A Virtual Biobank for Companion Animals: a Parvovirus Pilot Study.

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Abstract

There is a lack of national population data concerning infectious disease in companion animals. Here we piloted the feasibility of linking diagnostic laboratories, population surveillance and modern sequencing approaches to extract targeted diagnostic samples from laboratories before they were discarded, as a novel route to better understand national epidemiology of major small animal pathogens. Samples tested for canine or feline parvovirus were requested from a national veterinary diagnostic laboratory and Sanger Sequencing or whole genome sequencing. Together with associated metadata, sequences obtained from positive samples provided new insights into the recent geographical distribution of parvovirus strains in circulation in the United Kingdom (UK). This collaboration with industry represents a ‘National Virtual Biobank’ that can be called on, rapidly if necessary, to efficiently add new layers of epidemiological information of relevance to animal, and potentially human, population health.

Introduction

Infectious disease remains a major cause of ill health in companion animal populations. Whereas Public Health England and the Animal and Plant Health Agency largely exist for humans and food animals respectively, there is a lack of national population data for companion animals. To start to fill this gap, the Small Animal Veterinary Surveillance Network (SAVSNET) was established and collects electronic health records (EHRs) from a sentinel network of veterinary practices and commercial diagnostic laboratories across the UK; these data were recently used to respond to a vomiting outbreak in dogs (1). In the case of laboratories, these EHRs are based on clinical testing paid for by owners, the results being used by their veterinary surgeons to manage individual animal health. Most UK laboratories now collaborate with SAVSNET, supplying test results and associated anonymised metadata at scale from across the UK. When collated nationally, these data provide new information on temporal and spatial trends in infections in small animals and now contribute to regular surveillance reports including antimicrobial resistance, viral and protozoal disease (2, 3). However, as sequencing becomes cheaper, adding genetic information to these samples can add greater resolution through strain / variant typing, and allow for new insights into the epidemiology, pathogenesis and evolution of these pathogens. Although these technologies have significantly reduced in price, they tend not to be used by diagnostic laboratories as the information is of less clinical value to individual animals. Put simply, diagnostic laboratories have precious samples that are usually discarded before their value to animal health can be realised through enhanced phenotypic and genotypic analyses.

Here we piloted linking commercial diagnostic laboratories and SAVSNET to extract targeted samples and their associated metadata, before they were discarded, using parvoviruses as an exemplar. Parvoviruses are small non-enveloped, linear, single-stranded DNA viruses. They are an important cause of gastrointestinal disease in cats and dogs. Feline parvovirus (FPV) infects domestic and wild cats but does not infect domestic dogs. Modified live vaccines were developed in the 1960s. Canine parvovirus (CPV-2) first emerged in the late 1970s. It was called CPV-2 to distinguish it from canine minute virus (or CPV-1) which is genetically and antigenically distinguishable and not currently prevalent. Since its emergence, CPV-2 has been replaced in the field by new types, CPV-2a, -2b and -2c (4, 5).Whereas the original CPV-2 generally only infected dogs, the newer variants can infect cats as well as dogs. Disease in both species is largely controlled by vaccination using mostly live vaccines. The last survey of UK CPV diversity in dogs, using samples from 2006-2008, was published in 2011. At that time, CPV-2a and -2b were responsible for most canine UK infections, at least in the sampled population of clinically ill dogs with diarrhoea attending PDSA hospitals (6). In cats, a UK survey of parvoviruses in (asymptomatic) animals published in 2012, found only CPV-2a and -2b and no FPV (7). However, a study published in 2008, sequencing samples from cats with panleukopenia, analysed five from UK cats and all were FPV (8). Understanding the variation of these important viruses in the UK, the extent to which vaccine virus contributes to clinically detected disease, and whether new strains described elsewhere in the world have arrived in the UK remain important questions. In this study we piloted whether this type of information could be obtained from samples remaining after diagnostic testing by commercial laboratories, augmented with data from SAVSNET. In parallel, we sought to prove the utility of next-generation sequencing to obtain complete genome sequences from these clinic-derived diagnostic samples (9).

