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To cite this version:

J. Stavisky, G.L. Pinchbeck, A.J. German, S. Dawson, R.M. Gaskell, et al.. Prevalence of canine enteric coronavirus in a cross-sectional survey of dogs presenting at veterinary practices. Veterinary Microbiology, 2009, 140 (1-2), pp.18. 10.1016/j.vetmic.2009.07.012 . hal-00535916

HAL Id: hal-00535916

https://hal.science/hal-00535916v1

Submitted on 14 Nov 2010

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PII: S0378-1135(09)00330-7
DOI: doi:10.1016/j.vetmic.2009.07.012
Reference: VETMIC 4500

To appear in: *VETMIC*

Received date: 12-12-2008
Revised date: 24-6-2009
Accepted date: 3-7-2009

Please cite this article as: Stavisky, J., Pinchbeck, G.L., German, A.J., Dawson, S., Gaskell, R.M., Ryvar, R., Radford, A.D., Prevalence of canine enteric coronavirus in a cross-sectional survey of dogs presenting at veterinary practices, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2009.07.012

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Prevalence of canine enteric coronavirus in a cross-sectional survey of dogs presenting at veterinary practices

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Abstract

In order to determine the prevalence of canine enteric coronavirus (CECoV) in the general dog population, faecal samples were obtained in a cross-sectional study of 249 dogs presenting for any reason at veterinary practices randomly selected from across the UK. Demographic and clinical data was obtained for each of the samples, including signalment, number of dogs in the household, reason for visiting the practice, and any recent history of diarrhoea. The samples were tested by RT-PCR for the presence of both type I and type II CECoV. Seven samples were positive (three from dogs in the same household), a prevalence of 2.8% (95% confidence intervals 1.1-5.7). Phylogenetic analysis of partial M gene sequences revealed that all seven positive samples grouped with type I CECoV, the first report of this virus in the UK. None of the positive dogs presented for gastrointestinal disease. Interestingly five of the positive dogs from three separate households were aged over

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six years, suggesting that older dogs may play an important role in the persistence of CECoV in such populations.

Key Words

Canine coronavirus; prevalence; type I; RT-PCR; cross-sectional.

Introduction

Canine enteric coronavirus (CECoV) is a common pathogen of dogs. Disease is typified by mild enteritis; however sporadic outbreaks, usually in puppies, of haemorrhagic and fatal enteritis have been attributed to CECoV (Buonavoglia et al., 2006; Evermann et al., 2005; Pratelli, 2005). Severe disease outbreaks appear to be associated with the evolution of novel highly pathogenic strains of CECoV (Decaro et al., 2008). Coinfection with other enteric pathogens such as canine parvovirus appears to have a synergistic effect (Pratelli et al., 1999b).

CECoV has recently been found to exist in two closely related forms. The original strain is now designated as type II CECoV, whereas the more recently identified strain, first reported in 2003, is known as type I CECoV (Pratelli et al., 2003a). The strains are named in this way due to their respective homologies to types II and I feline coronavirus (FCoV) (Herrewegh et al., 1998). Infection may occur with a single strain, however mixed infections with both types appear to be common (Decaro et al., 2005). CECoV is distinct from the newly-recognised canine respiratory coronavirus (CRCoV), which is implicated in the canine infectious respiratory disease complex and falls into a separate group of the coronavirus family (Erles et al., 2003).

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A range of methodologies have been used to assess the prevalence of both type I and II CECoV in different dog populations. Seroprevalence estimates using type II-based assays range between 16%-94% (Naylor et al., 2001; Yesilbag et al., 2004), with kennelled dogs tending to show a higher prevalence. In diarrhoeic dogs the prevalence of CECoV by RT-PCR has been reported to range from 15-42% in pet dogs (Bandai et al., 1999; Pratelli et al., 2000; Yesilbag et al., 2004) and up to 73% in kennelled dogs (Sokolow et al., 2005).

There is little data on the prevalence of CECoV infection in dogs without enteric disease, and what is available is based on virus isolation, which can only detect type II CECoV, or electron microscopy, which is relatively insensitive (Tennant et al., 1993) (Schulz et al., 2008). This leaves a significant gap in our understanding of the epidemiology of this disease in the dog population as a whole. In order to address this deficiency, we have carried out a cross-sectional study of CECoV carriage as determined by RT-PCR in dogs presenting for any reason at randomly-selected veterinary practices across the UK.

Materials and Methods

One veterinary practice was recruited by random selection from each of the twenty-three regions of the United Kingdom (as defined by the Royal College of Veterinary Surgeons register) in order to source a wide geographical distribution of samples.

Participating veterinarians were requested to enrol a cross-section of twenty-five dogs visiting for any reason to reflect the clientele and caseload of their surgery: it was not feasible to select the study dogs randomly whilst maintaining compliance. From each sampled dog, a

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faecal sample was collected and sent by post, along with a brief questionnaire to elicit information such as age, breed, sex, reason for visit and any recent history of enteric disease.

At the laboratory, samples were homogenised in a 10% dilution of minimum essential medium with 10% foetal calf serum, clarified by centrifugation and genetic material extracted using the Qiamp Viral RNA mini kit (Qiagen) as recommended by the manufacturer. Reverse transcription was carried out using random primers and Superscript III MuLV Reverse Transcriptase (Invitrogen), according to the manufacturer's instructions.

A 409bp fragment of the M gene was amplified by PCR, using Thermoprime Plus DNA polymerase with 10X ReddyMix PCR buffer (Abgene), and the primer pair CCOV1/ CCOV2 (Pratelli et al., 1999a). These primers have previously been successfully used to detect both types I and II CECoV (Benetka et al., 2006). Negative controls and at least one positive control were included at every stage, from RNA extraction to PCR. The positive control comprised a faecal sample, either spiked with CECoV C54 (type II, available from the start of the study) or naturally infected with CECoV 07-019 (type I, became available during the course of the study).

The PCR products were purified (QIAquick PCR purification kit; Qiagen) and sequenced bi-directionally using primers CCOV1 and CCOV2 according to standard protocols (ABI Prism BigDye terminators version 3.0 cycle sequencing kits; Applied Biosystems). For each amplicon, a consensus sequence was produced using ChromasPro version 1.32 (Technelysium Ptl Ltd). Ambiguities identified consistently in both strands were included in the consensus sequence. All primer sites were removed prior to analysis, resulting in a final useable sequence of 316 nucleotides, corresponding to nucleotides 379 to 694 of CCoV type I

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259/01 (accession af502583) and nucleotides 6782 to 7094 of CCoV type II INSAVC (accession D13096). Sequences have been submitted to Genbank (EU339175 - EU339181).

