presented as the PT number, but if strains reacted with phages but failed to conform to a recognised type, they were classified as RDNC (reacts, does not conform). Because of the cost, only a representative selection (approximately 10 per cent) of *E coli* O157 isolates from epidemiological groups or species on each premises were examined for VT genes. If VTEC O157 was confirmed in these, it was inferred that other *E coli* O157 isolates were also VT strains, on the basis of the findings of Paiba and others (2003) and Synge and others (2003), and a recent study by Ellis-Iversen and others (2009) in which 91.4 per cent of *E coli* O157 were VT-positive. The term VTEC O157

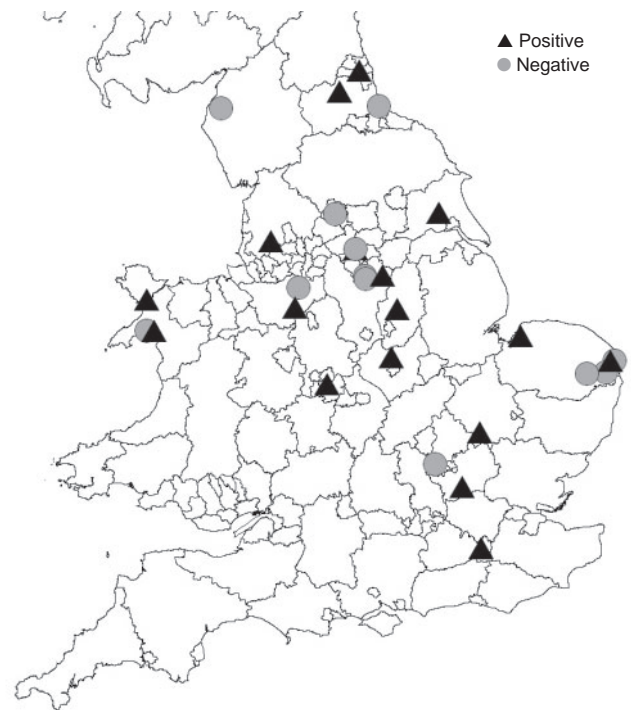


FIG 1: Geographical location and verocytotoxigenic *Escherichia coli* status of open farm premises investigated in England and Wales between 1997 and 2007 as potential sources of human cases

has therefore been used synonymously for all non-sorbitol-fermenting strains of *E coli* O157.

Statistical analysis

The numbers of staff and the annual numbers of visitors, and the numbers and types of animal species, were used to subdivide the premises subjectively into small, medium and large, for simple comparative purposes. Quantifying the premises in a more precise way was not feasible because of the diversity of the enterprises. The species were classified as cattle, sheep, goats, pigs, horses, donkeys, llamas, alpacas, deer, poultry (chickens and turkeys), waterfowl (geese and ducks), other birds (parrots, quails, gamebirds and pigeons), dogs, cats, small pet mammals (guinea pigs, pet rabbits, rats, ferrets, chipmunks, and so on), wild rabbits, other free-living wild mammalian species (badgers, bats, foxes and otters) and reptiles and amphibians.

A farm was defined as positive if VTEC O157 was isolated from one or more samples (at least one *E coli* O157 isolate per positive premises was invariably confirmed as a VT by PCR). This was analysed at premises, group and species level. The within-group proportion of positive samples (an approximate guide to prevalence) was defined as the proportion of VTEC O157-positive samples from the total number of samples analysed at the level of interest. The location and VTEC O157 status of the premises was plotted using ARCGIS v9.1 (ESRI) to show their geographical distribution. Group ages (adult only or young/mixed ages) were compared for the major species, defining adult as 24 months or older for cattle, five months or more for pigs and 12 months or more for sheep and goats. Sample groups with missing ages were excluded. Individual characteristics were evaluated against status by Yates-corrected Chi-squared test, Fisher's exact P values and tests for linear trends as appropriate, using Epi Info 6 software (Dean and others 1995). Crude odds ratios (ORs) were used to measure the strength and direction of significant associations. Logistic regression was used to compare group ages, using STATA 9 (Stata Corp), including the cluster function to account for the clustering of samples at the premises level.

