Molecular epidemiology of rotavirus in UK cats

German AC\textsuperscript{a,b,}\#, Iturriza-Górrara M\textsuperscript{a}, Dove W\textsuperscript{a}, Sandrasegaram M\textsuperscript{a}, Nakagomi T\textsuperscript{c}, Nakagomi O\textsuperscript{c}, Cunliffe N\textsuperscript{a}, Radford AD\textsuperscript{a,b}, Morgan KL\textsuperscript{b,d}
ABSTRACT

Rotaviruses are leading causes of gastroenteritis in the young of many species. Molecular epidemiological studies in children suggest that interspecies transmission contributes to rotavirus strain diversity in people. However, population-based studies of rotaviruses in animals are few. We investigated the prevalence, risk factors for infection and genetic diversity of *Rotavirus A* in a cross-sectional survey of cats housed within twenty-five rescue catteries across the UK. Morning litter tray faecal samples were collected during winter and summer 2012 from all pens containing kittens and a random sample of those housing adult cats. Group A rotavirus RNA was detected by real-time reverse transcription polymerase chain reaction and positive samples were G and P genotyped using nested VP4 and VP7 PCR assays. A total of 1727 faecal samples were collected from 1105 pens. Overall, rotavirus prevalence was 3.0% (95% CI 1.2-4.9). 52% (13/25; CI 31.3-72.2) centres housed at least one rotavirus-positive cat. Prevalence was associated with season, odds ratio 14.8 (CI 1.1-200.4), p=0.04, but not age or diarrhoea. It was higher during the summer (4.7% CI 1.2-8.3) than winter (0.8%, CI 0.2-1.5). Asymptomatic epidemics of infection were detected in two centres. G genotypes were characterised for 19 (33.3%) of the 57 rotavirus positive samples and P genotypes for 36 (59.7%). Two rotavirus genotypes were identified: G3P[9] and G6P[9]. This is the first population-based study of rotavirus in cats and the first report of feline G6P[9], which questions the previous belief that G6P[9] in people was of bovine origin.

INTRODUCTION
Rotavirus A (RVA), a species in the Rotavirus genus, of the Reoviridae, is an important pathogen of acute diarrhea in the young of many animal species including people (1, 2). With the advent of modern sequencing techniques, the extent of the contribution of interspecies transmission and reassortment to rotavirus genetic diversity is increasingly being realised (3-5). Despite the many reports of potential zoonotic infections in people (6, 7), due to the scarcity of rotavirus surveillance programmes in animals, little is known about the prevalence of potential anthroponotic and zoonotic strains in animals. Particularly, our knowledge and understanding of rotaviruses circulating in companion animal populations is minimal, which is remiss when we consider the extent of contact that occurs between pets (especially cats and dogs) and children in developed countries.

Although infections with feline rotaviruses (FRVs) rarely cause severe illness in cats (8-11), FRVs have captured attention as perpetuating, albeit infrequent, sources of human disease. Human RVAs with genetic homology to feline RVAs have been isolated from widespread geographical locations including Japan (12, 13), Israel (14, 15), Tunisia (16) and America (17). Additionally, putative human/feline reassortant rotaviruses have been identified in children in Italy (18).

Despite the reports of FRV isolations from people, there have been no recent studies or surveillance of rotaviruses in cats. FRV infection was first identified by serology in cats in 1978 (19). Experimental infections have given inconsistent results, with some showing association between rotavirus and reduced faecal quality in kittens (8), whilst others failed to link infection with disease (9, 10, 20). Currently, rotavirus is considered
to play a minor role in clinical disease and is not routinely screened for in diarrhoeic cases in small animal veterinary practice (21).

The prevalence of rotavirus infection in cats has been investigated by serum antibody titres and faecal electron microscopy; seroprevalence studies indicated exposure ranged from 3.5% -100% (8-11, 22), whilst electron microscopy indicated 3-6% of cats were infected (9, 10, 20). These studies involved small numbers of cats and looked at convenience samples of sub-populations (e.g. veterinary hospital admissions, single premises and research colonies) rather than a representative sample of the national feline population.

The aim of this study was to examine, by a systematic, population-based approach irrespective of diarrhoea status, the prevalence and genotypes of RVAs circulating in domestic cats in the UK.