Materials and Methods

Ethical approval to collect electronic health data and physical samples from participating laboratories was granted by the Research Ethics Committee at the University of Liverpool (RETH000964 and VREC698). CPV samples were obtained from a SAVSNET participating laboratory by requesting excess faecal samples sent for CPV testing by PCR or antigen detection. Feline parvovirus samples were obtained by requesting faecal samples from the same laboratory that had been sent for testing by PCR for a panel of feline gastro-intestinal diseases including FPV. Apart from CPV/FPV status, no criteria were applied for the selection of samples. The samples we received were based on convenience for the contributing laboratory. As samples were received, they were linked to the electronic health data for that sample (such as breed and spatio-temporal data) held in the SAVSNET database using a unique anonymised identifier.

Once received in the authors laboratory, faeces were suspended in PBS and centrifuged at 1500 rpm for 5 min. DNA was extracted from the resulting supernatant using a QIAamp viral RNA mini kit (Qiagen); this extracts both viral RNA and DNA. For the canine samples, the major surface protein (VP2) was amplified by PCR and sequenced according to previously published methods (6) using Sanger sequencing. Feline nucleic acid was sent for Illumina sequencing at the Centre for Genomic Research (University of Liverpool). The nucleic acid was treated with RNase, fragment libraries prepared from the remaining DNA (NEBNext Ultra II Kit; ~350 bp inserts), and sequenced (Illumina HiSeq 4000; Paired-end, 2x150 bp sequencing). Adapter sequences were trimmed using Cutadapt (10)n and further trimmed using Sickle (https://github.com/najoshi/sickle) with a minimum window quality score of 20. Reads shorter than 20 bp, and those aligning to the cat genome using Bowtie 2 (11), were removed. Remaining reads were then assembled using SPAdes (12) and resulting contigs compared to sequences in the NCBI nucleotide database (blastn). Sequences of the VP2 gene from both cat and dog samples were then aligned (ClustalW) and phylogenetically compared using Neighbour-Joining as implemented in MEGA6 (13). VP2 sequences of vaccines and field isolates from dogs (collected between 2006 and 2008 in the UK) obtained in a previous study (6) and sequences from GenBank were included for comparison.

Results

Nineteen canine faeces that tested positive using antigen detection (ELISA) were received from the diagnostic laboratory between December 2016 and October 2017. Using PCR, 12 tested positive in the author’s laboratory and the VP2 gene was sequenced. Four FPV positive feline faeces were received between January and May 2019. DNA from these four samples was sent for Illumina sequencing; three were sequenced successfully resulting in near full length FPV genomes (between 4507 and 4963 nt). Phylogenetic analysis of the VP2 sequences of the CPV and FPV isolates obtained in this study is shown in Figure 1, along with the geographical location of the isolates.

CPVs sequenced in this study (Dogs 1-12 highlighted in blue in Figure 1) were geographically distributed across the UK and phylogenetically interspersed with previously published UK isolates (presented as asterisks). Based on previously identified amino acid residues used to type CPV (14), five of the 12 canine isolates were typed as CPV-2a and seven as CPV-2b. No CPV-2c were found in these samples. The three feline isolates sequenced in this study all typed as FPV and clustered with previously published FPV.

Discussion

In this pilot study, the first of its kind, we have shown that we can link electronic health surveillance data to physical samples that have been ethically and practically extracted from diagnostic laboratories in a timely and actionable fashion. Importantly, we have shown that we can add new layers of information of relevance to animal health on top of more basic diagnostic data; in this case sequencing to identify variants of canine and feline parvoviruses .