Sequence alignments, nucleotide distance calculations (Jukes-Cantor), and phylogenetic analysis were performed using MEGA version 4.0 (Tamura et al., 2007). Support for individual nodes was sought by bootstrap analysis using 1000 repetitions.
Statistical analysis was performed using SPSS for Windows, Rel. 14.0.0 2005. Chicago SPSS Inc.. Data were analysed using Chi Squared and Fisher's Exact Tests.

Results

Two hundred and forty-nine samples were obtained from twenty practices (figure 1); numbers submitted varied from 3-33, with a mean of 12 per practice.

Characteristics of the sample population

Of the 249 dogs sampled, 111 (44.6%) were male, 134 (53.8%) female and for four (1.6%) the sex was not specified. Dogs ranging from two months to eighteen years of age were sampled. The age distribution was right-skewed (ie the tail is on the right) (figure 2). This contrasts with findings from a door-to-door questionnaire study in which the age distribution of the pet dogs formed a normal distribution (Westgarth C, 2008). This contrast may reflect the large number of routine veterinary visits which many dogs undergo early in life for puppy checks, primary vaccination and neutering; however it may also reflect possible bias in the selection of cases by veterinarians.

The majority of the dogs (144, 57.8%) were from single-dog households. Fifty-four (21.7%) were from two-dog households, and the remaining 51 (20.5%) came from households

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containing three or more dogs. This contrasts with previous data from a community-based survey of dog-ownership in which 77% of the households had a single dog, with 20% and 3% having two dogs and three or more dogs respectively (Westgarth et al., 2007). This may reflect the fact that a domestic door-to-door questionnaire would be likely to miss farms, commercial kennels and breeding premises. The breeds sampled are shown in figure 3. Apart from cross-breeds and Great Danes, all of the breeds most commonly sampled correspond to the 20 breeds most frequently registered in 2006 (KennelClub, 2007).

Numerous conditions were given as the primary reason for taking the dog to the veterinary surgeon. The most common reasons in the 228 dogs for which a specific reason was given are shown in figure 4. The most common reason was vaccination (18.4%, 42/228), followed by 'routine' (which included weight check, claw clip, parasite treatment or social reasons such as accompanying another dog) and ear/ skin problems, each of which were cited as the reason for visit in 12.7% (29/228) of the dogs surveyed. 7% (16/228) presented for diarrhoea, with or without vomiting.

Prevalence of CECoV

Of the 249 samples obtained, seven were positive for CECoV, a prevalence of 2.8% (95% confidence intervals 1.1-5.7). Three of the positive samples (sample numbers 01-033, 01-039 and 01-046) were obtained from dogs from the same multi-dog breeding household (fig 1). The other four were from different households with a wide geographical distribution. Samples 12-020 and 12-030 were from pups of less than 6 months of age; all of the other positive samples were from dogs aged 6-11 years. Three of the seven positive dogs (sample numbers 06-018, 07-019 and 12-020) had a history of diarrhoea within the previous month (Table 1), although none of the seven presented at the surgery for diarrhoea. There was no

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significant association between either presenting for, or having a history of, recent diarrhoea and being CECoV positive (p=0.4) (Table 1), although with such a small number of positive samples, it would have been difficult to detect a statistically significant association.

When sequenced, all seven positive samples grouped with type I CECoV in the M gene region (figure 1). No type II CECoV was obtained from any of the samples. Sequence analysis and retrospective testing of the positive samples with a real time PCR assay (adapted for use in our laboratory (Decaro et al., 2005) confirmed that none of the positive samples contained mixed type I/type II infections.

As the prevalence of infection was lower than anticipated, further investigations were carried out to confirm the validity of the methods used. In the 233/249 samples for which the data was available, 93.1% of the samples were received by the laboratory within four days, with a median time in transit in the post of 2 days (figure 5). A loss of titre during transportation was therefore considered possible. However in pilot experiments, a <10-fold loss in sensitivity was found, using the conventional PCR assay, when CECoV spiked faecal samples were left at room temperature for seven days (figure 6). As 98.3% of the samples were received at the laboratory within 7 days of collection, this time delay is unlikely to have been a major contributory factor to the low prevalence detected, although some potential effect cannot be ruled out in those few samples which were delayed in the post for longer periods.

The sensitivity of the conventional PCR assay used was also examined in relation to the real-time PCR assay of Decaro et al, 2005, and was found to have a similar sensitivity for the detection of type I, type II, and mixed infections. The sensitivity was equivalent to 100-1000 RNA copies per μl of cDNA for the conventional PCR, as compared to 100 RNA copies per

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μl of cDNA in the real-time PCR. In addition, both assays (real time and conventional) have subsequently been used on samples from 166 kennelled dogs, where the prevalence of CECoV carriage was expected to be high. Overall concordance between the two assays was 96% (160/166). Of these, 30 were CECoV positive by both methods (9 type I, 6 type II and 15 mixed), four by real-time PCR alone (2 type I, 1 type II, 1 mixed) and two by conventional PCR alone, presumably due to primer or probe binding site mismatches.

It was therefore concluded that the sensitivity and specificity of the methods used were broadly comparable to that of the real-time PCR.

Discussion

Although the importance of CECoV as a canine pathogen is being increasingly recognised, its prevalence is poorly understood. Most studies have focussed on diarrhoeic or kennelled dogs, and there is little data on the prevalence of CECoV in the wider dog population, including healthy pet dogs. Such information is, however, important in increasing our understanding of the epidemiology of the disease. In the present study, we have targeted this population of both asymptomatic and diarrhoeic dogs through a cross-sectional study survey of randomly selected veterinary practices from throughout the UK. In order to maximise compliance, random selection was not used at the client level, and it is possible that this may have caused some bias.

Despite the large number of dogs sampled in our study, the prevalence estimate obtained was relatively low (2.8%). There are few studies on prevalence in comparable dog populations, but in a small study in Liverpool using virus isolation, no CECoV was obtained from 26 healthy pet dogs in a boarding kennel, although eight of 32 dogs with acute diarrhoea were positive for type II CECoV in the same study (Tennant et al., 1993). This contrasts with a

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more recent study in which a prevalence of 17.5% was detected by electronmicroscopy in 200 healthy dogs in Germany (Schulz et al., 2008). The variability of prevalence estimates depends on factors such as the detection methods used and the characteristics and disease status of the dog population under study. In addition, it is possible that the prevalence of CECoV in a population might fluctuate over time and that any prevalence estimate will be dependent on the timing of sampling.