MATERIALS AND METHODS

STUDY POPULATION

The study population comprised cats held in the twenty-five UK rehoming or adoption centres run by Cats Protection, the UK’s largest feline welfare charity. Each year it rehomes or reunites with owners approximately 50,000 cats. These centres are widely distributed geographically with twenty-one in England, two in Wales, one in Northern Ireland and one in Scotland (Figure 1a). The centres vary in size and construction. The
number of cat accommodation spaces (pens) are fixed and range from 16 in a specially
adapted city house, to 202 in the purpose built National Cat Adoption Centre (NCAC).

The population of cats in the centres is in constant flux. The relinquishment and
adoption rates are such that centres operate at capacity throughout the year, although
stocking density (cats per pen) will vary. The demographic pattern in the centres
changes with the seasonal breeding pattern of cats, such that a higher proportion of the
population are kittens during the summer months (June-Aug) (Figure 2). Additionally,
the centres will not put cats originating from different sources into the same pen, so
relinquishment events (single cats or multicat households) will also influence the total
population size and stocking density in each centre.

STUDY DESIGN

Two cross sectional studies were undertaken to account for the seasonal changes in
demography. The first was in the UK winter months (3rd Feb – 30th Mar 2012), the
second was in the summer months (29th May – 17th Aug 2012). The centres were
stratified by size (small, medium and large) and randomly allocated to summer and
winter collection periods (Figure 1a).

The unit of sampling was the pen. These were selected from pen occupancy data
obtained the day prior to sampling. All pens containing at least one kitten were selected.
In addition, a random sample of those housing one or more adult cats was also
selected; the sample size was chosen to allow 95% confidence of detecting one positive
pen if the prevalence of faecal shedding was 2%, assuming a test with 100% specificity and 95% sensitivity. This also allowed a prevalence of 2% to be estimated with 95% confidence and 1.3-1.9% precision.

Recording sheets were used to transcribe demographic data from a number of sources including centre admission records, pen data recording sheets, veterinary paper records, Cats Protection internal database (“PAWS”) and from observation of pen content and construction, unit structure, hygiene precautions and centre management.

SAMPLE COLLECTION AND PROCESSING

Faecal samples were collected from litter trays and where necessary the pen floor between 6.30am and 12.00pm (noon) on the first day of the study visit; where faeces were not present, cats were monitored through the rest of the working day and a collection made if faeces were passed. In large centres, where the number of cats necessitated a longer visit for data transcription, cats who only defecated once every two to three days were observed and samples were collected from these individuals when faeces were eventually passed.

Faecal samples were collected in sterile 30 ml universal tubes using either individual disposable gloves or wooden applicator sticks. The colour, consistency and number of complete deposits of faeces in and outside the litter tray were recorded. Colour was recorded as brown, green, yellow, black or other (described). Consistency was graded 1 (watery) - 6 (hard, dry) using a modified version of the Bristol Stool Scale (Meyers...
Scale) (23). Where the number of faecal deposits was equal to the number of cats it was assumed that each was from a different cat (as an adult cat is highly unlikely to defecate twice in the morning unless it has large intestinal diarrhoea or diffuse gastrointestinal disease resulting in high volume faeces); the deposits were then randomly assigned to each cat. Occasionally cats were observed defecating, or dual occupancy pens contained cats who reliably produced differently graded faeces; these deposits were specifically assigned to an individual. In maternity pens, adult stools were easily differentiated from kitten stools by their size. In single occupancy pens, where there was more than one deposit, a sample was taken from each and the sample recorded as “pooled from an individual”. In multiple occupancy pens, where the number of samples exceeded the number of cats, the sample was recorded as “pooled sample from more than one cat”. Faeces were also examined for the presence or absence of worms, mucus and blood. Samples were transferred to +4°C or -20°C when all pens in a particular building had been sampled. They were transported to the laboratory at this temperature and either aliquoted into two 1.5ml cryovials the following day or kept at -80°C until aliquoted. Samples were kept at -80°C until tested.