Although only 15 samples were sequenced we were able to make several observations of relevance to parvoviruses in cats and dogs. We found only CPV-2a and -2b in the canine samples, not -2c. This is consistent with earlier studies (6, 19). However, CPV-2c is highly prevalent in other parts of the world (8, 15-18), and has been detected in mainland Europe (16, 23, 24) and in one dog in the UK (4). A larger study of this kind could determine whether CPV-2c does circulate in the UK. In addition, we found no CPV-2 in these samples. This original viral variant is now thought to occur only sporadically, and as a result of vaccination with live vaccines; CPV DNA is detectable for up to 28 days after vaccination, albeit at low levels (21). Live vaccines are frequently used in in the UK (20), and CPV-2 has been reported in a recently vaccinated UK dog (4). Furthermore, of the three FPVs sequenced in this study, one was identical to vaccine virus. Again, the FPV vaccine is a modified live virus and shedding is known to occur in recently vaccinated cats (22). Although it is unlikely such vaccine-derived strains contribute significantly to disease, a larger study of the kind reported here could determine the extent to they contribute to laboratory diagnosed parvovirus infections in cats and dogs.

Our ability to link electronic health data with samples and further produce sequence data concerning those samples, gives us confidence to develop this model further for parvoviruses and other pathogens. We have termed the collaboration between academic and diagnostic laboratories a “National Virtual Biobank” – reflecting the dispersed and transient nature of the samples held by participating laboratories prior to their targeted selection for more detailed genomic analysis; as demonstrated here. In ongoing studies we are using this Virtual Biobank for molecular surveillance of bacterial pathogens from companion animals showing resistance to critical antimicrobials, with new opportunities to compare human and animal resistance that come with sequence data. The National Virtual Biobank may also be used to monitor any future changing patterns in companion animal infection as and when they occur, providing a first step to filling a microbiological surveillance gap in national companion animal health.

In addition to targeted PCR sequencing, we have also demonstrated the ability to sequence near whole viral genomes from diagnostic samples without prior targeted enrichment, despite their convoluted journey from practitioner, to diagnostic laboratory and then to University of Liverpool. This provides the capability to also identify potential new pathogens (25), particularly applicable to future disease outbreaks, or to identify potential pathogens in samples that test negative for known pathogens.

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References

1. Radford AD, Singleton DA, Jewell C, Appleton C, Rowlingson B, Hale AC, et al. Outbreak of Severe Vomiting in Dogs Associated with a Canine Enteric Coronavirus, United Kingdom. Emerg Infect Dis. 2021;27(2):517-28.

2. Singleton DA, Stavisky J, Jewell C, Smyth S, Brant B, Sanchez-Vizcaino F, et al. Small animal disease surveillance 2019: respiratory disease, antibiotic prescription and canine infectious respiratory disease complex. Vet Rec. 2019;184(21):640-5.

3. Singleton DA, Arsevska E, Smyth S, Barker EN, Jewell C, Brant B, et al. Small animal disease surveillance: gastrointestinal disease, antibacterial prescription and Tritrichomonas foetus. Vet Rec. 2019;184(7):211-6.

4. Decaro N, Desario C, Addie DD, Martella V, Vieira MJ, Elia G, et al. The study molecular epidemiology of canine parvovirus, Europe. Emerg Infect Dis. 2007;13(8):1222-4.

5. Allison AB, Kohler DJ, Fox KA, Brown JD, Gerhold RW, Shearn-Bochsler VI, et al. Frequent cross-species transmission of parvoviruses among diverse carnivore hosts. J Virol. 2013;87(4):2342-7.

6. Clegg SR, Coyne KP, Parker J, Dawson S, Godsall SA, Pinchbeck G, et al. Molecular epidemiology and phylogeny reveal complex spatial dynamics in areas where canine parvovirus is endemic. J Virol. 2011;85(15):7892-9.

7. Clegg SR, Coyne KP, Dawson S, Spibey N, Gaskell RM, Radford AD. Canine parvovirus in asymptomatic feline carriers. Vet Microbiol. 2012;157(1-2):78-85.

8. Decaro N, Desario C, Miccolupo A, Campolo M, Parisi A, Martella V, et al. Genetic analysis of feline panleukopenia viruses from cats with gastroenteritis. J Gen Virol. 2008;89(Pt 9):2290-8.

9. Radford AD, Chapman D, Dixon L, Chantrey J, Darby AC, Hall N. Application of next-generation sequencing technologies in virology. J Gen Virol. 2012;93(Pt 9):1853-68.