Although 28.6% of the 248 dogs for which data was available either had diarrhoea at the time of sampling or a recent history of diarrhoea, no significant association was detected with CECoV shedding when compared to animals with no history of diarrhoea. However, the number of CECoV positive dogs in our study was small, and this makes the power to detect an association low. There are a number of possible reasons why relatively few diarrhoeic dogs were positive for CECoV in our study. Mild diarrhoea in dogs is very often non-infectious in origin and in some cases may simply be a natural sequel to dietary indiscretion. Alternatively, other pathogens may have been involved, or if the diarrhoea had been coronaviral in origin, virus shedding may have stopped by the time of sampling.

Interestingly however, although overall there was no significant association between being CECoV positive and having diarrhoea, three of the seven positive dogs (all three coming from different households) had had diarrhoea within the previous month. It is not clear whether these seven dogs were undergoing acute asymptomatic infection or more chronic shedding. Dogs have been shown to shed CECoV for a variable but potentially long time following infection and clinical resolution (Decaro et al., 2005). In one natural infection study, one animal was reported to shed CECoV for up to 156 days even though signs of clinical disease only lasted for 10 days post-infection (Pratelli et al., 2002). Such clinically

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normal carriers are likely to be important in maintaining infection in the general dog population.

This study reports the first identification of type I CECoV in the UK. No type II CECoV was identified from any of the samples in this study, although the same assay has been successfully used in our laboratory to detect both types I and II CECoV in other samples from UK dogs. There is some suggestion that type I CECoV may be shed at higher titres and for a longer period than type II, and if this is indeed the case this could lead to an enhanced probability of detecting type I CECoV in a cross-sectional study of this kind (Decaro et al., 2005). Type 1 CECoV virus was first described in 2003 in Italy (Pratelli et al., 2003b) and has since been reported in other countries. (Yesilbag et al., 2004), (Rennhofer et al., 2005). Recent evidence, based on the discovery of a new open reading frame present in type I CECoV (Lorusso et al., 2008a; Stavisky et al., 2008), with residual fragments in type II CECoV, and the closely related feline coronavirus and transmissible gastroenteritis virus, suggests that type I CECoV may actually represent the ancestral virus of this group (Lorusso et al., 2008b).

The clinical significance of the distinction between type I and II CECoV is not clear. Although classic CECoV infection is considered to cause only mild enteric disease (Tennant et al., 1991), there are several reports where type II CECoV has been associated with more severe haemorrhagic diarrhoea and occasional death. (Binn et al., 1974; Buonavoglia et al., 2006; Zappulli et al., 2007). It has also been suggested that such signs can be seen with type I (Benetka et al., 2006). Since more virulent strains appear to evolve spontaneously (Decaro et al., 2008; Zappulli et al., 2007), it may be that both types I and II have the ability to cause a wide spectrum of clinical manifestations, and this variation in virulence may depend on both

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viral evolution and host factors such as age and immune status. Further work is needed on different populations of dogs to help clarify this.

Recognition of CECoV in practice is hampered by the fact that commercial diagnostic tests suitable for the detection of types I and II CECoV are not widely available. Electron microscopy is relatively insensitive, and serological assays are based on type II CECoV, which limits their use as they have unknown efficacy in detecting type I. In addition, since seroprevalence in the general dog population is relatively high, such tests are difficult to interpret in relation to diagnosing acute disease unless paired samples are taken. The efficacy of currently available CECoV vaccines, which are all based on type II CECoV, is also unclear. The only data on cross protection available to date is based on *in vitro* work and suggests that the level of antigenic cross reactivity between the two types may be limited (Pratelli et al., 2004). Clearly, this should also be assessed in challenge experiments in vivo.

In conclusion, we have shown that the newly-recognised type I CECoV is circulating at a low prevalence among clinically normal adult dogs in the UK. Previous studies have concentrated on kennelled dogs, and dogs with disease, which are known to be at high risk of infection (Naylor et al., 2001; Sokolow et al., 2005). Subclinically infected animals within the general dog population, as identified by this study, may represent an important reservoir of infection, playing a significant role in the epidemiology of this disease and the evolution of the virus.

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Acknowledgements

We acknowledge all of the staff from all of the participating veterinary practices. Thanks also to Bryony Parsons and Carol Porter for their generous assistance in the laboratory.

This work was made possible by a grant from Intervet Schering-Plough Animal Health.

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References

Bandai, C., Ishiguro, S., Masuya, N., Hohdatsu, T., Mochizuki, M., 1999, Canine coronavirus infections in Japan: virological and epidemiological aspects. Journal of Veterinary Medical Science 61, 731-736.

Benetka, V., Kolodziejek, J., Walk, K., Rennhofer, M., Mostl, K., 2006, M gene analysis of atypical strains of feline and canine coronavirus circulating in an Austrian animal shelter. Vet Rec. 159, 170-174.

Binn, L.N., Lazar, E.C., Keenan, K.P., Huxsoll, D.L., Marchwicki, R.H., Strano, A.J., 1974, Recovery and characterization of a coronavirus from military dogs with diarrhea. Proceedings, annual meeting of the United States Animal Health Association, 359.

Buonavoglia, C., Decaro, N., Martella, V., Elia, G., Campolo, M., Desario, C., Castagnaro, M., Tempesta, M., 2006, Canine coronavirus highly pathogenic for dogs. Emerging Infectious Diseases 12, 492-494.

Decaro, N., Campolo, M., Lorusso, A., Desario, C., Mari, V., Colaianni, M.L., Elia, G., Martella, V., Buonavoglia, C., 2008, Experimental infection of dogs with a novel strain of canine coronavirus causing systemic disease and lymphopenia. Veterinary Microbiology 128, 253-260.

Decaro, N., Martella, V., Ricci, D., Elia, G., Desario, C., Campolo, M., Cavaliere, N., Di Trani, L., Tempesta, M., Buonavoglia, C., 2005, Genotype-specific fluorogenic RT-PCR assays for the detection and quantitation of canine coronavirus type I and type II RNA in faecal samples of dogs. J Virol Methods 130, 72-78.

Erles, K., Toomey, C., Brooks, H.W., Brownlie, J., 2003, Detection of a group 2 coronavirus in dogs with canine infectious respiratory disease. Virology 310, 216-223.

Accepted Manuscript

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Evermann, J.F., Abbott, J.R., Han, S., 2005, Canine coronavirus-associated puppy mortality without evidence of concurrent canine parvovirus infection. Journal Of Veterinary Diagnostic Investigation 17, 610-614.

Herrewegh, A.A.P.M., Smeenk, I., Horzinek, M.C., Rottier, P.J.M., Groot, R.J.d., 1998, Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. Journal of Virology 72, 4508-4514.