Results

Investigation and premises

Thirty-one investigations were undertaken between 1997 and 2007 (between one and six premises a year); all of the premises (apart from

TABLE 1: Numbers and proportions of VTEC O157-positive samples from different animal species sampled on 31 open farm premises

Species	Number of premises sampled	Total number of samples collected on all premises*	Number (%) of positive samples on all premises	Number (%) of positive premises	Mean within-group proportion (%) of positive samples on positive premises	Median within-group proportion (%) of positive samples on positive premises
Cattle	21	365	87 (23.8)	14 (67)	29.0	20
Sheep	19	427	120 (28.1)	11 (58)	24.4	12.5
Goats	27	281	25 (8.9)	12 (44)	9.9	0
Pigs	22	168	30 (17.9)	10 (46)	14.3	0
Horses	19	85	10 (11.8)	7 (37)	12.3	0
Donkeys	8	26	6 (23.1)	2 (25)	14.6	0
Alpacas	1	4	4 (100)	1 (100)	100	100
Llamas	3	13	9 (69.2)	2 (67)	40.7	33.3
Deer	4	27	1 (3.7)	1 (25)	1.5	0
Poultry	15	45	1 (2.2) [†]	1 (7)	6.7	0
Waterfowl	11	20	0	0	0	0
Other birds	4	44	0	0	0	0
Dogs	6	12	0	0	0	0
Cats	3	7	0	0	0	0
Small pet mammals	19	129	0	0	0	0
Wild rabbits	3	32	12 (37.5)	1 (33)	15.8	0
Other wild mammals	3	28	0	0	0	0
Lizards and amphibians	1	2	0	0	0	0

* Includes individual and pooled samples

[†] Chicken

one in January) were sampled between April and October, with most (12 premises) in July and August. Three premises were investigated twice, several years apart, and for the purposes of the analyses these investigations were classed as being on different premises because the livestock populations and other farm characteristics had changed significantly during the intervening periods. There was an average delay of 24 days (range five to 70 days) between the identification of the primary human case and the request for a field investigation. Twenty-six of the investigations were on typical open farms, of which 14 had a shop or cafeteria, two operated a campsite and three had associated commercial farms. The other five premises were essentially livery stables or farm campsites containing a number of animal species to which the public had general access. On average, there were seven different species on each premises, with a median of eight and a range from two to 12. The average area of the premises was 87.8 ha with a median of 10.1 ha and a range from 0.4 to 1012 ha, which often included land to which the public had little or no access, such as that used for commercial agriculture. The farms had an average number of 156,000 visitors annually (median 68,000, range 175 to 900,000), were open for an average of 344 days each year (median 365) and had an average of 14 full-time staff members (median six). Seventeen of the investigations were undertaken on small premises, 11 were on medium-sized premises and three were on large premises.

VTEC O157 status of the premises

VTEC O157 was identified on 19 (61.3 per cent) of the 31 premises. The time interval between the identification of the primary case and the investigation had no significant effect on the status of the premises. Large premises were more likely to be positive than smaller premises ($P=0.051$). The geographical distribution and VTEC O157 status of the 28 different locations investigated (including the three premises that were investigated twice) are shown in Fig 1; they covered most of England and Wales, but there were none in south Wales or south-west England.

An average of 55 samples (range two to 189) from six different species was collected per investigation, with wide variations depending on putative contact as perceived by the investigators of the outbreak on the basis of the case history. The probability of detecting VTEC O157 on a premises was greater when more than three species

TABLE 2: Numbers and percentages of VTEC O157-positive samples taken from farm animal species kept on open farms, by age, adjusted for clustering on premises

Species	Age	Number of samples	Number (%) of positives	P
Cattle	Adult	125	13 (10.4)	0.054
	Young or mixed age	227	69 (30.4)	
Goats	Adult	113	13 (11.5)	0.379
	Young or mixed age	165	12 (7.3)	
Pigs	Adult	60	18 (30.0)	0.013
	Young or mixed age	91	12 (13.2)	
Sheep	Adult	83	20 (24.1)	0.731
	Young or mixed age	317	89 (28.1)	

were sampled (linear $P=0.045$). When four to six species were sampled on eight premises, five (62.5 per cent) premises were positive (OR 4.2, $P=0.315$), and when seven or more species were sampled on 16 premises, 12 (75.0 per cent) premises were positive (OR 7.5, $P=0.066$).

VTEC O157 status by species

Although the samples were collected from epidemiological groups, for the purpose of this study the analyses were completed at sample level due to difficulties in consistently assigning the samples to groups. *E coli* O157 was isolated from 305 (17.8 per cent) of the 1715 samples collected from all premises; 187 (10.9 per cent) were examined for VT genes and 184 (98.4 per cent) were confirmed as VTEC O157. All but two of these contained the VT2 gene; the exceptions were classified as VT1+2, a sheep isolate that was RDNC and a deer isolate that was PT8. VTEC O157 was found in more than one species on 15 of the 19 positive farms. The three VTEC O157-negative isolates were from two premises, on both of which VTEC O157 was definitively confirmed by PCR in other species.

