Faecal proteome in clinically healthy dogs and cats: Findings in pooled faeces from 10 cats and 10 dogs

Matteo Cerquetella1 | Andrea Marchegiani1 | Sara Mangiaterra1 | Giacomo Rossi1 | Alessandra Gavazza1 | Beniamino Tesei1 | Andrea Spaterna1 | Gianni Sagratini2 | Massimo Ricciutelli2 | Valeria Polzonetti1,3 | Stefania Pucciarelli1,3 | Silvia Vincenzetti1

1 School of Biosciences and Veterinary Medicine, University of Camerino, Matelica, MC, Italy
2 School of Pharmacy, University of Camerino, Camerino, MC, Italy
3 School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, MC, Italy

Correspondence
Matteo Cerquetella, School of Biosciences and Veterinary Medicine, University of Camerino, Via Circonvallazione 93/95, Matelica, MC, 62024 Italy.
Email:

Abstract
Background: In the scientific literature, there are only a few manuscripts available on small animal faecal proteomics.

Methods: In the present pilot study, this evaluation was performed using pooled faecal samples from 10 clinically healthy dogs and, for the first time, in 10 clinically healthy cats by means of two-dimensional electrophoresis followed by liquid chromatography-tandem mass spectrometry.

Results: Our results showed the presence of nine (albumin, alkaline phosphatase, chymotrypsin-C-like, cytosol aminopeptidase, elastase-3B/proteinase E, immunoglobulins and nuclear pore membrane glycoprotein 210) and 14 (albumin, caspase recruitment domain-containing protein, chymotrypsin-like, deleted in malignant brain tumours 1 protein-like, hypothetical protein LOC107375, immunoglobulin, kallikrein-1, superoxide dismutase, transthyretin precursor, interstitial collagenase-like) different proteins in canine and feline faeces, respectively.

Conclusion: These preliminary findings document the presence of a range of proteins in the faeces of apparently healthy dogs and cats and may serve as a basis for larger, prospective studies to establish reference proteomic data against which diseased populations can be compared.

KEYWORDS
cat, dog, faecal proteomics, healthy pattern

INTRODUCTION
Faecal proteomic analysis has been recently introduced in dogs,1,2 with the long-term objective of investigating new faecal markers that could be helpful in monitoring and/or diagnosing gastrointestinal (GI) diseases. Proteomics is a science aiming at analysing protein structure, function and localization in different anatomic regions.1,3 In human medicine, the faecal proteome has been investigated in GI diseases such as colorectal cancer or other diseases like cystic fibrosis. In such cases, and in other conditions like food allergies, proteomics has been shown to be a very promising tool for finding potential markers that may be useful in diagnosing and monitoring these conditions.3–5 Besides, proteomics has the advantage, compared to other ‘-omics’ (e.g., genomics), of describing the real situation of the metabolic and functional state of a certain tissue.3

At the moment, only a few studies in the dog are available in the literature on faecal proteomics to draw any conclusions. In one study, the faecal proteome of healthy Boxer dogs was investigated2; while in another study the faecal proteome of healthy dogs was compared to that of dogs suffering from food responsive diarrhoea.1 In the present paper, our aim was to describe the proteins present within the faeces of a population of apparently normal dogs and cats. Besides, to the authors’ knowledge, this is the first proteomic study on feline faeces.

MATERIALS AND METHODS
Patients and faecal sample collection
Naturally voided faecal samples from 10 clinically healthy dogs and 10 clinically healthy cats were collected and used for
the study; all owners gave informed consent. Patients were considered clinically healthy based on the absence of clinical signs of any disease at the time of sampling, absence of episodes of diarrhoea in the last 3 months, absence of diagnosis of any ongoing chronic disease (e.g., dermatopathies, endocrinopathies, cardiac disorders) according to patients’ clinical history and on the absence of any drug administered in the last 3 months (including antimicrobials, pre-probiotics and anti-inflammatory drugs). Additionally, all included cases were negative for intestinal parasitism based on centrifugation and flotation, Lugol staining and *Giardia* antigen testing from 3-day pooled faecal samples.

**Sample preparation**

The protein extraction was performed as described previously. Briefly, faecal samples were collected separately, immediately after production, and stored at −20°C until use. Two grams of frozen faeces from each clinically healthy animal were weighed and pooled together in order to have a total of 20.0 grams of faeces from both the healthy dogs’ and cats’ groups.

The experimental design of the present work is based on the complete sample pooling strategy as previously described, where the samples from each group of dogs and cats were pooled, and the replicates (repeated measurements of the same sample) were the technical replicates of the group.

Sixty millilitres of phosphate-buffered saline (PBS) containing a 1:100 diluted protease inhibitor cocktail (Sigma-Aldrich, Saint Louis, MO, USA) were added to each pooled sample. Both samples were subjected to agitation through a magnetic stirrer for 60 min on ice. Subsequently, the two mixtures were centrifuged at 10000 rpm for 20 min, at 4°C. After centrifugation, the two supernatants (from both groups) were collected, filtered three times with a paper filter, and after 30 min incubation on ice, the samples were centrifuged at 10000 rpm for 30 min at 4°C. Each resulting pellet was resuspended in 500 microliters PBS, and the total protein content was determined by the Bradford method.