KennelClub 2007. 2006 Top Breed Registrations. In Kennel Club.

Lorusso, A., Decaro, N., Schellen, P., Rottier, P.J.M., Buonavoglia, C., Haijema, B.-J., De Groot, R.J., 2008a. Gain, Preservation and Loss of a Group 1a Coronavirus Accessory Glycoprotein. In: Nidovirus XI, Oxford, 22/06/08, p. 45.

Lorusso, A., Decaro, N., Schellen, P., Rottier, P.J.M., Buonavoglia, C., Haijema, B.-J., de Groot, R.J., 2008b, Gain, Preservation, and Loss of a Group 1a Coronavirus Accessory Glycoprotein. J. Virol. 82, 10312-10317.

Naylor, M.J., Monckton, R.P., Lehrbach, P.R., Deane, E.M., 2001, Canine coronavirus in Australian dogs. Australian Veterinary Journal 79, 116-119.

Pratelli, A., 2005, Canine coronavirus. Recent advances in its biological, diagnostic, and prophylactic characteristics. Obiettivi e Documenti Veterinari 26, 27-34.

Pratelli, A., Buonavoglia, D., Martella, V., Tempesta, M., Lavazza, A., Buonavoglia, C. 2000. Diagnosis of canine coronavirus infection using nested-PCR. In Journal Of Virological Methods, pp. 91-94.

Pratelli, A., Elia, G., Decaro, N., Tola, S., Tinelli, A., Martella, V., Rocca, S., Tempesta, M., Buonavoglia, C., 2004, Cloning and expression of two fragments of the S gene of canine coronavirus type I. Journal Of Virological Methods 117, 61-65.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Pratelli, A., Elia, G., Martella, V., Tinelli, A., Decaro, N., Marsilio, F., Buonavoglia, D., Tempesta, M., Buonavoglia, C., 2002, M gene evolution of canine coronavirus in naturally infected dogs. Veterinary Record 151, 758-761.

Pratelli, A., Martella, V., Decaro, N., Tinelli, A., Camero, M., Cirone, F., Elia, G., Cavalli, A., Corrente, M., Greco, G., Buonavoglia, D., Gentile, M., Tempesta, M., Buonavoglia, C., 2003a, Genetic diversity of a canine coronavirus detected in pups with diarrhoea in Italy. Journal Of Virological Methods 110, 9-17.

Pratelli, A., Martella, V., Pistello, M., Elia, G., Decaro, N., Buonavoglia, D., Camero, M., Tempesta, M., Buonavoglia, C., 2003b, Identification of coronaviruses in dogs that segregate separately from the canine coronavirus genotype. Journal Of Virological Methods 107, 213-222.

Pratelli, A., Tempesta, M., Greco, G., Martella, V., Buonavoglia, C., 1999a, Development of a nested PCR assay for the detection of canine coronavirus. Journal Of Virological Methods 80, 11-15.

Pratelli, A., Tempesta, M., Roperto, F.P., Sagazio, P., Carmichael, L., Buonavoglia, C., 1999b, Fatal coronavirus infection in puppies following canine parvovirus 2b infection. Journal of Veterinary Diagnostic Investigation 11, 550-553.

Rennhofer, M., Benetka, V., Sommerfeld-Stur, I., Möstl, K., 2005, Epidemiological investigations on coronavirus infections in dogs and cats in an animal shelter. Wiener Tierärztliche Monatsschrift 92, 21-27.

Schulz, B.S., Strauch, C., Mueller, R.S., Eichhorn, W., Hartmann, K., 2008, Comparison of the prevalence of enteric viruses in healthy dogs and those with acute haemorrhagic diarrhoea by electron microscopy. Journal of Small Animal Practice 49, 84-88.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Sokolow, S.H., Rand, C., Marks, S.L., Drazenovich, N.L., Kather, E.J., Foley, J.E., 2005, Epidemiologic evaluation of diarrhea in dogs in an animal shelter. American Journal Of Veterinary Research 66, 1018-1024.

Stavisky, J., Robinson, O., Pinchbeck, G., German, A.C., Dawson, S., Gaskell, R.M., Ryvar, R., Radford, A.D., 2008. Investigation of the Distribution and Recent Evolution of Canine Enteric Coronavirus. In: Nidovirus XI, Oxford, 22/06/08, p. 80.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007, MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol Biol Evol 24, 1596-1599.

Tennant, B., Gaskell, R.M., Kelly, D.F., Carter, S.D., Gaskell, C.J., 1991, Canine Coronavirus infection in the dog following oronasal inoculation. Research in Veterinary Science, 11-18.

Tennant, B.J., Gaskell, R.M., Jones, R.C., Gaskell, C.J., 1993, Studies on the epizootiology of canine coronavirus. Vet Rec. 132, 7-11.

Westgarth C, P.G., Bradshaw JWS, Dawson S, Gaskell RM, Christley RM, 2008, Dog-human and dog-dog interactions in a community of 260 dog-owning households in Cheshire. . Veterinary Record In Press.

Westgarth, C., Pinchbeck, G., Bradshaw, J., Dawson, S., Gaskell, R., Christley, R., 2007, Factors associated with dog ownership and contact with dogs in a UK community. BMC Veterinary Research 3, 5.

Yesilbag, K., Yilmaz, Z., Torun, S., Pratelli, A., 2004, Canine coronavirus infection in Turkish dog population. Journal Of Veterinary Medicine Series B-Infectious Diseases And Veterinary Public Health 51, 353-355.

Zappulli, V., Caliari, D., Cavicchioli, L., Tinelli, A., Castagnaro, M., 2007, Systemic fatal type II coronavirus infection in a dog: Pathological findings and immunohistochemistry. Res Vet Sci.

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Table 1: Numbers of dogs with and without recent diarrhoea sampled in a cross-sectional study of 249 dogs from 20 UK veterinary practices.

Figure 1: Map of the United Kingdom. Squares show approximate locations of participating practices. Black squares indicate practices which submitted positive samples; grey squares indicate all other participating practices.

Rooted neighbour-joining tree of the seven partial M gene sequences generated in this study and published reference canine, feline and human coronaviruses (outgroup). All branch lengths are proportional to distances established using the Jukes-Cantor method. Bootstraps are expressed as percentages, and only included where greater than 75%.

Key: HCoV NL63 accession DQ445912, FCoV C1Je accession DQ848678; FCoV 1146 accession DQ010921; FCoV DF-2 accession DQ286389; CECoV BGF10 accession AY342160; CECoV 259/01 accession af502583; CECoV 23/03 accession AY548235; CECoV INSAVC accession D13096. Samples from this study are coded CECoV x-y, where x is the internally designated practice number, and y the individual dog reference.