Table 1 shows the proportions of VTEC O157-positive samples by species, both overall and on positive premises only. Multiple isolates of VTEC O157 were obtained from cattle, sheep, goats, pigs, horses, donkeys and llamas on more than one premises. Of the traditional domesticated animal species, the highest mean proportion of positive samples on positive premises (29.0 per cent) was detected for cattle, followed by sheep (24.4 per cent), donkeys (14.6 per cent), pigs (14.3 per cent), horses (12.3 per cent) and goats (9.9 per cent); the ranking of the top two species was the same with the median results. Samples from camelids had a high proportion of positives; however, samples were collected on only three premises, of which two premises were positive, including one where *E coli* O157 was isolated from 12 of 13 samples from llamas and alpacas. There were multiple positive samples from wild rab-

bits on one of the three premises on which wild rabbits were sampled. Single positive samples, each from single premises, were obtained from a deer and a chicken. No VTEC O157 was isolated from dogs, cats, small pet mammals, waterfowl, other avian species, lizards and amphibians, or wildlife other than wild rabbits.

Age and VTEC O157

Samples taken from cattle in a group containing young cattle were more likely to be VTEC O157-positive than samples from groups containing only adult cattle. Conversely, samples from pigs were less likely to be positive if they were in a group containing young pigs rather than in a group of adults only (Table 2).

VTEC O157 phage types

A total of 174 VTEC O157 isolates (between one and 22 from each positive investigation) were phage-typed and 10 different PTs and RDNC types were identified. Fourteen (73.7 per cent) of the 19 positive premises had only a single PT or RDNC strain, despite the isolates originating from up to six different animal species. One premises had two different PTs in cattle and four premises had two or more PTs or RDNC strains in up to six different species. The most common was PT 2 (six premises), followed by PT 21/28 (five premises) and PT 32 (three premises). PT 8 and PT 4 were each detected on two premises, and PTs 14, 33, 34, 38 and 43 were all detected on single premises. Three premises had RDNC strains. Because of the small sample sizes it was not possible to assess any statistical associations between PT and animal species, but no obvious links were observed.

Discussion

The 31 premises were all sampled because of perceived links with human cases of VTEC O157 infection and were not part of a structured survey. It cannot therefore be assumed that any associations detected were representative of all open farms. Furthermore, it is likely that there was a significant cluster effect, whereby animals within groups and within farms were more alike. The influence of clustering on the effect of age was accounted for in the analyses but the data set was not suitable for multivariable analysis owing to the diversity of the premises. As a result, it cannot be concluded that any associations, such as with the number of species sampled, were causal or not confounded by other factors. Nonetheless, the study was one of the largest investigations into VTEC O157 among the wide range of species in mixed-species environments and has generated hypotheses that merit further investigation. The lack of premises in south Wales and south-west England did not appear to be associated with fewer human cases (Health Protection Agency [HPA] 2006, 2007) or fewer open farms in these areas (National Farm Attractions Network 2008). VT genes were detected in 98.4 per cent of 187 *E coli* O157 isolates examined by PCR, a slightly higher proportion than reported by other researchers in Great Britain (Paiba and others 2003, Syngé and others 2003, Ellis-Iversen and others 2008).

Over 60 per cent of the premises were infected, a much higher proportion than the between-herd prevalence of 40 per cent or less in cattle herds surveyed in England and Wales by Paiba and others (2003) and in Scotland by Syngé and others (2003). This result suggested either that the initial screening of cases carried out by public health authorities for possible association with animal contact was successful in identifying premises with a higher risk of being VTEC O157-positive, or that the open farms had an intrinsically higher prevalence of infection. However, causal associations with human cases, assessed by PT and PFGE profiles, were not confirmed in all the positive premises, and therefore some of the VTEC O157 infection detected was incidental. Sampling at least four different species significantly increased the farm-level sensitivity of detection, but this association may have been confounded by the number of species present, the size of the farm and the number of samples tested. A larger sample size would have made a multivariable analysis possible to separate the effect of each variable and achieve a more accurate conclusion.