DETECTION AND TypING OF ROTAVIRUS

10% faecal suspensions were prepared, clarified by centrifugation at 12000 rpm for 10 minutes and 250 μl used for RNA extraction (QIAamp RNA Kit, Qiagen, Manchester, UK), according to manufacturer’s instructions, and eluted into 50 μl RNase-free water. A 20 μl aliquot was used for reverse transcription using random primers and Superscript II Reverse Transcriptase (Invitrogen, Paisley, UK), following manufacturer’s instructions,
giving a final cDNA volume of 35 μl. Rotavirus NSP3-specific qPCR (24) was performed and samples with a CT value <40 were considered positive. The qPCR mix per reaction was 12.5µl Invitrogen Platinum Mastermix; 8 pmol NSP3-F/NSP3-R primers and 3 fmol NSP-3 probe with 2μl cDNA, made up to 25 μl with RNase free water. The assays were run on a RotorGene Q 6000 thermal cycler at 50°C for 2 minutes, 95°C for 2 minutes and then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

LIMIT OF DETECTION
The limit of detection of the NSP3 qPCR assay was investigated using a simian rotavirus positive control SA11 (25). This was diluted in a two-fold series from 90 infectious virus particles (IVP)/reaction to 3 IVP/reaction, and repeatability assessed with five replicates of a ten-fold dilution series from 1x10^5 IVP/reaction to 1x10^1 IVP/reaction and with two replicates of a two-fold dilution series from 1000 to 16 IVP/reaction. The assay was validated further for the limit of detection of feline RVs FRV-1 (26) and FRV-64 (27).

GENOTYPING
Rotavirus-positive samples were further characterised using hemi-nested VP7 (G-type) and VP4 (P-type) PCR assays, which included primers specific for G1, G2, G3, G4, G8, G9, G10 and G12, and P[4], P[6], P[8], P[9], P[10] and P[11] (28, 29). To increase the G-typing sensitivity, a fragment of the VP7 gene segment from culture-adapted FRV strains from this study was sequenced, using the consensus VP7 primers from the G-typing first amplification round. The sequence data generated was used to characterise
the G-types of the strains that could be propagated in cell culture, and also to design
feline G6- and G3-specific primers. These primers were subsequently used in single
typing assays to test all rotavirus-positive samples. A total of 20 pmol of primers G6F
AACGAGGATGATGGACTACA (nt 126-145) and G3R TARATAGATCCTGTTGGCC
(nt 347-329) were used in separate hemi-nested reactions, with VP7R or VP7F first
round consensus primers respectively, using Top Taq Master Mix (Qiagen, UK) and an
annealing temperature of 50°C.

**STATISTICAL ANALYSIS**

Prevalence was estimated at cat and centre levels for the combined and separate
collection periods. The overall prevalence at cat level was estimated using the `svy`
commands in STATA to adjust for stratification by season and clustering by centre and
pen (StataCorp 2009. Stata Statistical Software: Release 11. College Station TX:
StataCorp LP). The sampling weights were adjusted for the different sampling strategies
(i.e. for pens with kittens and those with adult cats) and for those cats that did not
defaecate on the day of collection. Prevalence at centre level was the proportion of
centres with at least one positive cat, with exact binomial confidence intervals. Fisher’s
exact tests were used to compare the centre prevalence in summer and winter.
Hierarchical univariable and multivariable logistic regression was used to examine
associations between infection and age, season and diarrhoea using the `melogit`
commands in STATA. A three-level model was used, incorporating centre, pen and
individual. Age was modelled as a continuous variable and also as a binary variable.
Three categories were used for kittens: cats aged 5 months or less, 3 months or less, or
2 months or less. Faecal score was also reduced to a binary variable with a cut off at both scores 2 and 3. Cats with a score equal to or below 2 or 3 were considered to have diarrhoea.

Geographical distribution maps were constructed using QGIS 2.0.1 (Dufour)

ETHICAL APPROVAL
This study was approved by the University of Liverpool Veterinary Research Ethics Committee (VREC20) and the Cats Protection Ethical Review Committee.