**Two-dimensional electrophoresis and liquid chromatography-tandem mass spectrometry**

According to the protocol previously described by Cerquetella et al., before being loaded onto the two-dimensional electrophoresis (2-DE), 1 milligram of total proteins was treated with the 2-D Clean-Up Kit (GE-Healthcare Life Sciences, Uppsala, Sweden). The first dimension consisted of an isoelectric focusing in a pH range of 3–10 (Immobiline DryStrip, IPG-strip, length 18 centimetres; IPGphor isoelectric focusing cell, GE-Healthcare), the second dimension was a 13% SDS-PAGE (Protein II apparatus, Bio-Rad, Hercules, CA, USA). After the electrophoresis, gels were stained (0.1% Coomassie Brilliant Blue R250, 50% CH₃OH; 10% CH₂COOH), de-stained (50% CH₃OH; 10% CH₂COOH), scanned at 600 dpi and finally subjected to image analysis using PDQuest software (Version 7.1.1; Bio-Rad Laboratories) which allows calculation of the isoelectric point (pI), molecular mass, and normalized quantity of each spot in the gel. Among the total spots found in the 2DE maps (of both clinically healthy dogs and cats), only those showing a normalised quantity greater than $20 \times 10^3$ were selected for subsequent analysis by mass spectrometry. Based on the authors’ experience, a normalised quantity value of $20 \times 10^3$ is the minimum necessary to obtain a protein identification by mass spectrometry analysis. The spots obtained from both gels (clinically healthy dogs and cats) were excised, and the proteins were treated with trypsin and then extracted from the gel by following the protocol described by Shevchenko et al.

After extraction, the peptides were subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) performed as previously described. The resulting spectra were extracted and analysed by the MASCOT software and by the SONAR software that use mass spectrometry data for the protein identification from a peptide sequence database. For protein identification, the following search parameters were used: database, SwissProt and NCBI; taxonomy: *Mammalia*; enzyme, trypsin; peptide tolerance, 1.2 Da; MS/MS tolerance, 0.6 Da and allowance of one missed cleavage.

**RESULTS**

The mean age of dogs included was 6.5 years (minimum 2 years - maximum 14 years), three were males and seven females. With regard to breeds, four were Mestizos, and one was a representative of each of the following breeds: German Shepherd, Labrador Retriever, Vizsla, Rottweiler, Weimaraner and French Bulldog. The mean age of cats was 5.5 years (minimum 2 years - maximum 9 years), five were males and five females; nine were European shorthair cats, and one was a crossbreed (European shorthair cat x Persian cat).

Results of 2-DE evaluations for clinically healthy dogs and healthy cats are reported in Figures 1 and 2, respectively. The proteins that were identified by LC-MS/MS followed by MASCOT and SONAR software analysis are reported in Tables 1 and 2.

Fourteen proteins were identified for dogs (nine after excluding overlapping findings) and 19 for cats (14 after excluding overlapping findings) (Tables 1 and 2).

With regard to dogs, the spots Y and Y1 were identified as serum albumin isoform XI. Spots V1 and V2 were identified as alkaline phosphatase. Spots G and G1 were identified as immunoglobulin $\lambda$–1 light chain, and the spots G2, G3 and G4, characterised by different molecular weights and (pI), were all identified as immunoglobulin $\lambda$-light chain, VLI region. Other proteins identified in the dogs’ faeces are reported in Table 2.

Similarly, in cats, multiple spots on the electrophoresis gel were found to correspond to the same protein, as identified by LC-MS/MS. They are spots G and I identified as IgA constant region, spots L1 and L2 identified as immunoglobulin kappa light chain, and spots L3 and L4 as immunoglobulin heavy chain variable region 3. Spots M and Q were recognised
FIGURE 1 2-DE map of the healthy dogs' faecal proteins. The spots that were identified by the mass spectrometry are evidenced in red. The proteins were separated on an immobilized pH 3–10 linear gradient strip and subsequently subjected to a 13% SDS-PAGE. The standards were Bio-Rad low molecular weight (phosphorylase b, 97.4 kDa; bovine serum albumin, 66.2 kDa; ovalbumin 45.0 kDa; carbonic anhydrase, 31.0 kDa; soybean trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa)

FIGURE 2 2-DE map of the healthy cats' faecal proteins. The spots that were identified by the mass spectrometry are evidenced in red. The proteins were separated on an immobilized pH 3–10 linear gradient strip and subsequently subjected to a 13% SDS-PAGE. The standards were Bio-Rad low molecular weight (phosphorylase b, 97.4 kDa; bovine serum albumin, 66.2 kDa; ovalbumin 45.0 kDa; carbonic anhydrase, 31.0 kDa; soybean trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa)

as kallikrein-1, and finally, spots R and S identified as deleted in malignant brain tumours 1 protein-like (DMBT-1). Other proteins identified in the faeces of cats are shown in Table 2.