10. M. M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetjournal. 2011;17(1):10-2.

11. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9(4):357-9.

12. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19(5):455-77.

13. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406-25.

14. Martella V, Decaro N, Buonavoglia C. Evolution of CPV-2 and implication for antigenic/genetic characterization. Virus Genes. 2006;33(1):11-3.

15. Woolford L, Crocker P, Bobrowski H, Baker T, Hemmatzadeh F. Detection of the Canine Parvovirus 2c Subtype in Australian Dogs. Viral Immunol. 2017;30(5):371-6.

16. Sutton D, Vinberg C, Gustafsson A, Pearce J, Greenwood N. Canine parvovirus type 2c identified from an outbreak of severe gastroenteritis in a litter in Sweden. Acta Vet Scand. 2013;55:64.

17. Castro TX, Costa EM, Leite JP, Labarthe NV, Cubel Garcia RC. Partial VP2 sequencing of canine parvovirus (CPV) strains circulating in the state of Rio de Janeiro, Brazil: detection of the new variant CPV-2c. Braz J Microbiol. 2010;41(4):1093-8.

18. Calderon MG, Romanutti C, A DA, Keller L, Mattion N, La Torre J. Evolution of canine parvovirus in Argentina between years 2003 and 2010: CPV2c has become the predominant variant affecting the domestic dog population. Virus Res. 2011;157(1):106-10.

19. Decaro N, Desario C, Billi M, Mari V, Elia G, Cavalli A, et al. Western European epidemiological survey for parvovirus and coronavirus infections in dogs. Vet J. 2011;187(2):195-9.

20. Sanchez-Vizcaino F, Muniesa A, Singleton DA, Jones PH, Noble PJ, Gaskell RM, et al. Use of vaccines and factors associated with their uptake variability in dogs, cats and rabbits attending a large sentinel network of veterinary practices across Great Britain. Epidemiol Infect. 2018;146(7):895-903.

21. Freisl M, Speck S, Truyen U, Reese S, Proksch AL, Hartmann K. Faecal shedding of canine parvovirus after modified-live vaccination in healthy adult dogs. Vet J. 2017;219:15-21.

22. Bergmann M, Schwertler S, Speck S, Truyen U, Reese S, Hartmann K. Faecal shedding of parvovirus deoxyribonucleic acid following modified live feline panleucopenia virus vaccination in healthy cats. Vet Rec. 2019;185(3):83.

23. Mira F, Purpari G, Lorusso E, Di Bella S, Gucciardi F, Desario C, et al. Introduction of Asian canine parvovirus in Europe through dog importation. Transbound Emerg Dis. 2018;65(1):16-21.

24. Mira F, Purpari G, Di Bella S, Colaianni ML, Schiro G, Chiaramonte G, et al. Spreading of canine parvovirus type 2c mutants of Asian origin in southern Italy. Transbound Emerg Dis. 2019;66(6):2297-304.

25. Carmona-Vicente N, Buesa J, Brown PA, Merga JY, Darby AC, Stavisky J, et al. Phylogeny and prevalence of kobuviruses in dogs and cats in the UK. Vet Microbiol. 2013;164(3-4):246-52.

26. Felsenstein J. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. Evolution. 1985;39(4):783-91.

27. Nei M, Kumar S. Molecular evolution and phylogenetics. Oxford ; New York: Oxford University Press; 2000. xiv, 333 p. p.

Figure Legend

Figure 1. VP2 gene sequences of UK canine and feline parvovirus isolates. A: Neighbour-Joining tree (13). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (26), only values of >70 are shown. The evolutionary distances were computed using the p-distance method (27) and are in the units of the number of base differences per site. The analysis involved 76 nucleotide sequences. CPV and FPV isolates from this study are labelled in blue and green, respectively; CPV sequences from a previous UK study (6) are indicated by an asterisk\*. CPV and FPV sequences retrieved from GenBank are labelled in black. B: The geographic location of viruses sequenced in this study. Colours correspond to the sequences in A.