Figure 2: Histogram to show age distribution of dogs sampled in a cross-sectional study of 249 dogs from 20 UK veterinary practices. Data missing from one dog, therefore total number of dogs included is 248.

Figure 3: Histogram to show distribution of most common breeds of dog sampled in a cross-sectional study of 249 dogs from 20 UK veterinary practices. 202 dogs are included; breed was not specified for 47 dogs. Breeds with less than 4 dogs sampled are grouped under 'other breeds'.

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Figure 4: Bar chart to show the reasons for visit cited by study participants. This data was available for 228 dogs. *Routine includes weight check, claw clip, flea/ worm treatment and social reasons for visiting the surgery. # Sick dog included a broad range of diagnoses such as pyometra, false pregnancy, and convulsions. Conditions classified as 'misc' (miscellaneous) were those where the health status of the dog was impossible to determine, eg 'operation' which could refer to an elective or emergency surgery.

Figure 5: Histogram to show the length of time between collection of each faecal sample and receipt at the laboratory. Data was available for 233/249 samples submitted.

Figure 6: Faeces spiked with CECoV-C54 were kept at room temperature for a number of days and then underwent RT-PCR using the protocol described. It can be seen that even after 7 days a faint band was visible at the -3 dilution, suggesting a loss in titre of less than tenfold.

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Table 1

	CECoV positive	CECoV negative	Total
Diarrhoea as presenting complaint	0	16	16
Owner-reported diarrhoea within previous month	3	52	55
No history of recent diarrhoea	4	173	177
Total	7	241	248*

* One negative sample had missing questionnaire data

Figure 1

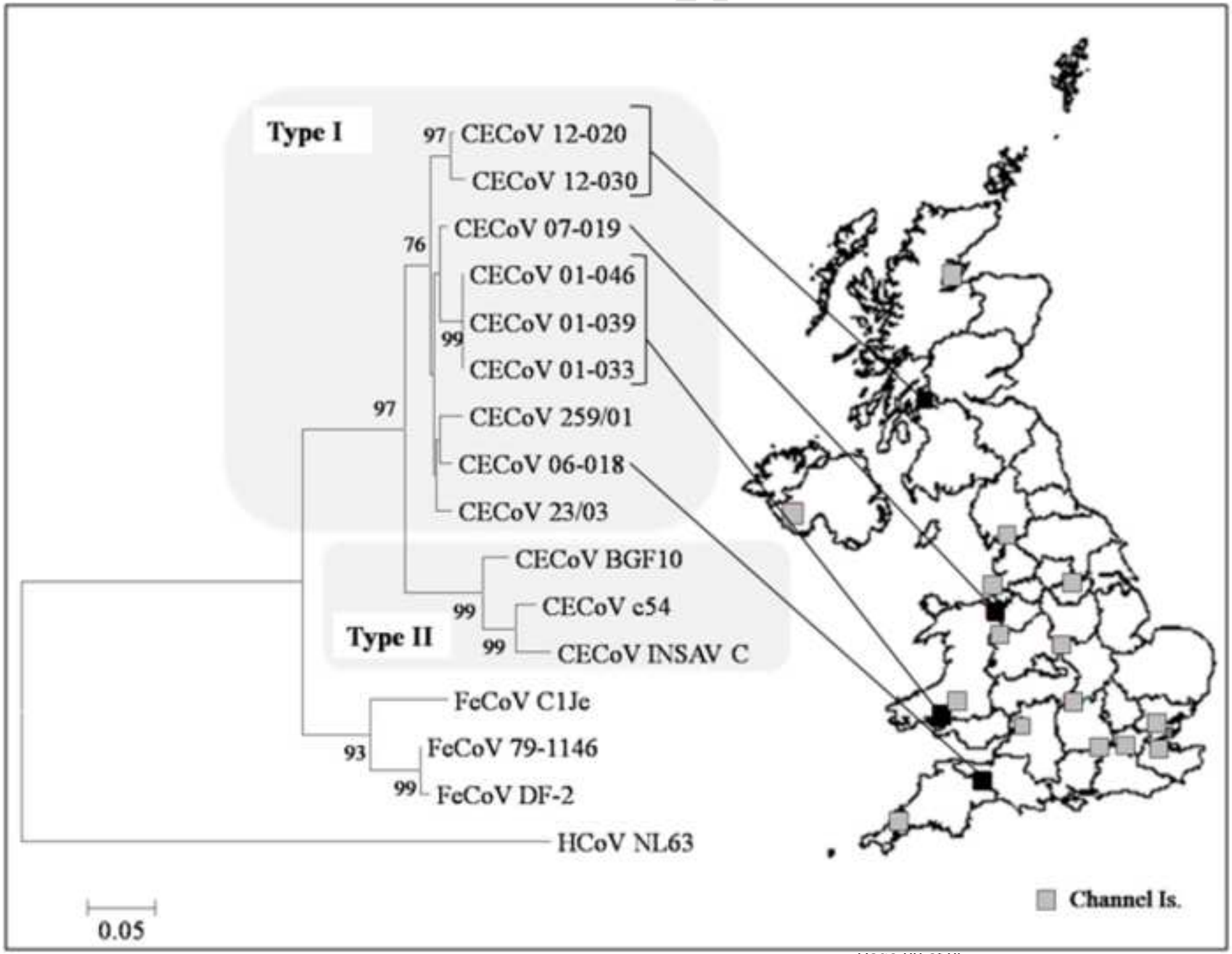

Type I
97
CECoV 12-020
CECoV 12-030
76
CECoV 07-019
CECoV 01-046
99
CECoV 01-039
CECoV 01-033
97
CECoV 259/01
CECoV 06-018
CECoV 23/03
CECoV BGF10
99
CECoV c54
Type II
99
CECoV INSAV C
FcCoV C1Je
93
FcCoV 79-1146
99
FcCoV DF-2
HCoV NL63
0.05
Channel Is.