RESULTS
POPULATION STRUCTURE
A total of 1727 faecal samples were collected from 1105 pens across the twenty-five centres. The median number of occupied pens per centre was 41 (interquartile range, IQR 30-79). The number of samples from each centre varied from 8 in the North London Centre to 224 in Bridgend. The age of cats sampled ranged from one week to 21.5 years of age. Overall, a third (30.9% 683/2213; CI 29.0-32.8%) of the Cats Protection Adoption Centre population was kittens (<6 months). The proportion of kittens was significantly greater during the summer collection period (Figure 2), 44.6% compared with 13.9% in the winter period (P<0.0005). This was also reflected in the sample, where the proportion of kittens was 33.6% overall (581/1727; CI31.4-35.9); 48.2% in summer and 11.6% in winter. The median age of cats sampled in summer was 9.0
months (IQR 3.0-38.0) compared with 36 months (IQR 10.5-84.0) for the winter-sampled population (p<0.0005).

A total of 85.6% (83.6-87.8%) of cats defaecated during the collection period. These cats were of similar age to those that did not defaecate. The median ages were 16.5 months (IQR 3.3-62) and 26.5 months (IQR 1.5-84.5) respectively. Of faecal samples were scored for consistency. The median faecal score was 5 (ideal) (IQR 4.0-5.0). 5.6% (95/1686) of samples had a faecal score of 6 (dry/hard), 11.9% (201/1686) had a score of 3 or less (diarrhoea) and 2.4% (41/1686) were 2 or less (watery diarrhoea). 60.2% (1040/1727) of samples were collected during the summer.

ROTAVIRUS PREVALENCE

The overall estimated prevalence of rotavirus qPCR positive faecal samples in the population was 3.0% (57/1727, 95% CI 1.2-4.9) and 52% of centres (13/25, CI 31.3-72.2) housed at least one positive cat (Figure 1a). There were differences in prevalence between the two collection periods and individual centres (Table 1). The prevalence was 4.7% (CI 1.2-8.3) in the summer collection period compared with 0.8% (95% CI 0.2-1.5) in the winter period, p<0.0001. The proportion of centres with rotavirus positive cats was also higher during summer (69.2%, CI 44.3-89.4) than winter (33.3%, CI 11.6-62.3) but this difference was not statistically significant, p=0.16.

The higher prevalence in the summer was a reflection of two centres (Table 1), Truro and Birmingham, in which the prevalence was 61.2% (CI 57.5-65.1) and 10.2% (7.9-
12.5% respectively. These prevalences were significantly higher than those in other centres (p<0.0001), and were considered to be epidemics of infection. The prevalence in the remaining centres ranged from 0-8.9%. There was no significant difference in the prevalence of the centres sampled in the winter period, which ranged from 0-2.9%.

LACK OF ASSOCIATION BETWEEN ROTAVIRUS AND DIARRHOEA

The presence of rotavirus RNA in faeces was not associated with diarrhoea. The median faecal score for rotavirus positive faeces was 5.5 (IQR 4-5) compared with 5 (IQR 4-5) for negative faeces. This difference was not statistically significant (p=0.17). When a binary variable was created, with a faecal score of either 3 or 2 or less considered as diarrhoea, there was still no association with the presence of rotavirus. The odds ratios (OR) were 1.83 (95% CI 0.60-5.53, p=0.28) and 4.48 (CI 0.79-25.49, p=0.09) respectively. Intraclass correlation coefficients indicated that diarrhoea was highly correlated with the same pen within a centre; 53 and 60% of the variation in the probability of having diarrhoea score 3 or 2 was associated with pens in the same centre. In contrast it was only slightly correlated with the same centre alone, accounting for 7 and 10% of the total residual variance respectively.

RISK FACTORS FOR FAECAL ROTAVIRUS

In univariable analysis the presence of rotavirus in faeces was associated with season. The OR for rotavirus presence in the summer collection period was 12.3 (CI 1.07-141.5, p=0.04) when compared with the winter period. There was no association with age, entered in the model either as a continuous variable in months (OR 1.0, CI 0.99-1.02,
p=0.8) or as a binary variable defining a kitten of 5 months (OR 1.41, CI 0.52-3.85, 
p=0.5), 3 months (OR 1.32, CI 0.46-3.80, p=0.6) or 2 months (OR 1.17, CI 0.30-4.64, 
p=0.82) of age or less. When season, kittens and an interaction term between season 
and kittens were entered into a multilevel model, only season remained significant (OR 
14.8, CI 1.1-200.4, p=0.04) (Table 2). Intraclass correlation coefficients, estimated by 
including season, centre and pen in four level intercept only models, indicated that 76% 
of the variance was associated with pens within centres, 41% with centres within 
seasons and 6.3% with season alone.