The high proportion of VTEC O157-positive samples (a crude estimate of prevalence) on the positive farms in this study could have

been associated with different husbandry and management practices on open farms compared with the typical commercial farms surveyed previously. Open farms are more likely to have higher stocking rates, more movements of staff and equipment, less stable animal group structures (Ellis-Iversen and others 2008) and may comply less carefully with biosecurity precautions (Pritchard and others 2005). Furthermore, open farms often introduce lambs, calves or other species to the resident animal population, depending on the season and their availability, which can increase the risk of introducing infection. The larger premises may have been more likely to be infected than the smaller premises because they maintained larger animal groups (Ellis-Iversen and others 2008), kept more staff and had more visitors, leading to an increased risk of introducing the organism via fomites and a greater risk of within-farm transmission. There was no evidence that the period from the identification of a human case to sampling the farm influenced the detection of VTEC O157. However, Pritchard and others (2000) detected a much lower prevalence of infection during the 12 months after an associated VTEC O157 outbreak on an open farm than during the initial investigation, and it is possible that the prevalence of VTEC O157 fluctuates on open farms and that periods of higher prevalence may contribute to outbreaks. However, further longitudinal studies are needed to investigate this hypothesis.

The findings suggest that young cattle were a significant risk factor, as is the case on commercial cattle farms (Paiba and others 2003). However, there was less supporting evidence for the association with older pigs housed without younger pigs. This could have been associated either with the fact that many of the farms housed pigs of different ages together, to increase visitor interest, or with the fact that the adult pigs were often tame Vietnamese pot-bellied pet pigs, which may have had more contact with all the other animal groups. The data were inconclusive.

The highest proportion of positive samples in farm species on positive premises was detected in cattle, followed by sheep, donkeys, pigs, horses and goats, although the greatest proportion of positive samples from all premises (28.1 per cent) was in sheep. VTEC O157 infection has been described in all these species (Trevéna and others 1996, Chalmers and others 1997, Milne and others 1999, Chapman 2000). An unexpectedly high proportion of positive samples was detected in camelids, although they were sampled on only three premises. This appears to be the first report of VTEC O157 infection in these species and further studies are needed to determine whether they constitute a particular zoonotic hazard on open farms or elsewhere. VTEC O157 was isolated from a deer on one of the farms, and has also been described in deer by Chapman and Ackroyd (1997). VTEC O157 was detected in one chicken and the organism has been reported in laying hens in Italy (Dipineto and others 2006). However, the single isolate from birds suggested that they constitute only a minimal risk to visitors, despite reports from elsewhere of infection in various avian species (Wallace and others 1997, Syngé 1999, Ejidokun and others 2006). The extensive infection in wild rabbits on one premises was described in detail by Pritchard and others (2001) and followed up by Scaife and others (2006). Domesticated rabbits can be infected experimentally with VTEC O157 (Ashkenazi and others 1992), but there was no evidence of the infection in pet rabbits or other small pet mammals in the many samples taken on 19 premises, which suggests that there is no significant risk of acquiring the infection from these species. The infection was not detected in any of the dogs or cats, but VTEC O157 infection has been described in farm dogs by Trevéna and others (1996) and Hogg and others (2009).

A single PT or RDNC strain was found on almost 75 per cent of the positive premises and was often isolated from several different species. Where multiple PTs were present, they usually occurred in different species. The prevalent PTs (2 and 21/28) were the same as those most commonly isolated from human infections in England and Wales over the same period (HPA 2002, 2003, 2004, 2005, 2006, 2007). There was no apparent link between the animal species and the PT, and the PTs appeared to be associated with individual farms rather than species, suggesting that within-farm transmission was more common than the introduction of new strains.

McMillan and others (2007) and Weese and others (2007) described high-risk behaviour and poor hygienic practices by visitors to animal amenity premises. However, despite the high prevalence of VTEC O157 detected, the risk of acquiring the infection on open farms and other animal premises is very small in relation to the large numbers of visitors each year. It is also necessary to balance this small risk against the undoubted benefits of allowing the public to interact with farm animals (Pritchard and others 2000). The risk of people acquiring an infection from animals depends more on the degree of contact and the precautions adopted than the prevalence of infection in a particular species. Procedures to prevent the faecal-oral transmission of VTEC O157 and other zoonotic infections to people visiting open farms have been described (Health and Safety Executive [HSE] 2002). Strain typing can be used to confirm or refute a causal association between a human infection and an animal source, as has been described by Milne and others (1999) and Pritchard and others (2001).

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