Figure 2

ACCEPTED MANUSCRIPT

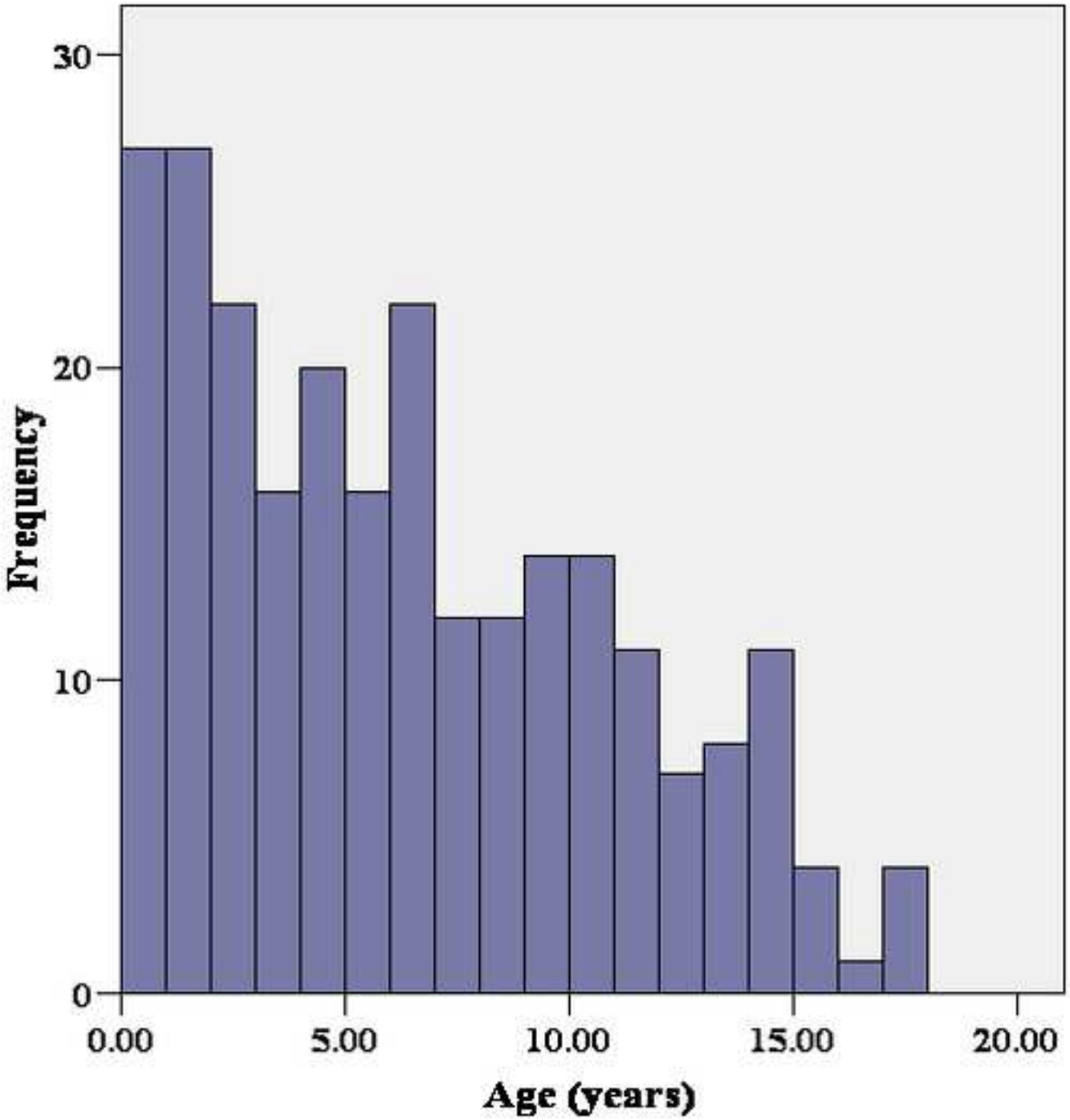


Figure 3

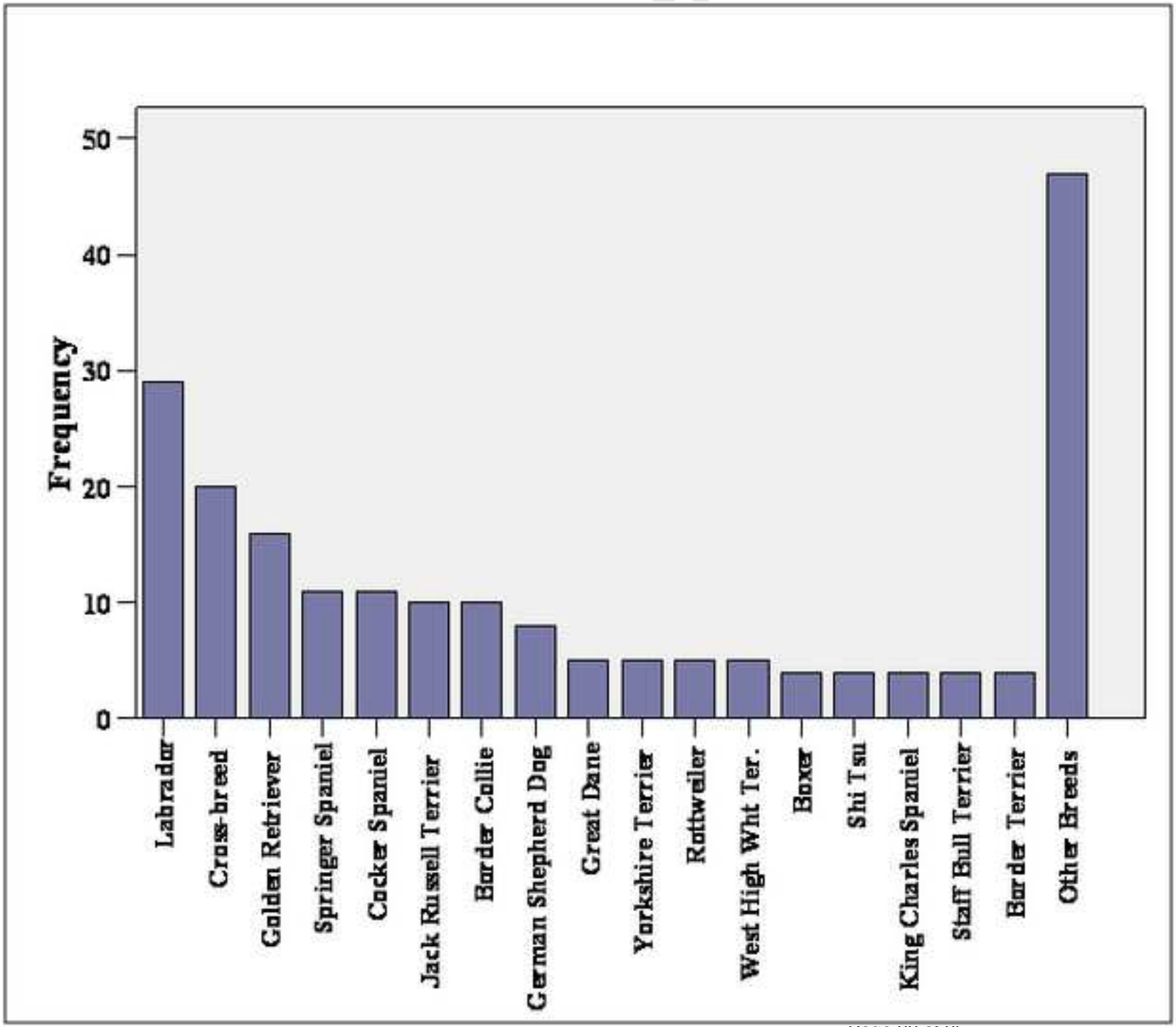

Frequency
0
10
20
30
40
50
Labrador
Cross-breed
Golden Retriever
Springer Spaniel
Cocker Spaniel
Jack Russell Terrier
Border Collie