LIMIT OF DETECTION
The limit of detection for SA11 in the two-step NSP3 qPCR was between 16 and 32 
IVP/reaction. Intra-assay repeatability was good, with a coefficient of variation of 0.015. 
The limits of detection for feline rotavirus genomes belonging to the FRV-64 genogroup 
(K9 genogroup) and the FRV-1 genogroup were similar.

MOLECULAR CHARACTERISATION
G genotypes were characterised for 19 (34%) of the positive samples and P genotypes 
for 34 (61%). Two G genotypes were identified; G6 (84%; 16) and G3 (16%; 3). A single 
P genotype was recognised, P[9]. Combined G and P genotypes were found for 13 of 
the positive samples (23%); two of these were G3P[9] and eleven were G6P[9]. Higher 
NSP3 CT values correlated with a reduced likelihood to genotype (Figure 3). Culture 
and sequencing of representative feline rotavirus strains, followed by primer and probe 
redesign, were essential to genotype both G6P[9] and G3P[9] genotypes.
Genotype G6 was isolated in both winter and summer collection periods from three centres: Glasgow, Birmingham and Truro, which span the length of the UK. Truro and Birmingham were centres at which epidemics of infection were observed. G3 was isolated from Exeter (winter) and Bridgend (summer) (Figure 1b and Table 3).

**DISCUSSION**

This study represents one of the first population-based studies of rotavirus prevalence in any animal species. Feline rotaviruses (G6P[9] and G3P[9]) were detected at low prevalence (3.0%) in the UK cat population. This is similar to estimates from other countries (9, 10, 20), but showed interesting regional and seasonal variations. We report the first detection of epidemics of asymptomatic rotavirus infection (prevalences 61.2% and 10.2%) in cats in spatially and temporally distinct locations. Full details of these will be reported elsewhere. The low frequency of rotavirus epidemics in the cat population could be due to a combination of the low national prevalence of infection (3.0%) and the “snapshot” view that cross-sectional sampling affords. Longitudinal sampling strategies based on this prevalence could investigate transmission dynamics in more detail. Biosecurity measures are of high standard across the country for Cats Protection Adoption Centres, as they all adhere to strict hygiene guidelines. However, it is known in human hospitals that viral diseases, particularly gastrointestinal pathogens, are not easily contained, with rotavirus being one of the most frequent pathogens to transmit nosocomially and cause outbreaks, particularly in childrens and neonatal wards (30). A return to Truro six weeks later found a complete turnover of cats and a reduction...
of the centre prevalence from 61.2% (21/34) to 2.5% (1/40), despite the continued influx of new individuals, which might be thought to maintain epidemic infection. Therefore, it is unknown whether the observed epidemics arose from spread within a centre or reflected intake of cats already infected with rotavirus due to an outbreak in the local area. Diversity and prevalence of feline calicivirus strains in UK cat shelters has been suggested to arise from cats sampling local strain diversity prior to entering a shelter, rather than spread within a shelter (31).

The absence of an association between rotavirus infection and diarrhoea in cats is in stark contrast to people, where it is a major cause of morbidity and mortality. Infection has also been associated with diarrhoea and decreased productivity in cows (32, 33), suckling pigs (34-36) and horses (37, 38). Asymptomatic infections are reported, although their importance in transmission is not well understood due to the lack of population-based studies. The lack of association between feline rotavirus infection and diarrhoea may reflect cat behaviour. Queens clean the perineal region of their kittens to stimulate defecation and consume the faeces that are produced. Maternal coprophagy may reduce the risk of kitten diarrhoea by preventing transmission within and between litters and by “orally vaccinating” the queen, increasing the titres of milk anti-rotavirus IgA antibodies.