German Shepherd Dog
Great Dane
Yorkshire Terrier
Rottweiler
West High Wht Ter.
Boxer
Shi Tsu
King Charles Spaniel
Staff Bull Terrier
Border Terrier
Other Breeds

Figure 4

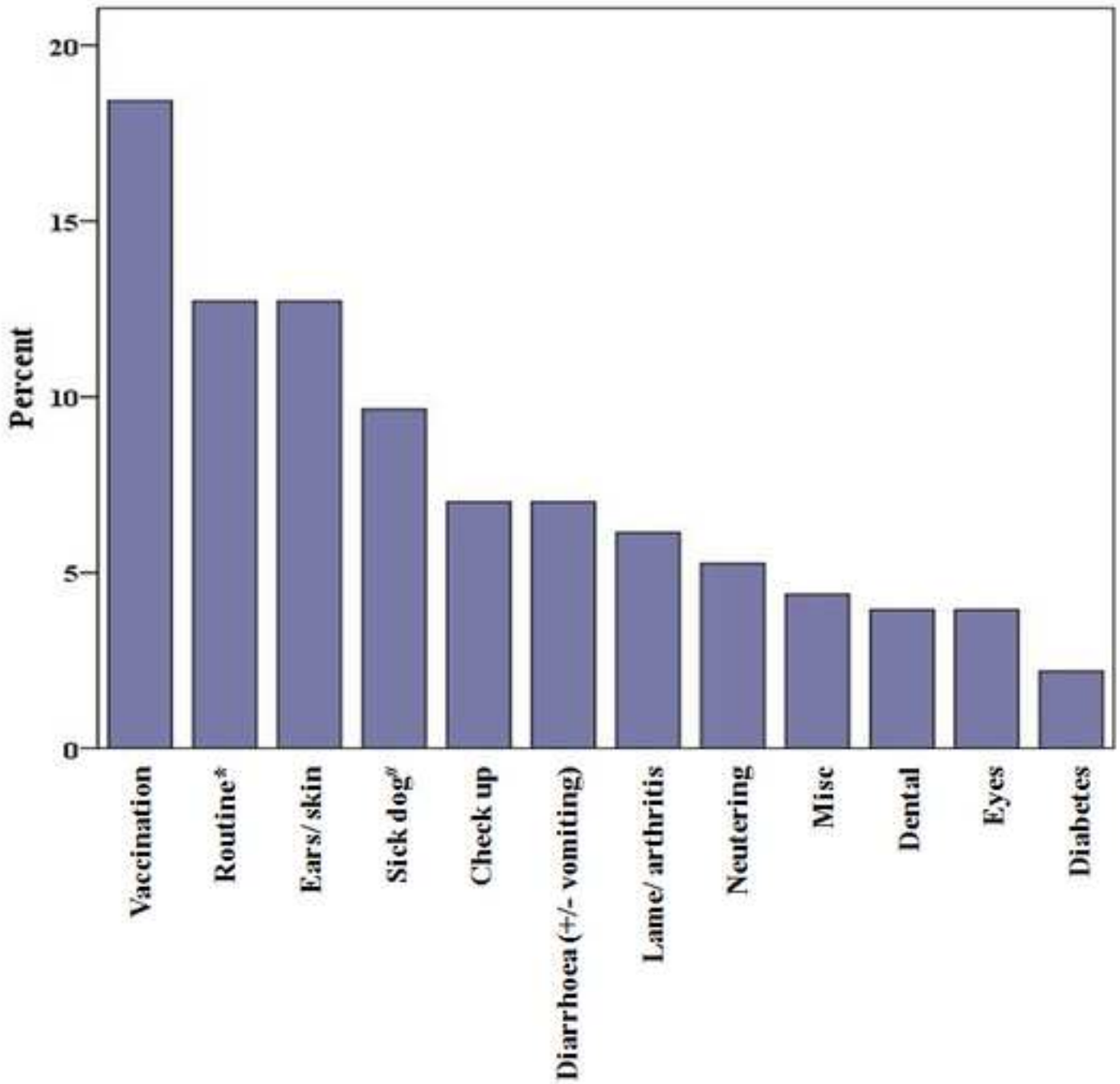
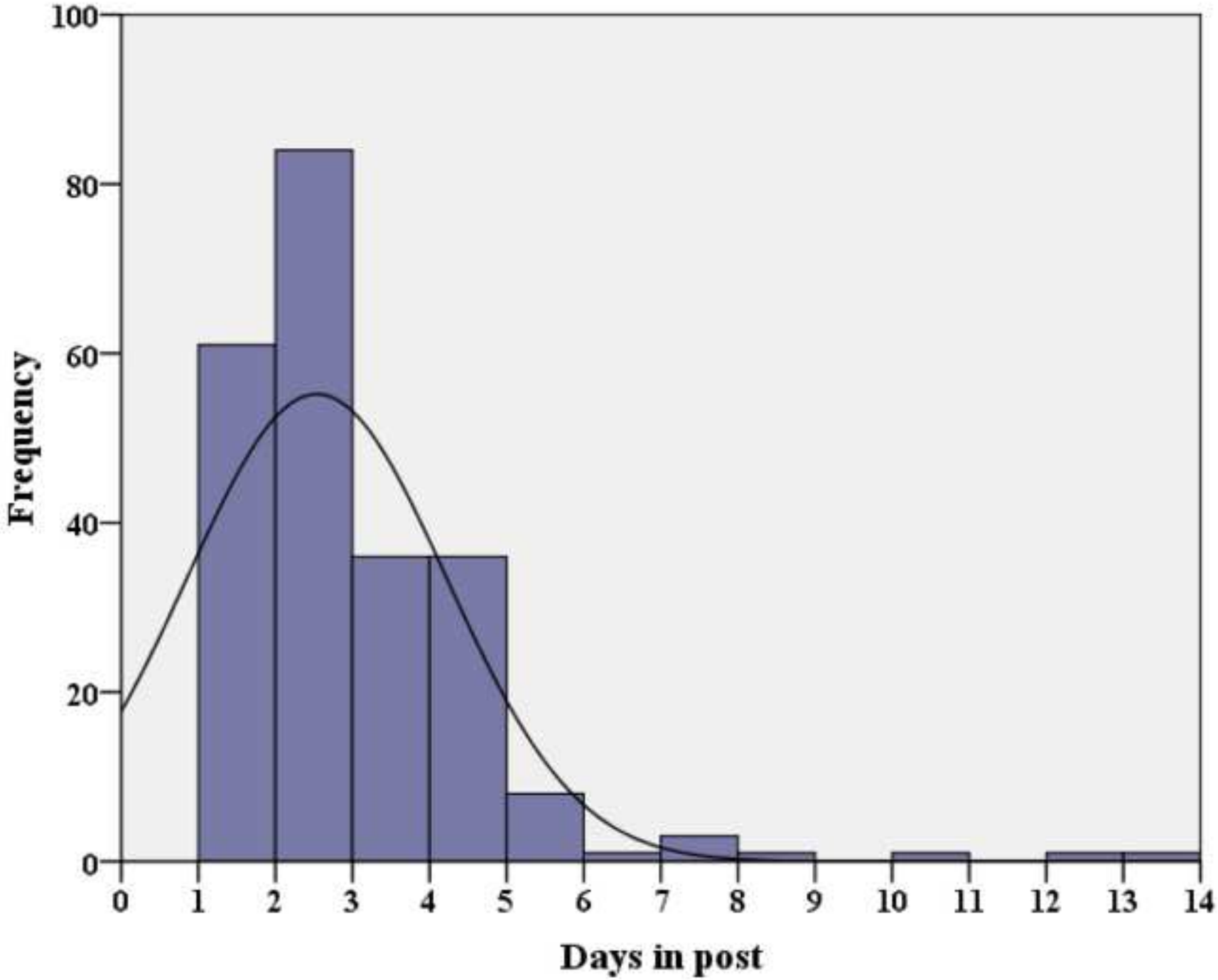