Low viral titres and virus strain may also be a reason for the lack of association of feline rotavirus with diarrhoea. Many of the CT values obtained for rotavirus positive feline faecal samples, even in the epidemics of infection observed in this study, fell outwith the
clinically relevant range consistent with human symptomatic rotavirus infection (39). In 338 cows, asymptomatic individuals shed similar viral titres to clinically infected individuals and the roles of virulent and avirulent strains of rotavirus have been postulated (40, 41). In dogs and pigs, species where asymptomatic infections are more frequently recognised (35, 42-45) no population-based studies of sufficient power exist to truly characterise the role of asymptomatic infection. Asymptomatic infection may be a reflection of the nature of the relationship of G6P[9] and G3P[9] with the feline host. It is possible that zoonotic infection with these strains is similarly associated with asymptomatic carriage or mild clinical signs in people, and if population studies of adults and children, particularly the pet-owning population and those working in contact with cats were performed, we may see a greater prevalence than we currently accept.

Rotavirus most commonly infects neonatal and young people, cattle and pigs (6, 7, 46). However, this was not the case for the cat population in this study. Kittens may play a role as a multiplier of infection without this being detected because of maternal coprophagy. Kitten faeces are not found in litter trays until they reach approximately four weeks of age. Ethically, per rectum faecal sampling requires a Home Office Licence in the UK and was not possible. This age group, the equivalent of very young children, is not represented and represents a systematic bias in our study. However, rotavirus was not detected in faeces from any of the nursing queens.

FRV infection was strongly associated with season OR 14.8 (CI 1.1-200.4), p=0.04. It was more common in the summer months, contrasting with the pattern observed in UK.
people and cattle, where rotavirus is considered a cold weather disease (32, 47, 48). In worldwide surveillance studies, country income level is a more reliable predictor of infection than latitude or geographical location. Wealthier countries have a seasonal peak (49), the timing and spread of which is influenced by birth rate (50); poorer countries experience year round disease associated with high transmission and birth rates (51). An increase in the susceptible population is therefore important, but it is possible that seasonality is also influenced by an increased density of individuals within housing and airspace. Cattle are brought into close contact by indoor housing over winter, and people spend more time indoors over winter in close proximity with others (52). In our cat population, although infection was not associated with age, it is possible that kittens play a role in seasonality but it is masked by population dynamics and cat behaviour. The cat is a seasonal and prolific breeder; an average adult queen can produce two litters of four kittens between spring and autumn (53, 54). During the breeding season, the increased number of animals per pen and the greater volume of stray kittens and pregnant queens could increase the risk for transmission of infection. We have identified two G and P genotype combinations carried by rotaviruses circulating in the UK cat population in 2012: G3P[9] and G6P[9]. Whilst G3P[9] is a recognised feline genotype (AU-1-like and BA222-like genotype constellations (13, 14, 55)), this is the first report of G6P[9] detection in cats. G6 was the more prevalent genotype (84%), and was detected in Scotland, the Midlands and Cornwall – geographically distinct regions encompassing the length of the UK. Further, it was identified in both the winter and summer collection periods. Although both rotavirus
genotypes did not coexist within a single centre, there is potential for spatial co-existence as G and P genotyping was incomplete. (This is a common finding in the molecular epidemiology of rotavirus and most likely reflects low virus titres, although the possibility that novel feline sequence reduced the efficiency of primer binding should also be considered). Both G3P[9] and G6P[9] have been isolated from people in other parts of the globe. Human G3P[9] is considered to be a direct result of transmission events from canine or feline rotaviruses and has been reported in human clinical surveillance samples worldwide, including Japan (13), Israel (56), Brazil (57), Thailand (58), Russia (59) and Hungary (60). G6P[9] was originally isolated from an Italian child with diarrhoea (61), and has subsequently been reported in America (62), Hungary (60, 63), Japan (64), Australia (65, 66) and Tunisia (16). Yamamoto and colleagues (64) considered that their isolates represented reassembly events between bovine-like human rotaviruses and human/feline AU-1-like rotaviruses. G6 is a common genotype in cattle/buffalo (67-70), sheep (71-73) and goat (74, 75) and has been identified sporadically or at low prevalence in rabbits (76) and pigs (77, 78). It is uncommon in people, and although a zoonotic origin is postulated (79-84), it has not yet been convincingly proven whether such zoonotic strains spread among people. More recently, with the advent of advanced genome sequencing techniques and a more robust classification system (85), the possibility of G6 feline origin at some historical point has been proposed (16). With clustering of published human G6 genotypes with our feline G6 genotypes, rather than with published bovine G6 genotypes (86), our work strongly suggests that G6P[9] genotypes are examples of zoonotic or anthropozoonotic transmission between cats and people. Whole genome sequencing to further explore
the relationship between G3P[9] and G6P[9] genotypes identified in this study and other human and animal rotaviruses is underway.

Interestingly, G3P[3] was not found in our study population. This genotype has been reported in cats (20, 87), people (14, 15, 17, 88-92), dogs (93-98) and other animals (99-103). This genotype may not be circulating in the UK cat population or may be at a prevalence too low to detect with our study (<2%). It is also possible that, similar to human RVA, feline RVA strain diversity and the prevalence of different strains may fluctuate between years, but this can only be confirmed through sustaining surveillance in consecutive years. Reassuringly, none of the common human rotaviruses were detected in cats and no reassortment was observed between human and feline rotaviruses.

An additional limitation of this study was using a shelter cat population as a sentinel for the UK cat population. Sampling a shelter cat population rather than an owned pet population may carry with it the additional stressors and opportunities for disease spread associated with mass housing of cats from different backgrounds and a high throughput environment. However, the majority of cats housed in Cats Protection Centres are healthy cats that have been relinquished from homes due to socioeconomic factors. True strays represent a minority of the population and feral cats are only occasionally and briefly housed for trap-neuter-release programmes. Although nosocomial spread of viral disease is difficult to prevent, hygiene standards were very good and diversity of feline calicivirus within these centres has been suggested to...
reflect local strain distribution rather than within-centre spread (31). Cats were also
relinquished from geographically widespread postcode locations, encompassing the UK.
Therefore, we considered the centres allowed a valid comparison to the UK pet cat
population.

CONCLUSION

G6P[9] is a relatively common feline rotavirus and, along with G3P[9], exists at low
prevalence in this UK cat population. Diarrhoea and age are not risk factors for infection,
although infection increases in prevalence over the summer. Transmission events
between cats and people in the UK likely exist, although they are infrequent and do not
cause outbreaks of disease. Surveillance of rotavirus in our domestic pet population is
important to investigate rotavirus genetic diversity, elucidate the role of asymptomatic
carriage, explore zoonotic risk, and monitor the potential role of non-human rotaviruses
in rotavirus evolution.

ACKNOWLEDGEMENTS

This work was supported by an RCVS Charitable Trust Blue Sky Award in Virology
(BSR11 1428) and internal funding from the Institute of Infection and Global Health,
University of Liverpool. The authors express their gratitude to the Cats Protection for
financial support of AC German’s lectureship and the co-operation of their staff and cats
with faecal sample and data collections in 2012. Additionally, thank you to everyone
involved with data entry, particularly Anna Edwards, Harriet Campbell, Anneka Summan
and Sabrina Knight.
Conflict of interests
NC has received grant support from GlaxoSmithKline (GSK) for rotavirus research, and
honoraria for participation in GSK rotavirus vaccine advisory board meetings. MIG has
received funding from GSK and Sanofi Pasteur MSD in the form of unrestricted
educational grants for research.

REFERENCES
record. 84:220-236.
3. Abe M, Ito N, Masatani T, Nakagawa K, Yamaoka S, Kanamaru Y, Suzuki H,
Shibano K, Arashi Y, Sugiyama M. 2011. Whole genome characterization of new
bovine rotavirus G21P[29] and G24P[33] strains provides evidence for interspecies
4. Matthijnssens J, Ciarlet M, Heiman E, Arijs I, Delbeke T, McDonald SM,
Palombo EA, Iturriza-Gómarra M, Maes P, Patton JT, Rahman M, Van Ranst M.
2008. Full Genome-Based Classification of Rotaviruses Reveals a Common Origin
between Human Wa-Like and Porcine Rotavirus Strains and Human DS-1-Like and
unusual human G6P[6] rotavirus isolate are of bovine origin. J. Gen. Virol. 89:2630-
2635.