Percent
20
15
10
5
0
Vaccination
Routine*
Ears/ skin
Sick dog#
Check up
Diarrhoea (+/- vomiting)
Lame/ arthritis
Neutering
Misc
Dental
Eyes
Diabetes

Figure 5


100
80
60
40
20
0
Frequency
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Days in post

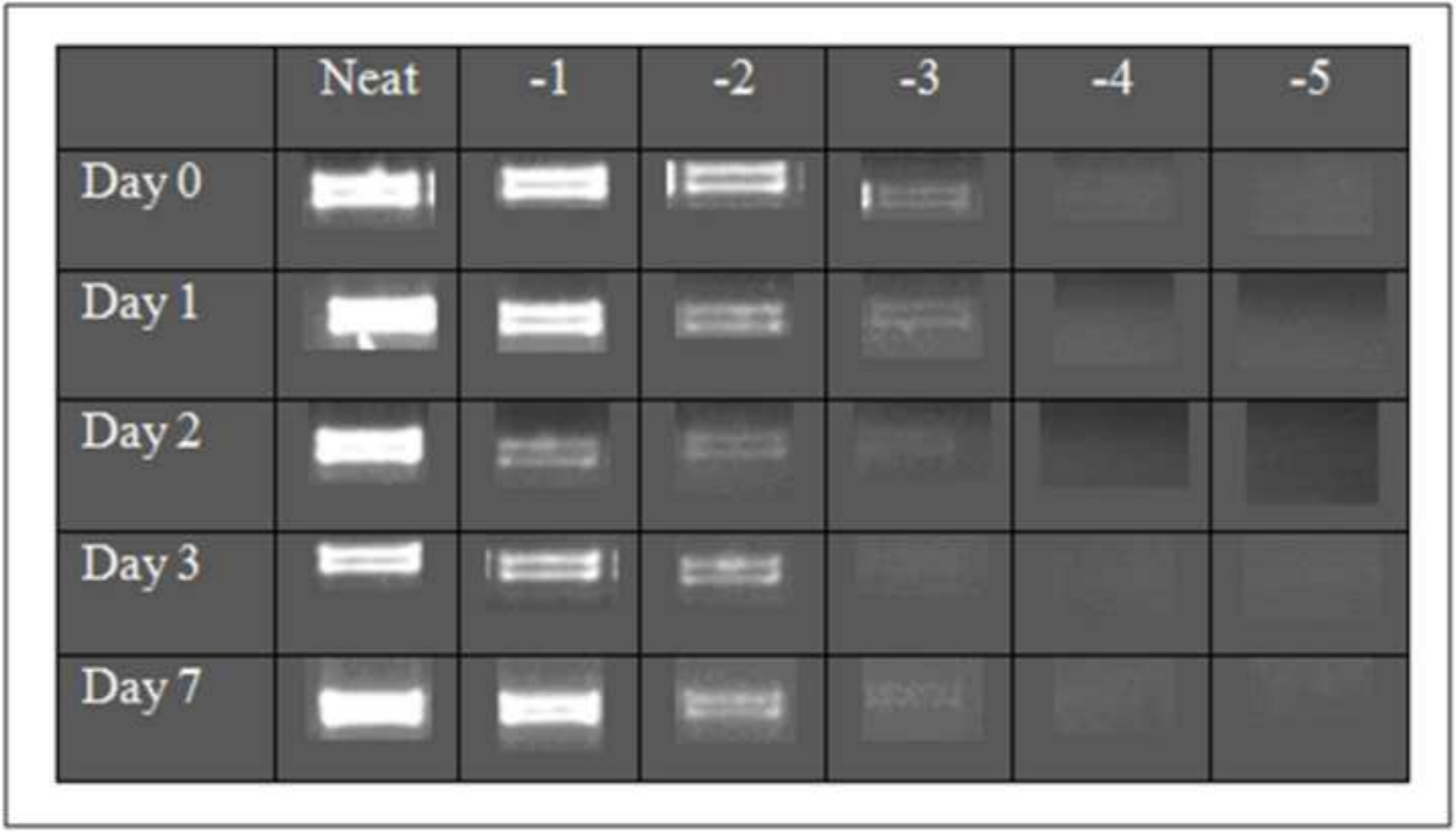



Figure 6