Table 1: The prevalence of faecal rotavirus in different centres. (* indicates centres with prevalence significantly higher than in other centres, p<0.0001. Centres in Ireland, Scotland and Wales are indicated; the rest of the centres are in England)

<table>
<thead>
<tr>
<th>CENTRE</th>
<th>PREVALENCE (Number)</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUMMER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIRMINGHAM</td>
<td>10.2 (18/176)*</td>
<td>8.2-12.7</td>
</tr>
<tr>
<td>BRIDGEN (WALES)</td>
<td>1.3 (4/224)</td>
<td>0.9-2.0</td>
</tr>
<tr>
<td>CHELMSFORD</td>
<td>3.5 (2/57)</td>
<td>1.5-8.1</td>
</tr>
<tr>
<td>DEREHAM</td>
<td>0 (0/75)</td>
<td>0-3.9</td>
</tr>
<tr>
<td>DOWNHAM MARKET</td>
<td>2.6 (2/85)</td>
<td>1.6-4.1</td>
</tr>
<tr>
<td>HASLEMEERE</td>
<td>3.9 (1/31)</td>
<td>1.2-11.9</td>
</tr>
<tr>
<td>HEREFORD</td>
<td>0 (0/43)</td>
<td>0-8.2</td>
</tr>
<tr>
<td>IOW</td>
<td>1.5 (1/73)</td>
<td>0.9-2.6</td>
</tr>
<tr>
<td>NCAC</td>
<td>0.6 (1/172)</td>
<td>0-2.1.7</td>
</tr>
<tr>
<td>NORTH LONDON</td>
<td>0 (0/8)</td>
<td>0-36.9</td>
</tr>
<tr>
<td>NOTTINGHAM</td>
<td>8.9 (2/24)</td>
<td>4.9-15.6</td>
</tr>
<tr>
<td>TRURO</td>
<td>61.3 (21/34)*</td>
<td>57.6-64.8</td>
</tr>
<tr>
<td>YORK</td>
<td>0 (0/38)</td>
<td>0-9.3</td>
</tr>
</tbody>
</table>
Table 2: Multivariable analysis of the effects of age and season on rotavirus shedding

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio (OR)</th>
<th>95% confidence interval (CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season (summer)</td>
<td>14.78</td>
<td>1.09-200.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Age (kittens)</td>
<td>3.82</td>
<td>(0.17-87.1)</td>
<td>0.4</td>
</tr>
<tr>
<td>Age,Season</td>
<td>0.29</td>
<td>0.11-7.67</td>
<td>0.46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>95% confidence interval (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centre</td>
<td>4.64</td>
</tr>
<tr>
<td>Centre&gt;Pen No.</td>
<td>5.35</td>
</tr>
</tbody>
</table>

LR test v logistic regression: chi²=111.5 prob.chi²=0.0000
Table 3: Distribution and genotypes of feline rotaviruses isolated from all FRV-positive Cats Protection Adoption Centres in the UK during 2012. X indicates G or P rotavirus genotypes that were unable to be elucidated.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Birmingham</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Bridgend</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Chelmsford</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Derby</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Downham Market</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Exeter Axhayes</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Glasgow</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Haslemere</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Isle of Wight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Mansfield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>NCAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Nottingham</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Truro</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>18</td>
<td>1</td>
<td>21</td>
</tr>
</tbody>
</table>

Total FRVs: 11 5 2 1 21 17 57
Figure 1: Cats Protection Adoption Centres and feline rotavirus distribution in the UK.

(a) Distribution of Cats Protection Adoption Centres across the UK and their feline rotavirus (FRV) status in 2012.

(b) Distribution of feline rotavirus (FRV) genotypes between the twelve FRV-positive Cats Protection Adoption Centres in the UK. The pie chart markers represent strain distribution within a centre, but their size does not indicate prevalence between centres. x = unable to determine genotype.

Maps generated using QGIS 2.0.1 (Dufour)

Figure 2: Seasonal demographics of the Cats Protection Adoption Centres’ cat population. Cats are seasonal breeders (spring-autumn), resulting in high numbers of kittens over the summer months. Demographic data was used from 2010 to plan the collection periods (indicated as winter and summer over the graph); this pattern remains relatively constant between years. The data shown is from 2012, indicating the true distribution of the cat population sampled.

Figure 3: Relationship between CT value and ability to genotype FRV strains. (Horizontal bar indicates average CT for each genotyping category). The ability to genotype improved as the CT value lowered. The majority of FRV isolates had a CT value of 30 or over.