It can be noted (Tables 1 and 2) that in some cases the identified proteins have been attributed to a different animal species. In fact, in dogs, eight proteins were ascribed to Canis lupus familiaris, while two to Felis catus, three to Homo sapiens, and one to Canis lupus dingo, whereas in cats 15 spots were ascribed to Felis catus, while two to Homo sapiens, one to Canis lupus familiaris, and one to Mus musculus. Since a specific taxonomy Canis lupus familiaris is not available in the database, in this analysis the taxonomy Mammalia has been used; therefore it could be possible that a protein is assigned to a different species. Furthermore, it should be noticed that the more peptides MASCOT identifies from a protein, the higher is the MASCOT score for that protein; in this study, proteins were identified with high MASCOT scores. In addition, if a sequence alignment of the identified proteins from different species is carried out, their high sequence homology with those from Canis lupus familiaris were observed (e.g., immunoglobulin kappa light chain from Canis lupus familiaris and Felis catus has 82.7% sequence homology), thus suggesting the reliability of the protein identification.

DISCUSSION

2-DE is a powerful tool that allows the separation, identification and quantification of proteins in complex mixtures when coupled with mass spectrometric analysis. This technique finds a particular application in the clinical field as it may help to provide pathophysiological insight, to iden-
The aim of the present work was the proteomic analysis of faecal proteome and more studies on a larger number of animals, divided by breed, are therefore required to investigate this further.

Other proteins that were found in this study included albumin (spots Y and Y2) and enzymes (spots V1, V2, H, H1 and F2). Reasons for these findings could include physiological enterocyte turnover (and associated cell death) and/or physiological enzymatic secretion associated with digestive processes, as both can lead to the release of intracellular content into the intestinal lumen. Examples of such an occurrence include when the protease elastase-3B (spot H1) is found in faeces from a population of apparently healthy dogs and cats: albumin (spot A) in cats and serum albumin isoform X1 (spot Y) in dogs, chymotrypsin-like elastase family member 3B (spot O2) in cats and chymotrypsin-C-like (spot H) in dogs and, finally, immunoglobulin kappa light chain in both cats (spots L1 and L2) and dogs (spot H3). When looking for proteins (SwissProt & NCBI databases, plus the CanisOme database) that had been previously found in canine faeces we only found one, the immunoglobulin lambda-1 light chain that partially overlaps with a protein found in a study of healthy Boxer dogs, although this previous study referred to the protein as immunoglobulin lambda-1 light chain isofrom X36.2 The fact that this is only a single partially similar result may be due to the use of different criteria for spots selection and that the present study did not include Boxers. This difference suggests that other factors, such as breed, may impact on the faecal proteome and more studies on a larger number of animals, divided by breed, are therefore required to investigate this further.

Other proteins that were found in this study included albumin (spots Y and Y2) and enzymes (spots V1, V2, H, H1 and F2). Reasons for these findings could include physiological enterocyte turnover (and associated cell death) and/or physiological enzymatic secretion associated with digestive processes, as both can lead to the release of intracellular content into the intestinal lumen. Examples of such an occurrence include when the protease elastase-3B (spot H1) is found in the human small intestine.18 and when alkaline phosphatase (spots V1 and V2) has been found in faeces of healthy dogs.19 It

| Spot ID* | Protein namea | Scorec | Mr (kDa)/pId | Mr (kDa)/pIe | Sequence | Normalized quantity* (x10⁴) |
|----------|---------------|---------|--------------|--------------|----------|--------------------------|
| Y        | Serum albumin isoform XI (Canis lupus familiaris) | 56      | 68.6/5.51    | 72 ± 4.6/5.8 ± 0.10 | LVAAQAALV | 115 ± 47                |
| Y2       | Serum albumin isoform XI (Canis lupus familiaris) | 41      | 68.6/5.51    | 63 ± 3.0/5.8 ± 0.15 | ADFAEISK | 79 ± 13                 |
| V1       | Alkaline phosphatase (Canis lupus familiaris) | 125     | 68.6/6.47    | 59 ± 1.3/6.6 ± 0.23 | ANYQTIGVSAAR | 174 ± 108             |
| V2       | Alkaline phosphatase (Canis lupus familiaris) | 117     | 68.6/6.47    | 58 ± 1.6/6.6 ± 0.15 | ANYQTIGVSAAR | 148 ± 51               |
| H        | Chymotrypsin-C-like (Canis lupus dingo) | 49      | 29.1/5.33    | 29 ± 1.0/5.6 ± 0.11 | LAEPVQLSDTIK | 290 ± 93              |
| H1       | Elastase-3B, Proteinase E (Canis lupus familiaris) | 40      | 28.8/5.27    | 29 ± 0.8/5.2 ± 0.11 | VSAFNDDWIEEVMSH | 585 ± 139             |
| H3       | Immunoglobulin kappa light chain (Felis catus) | 41      | 26.7/6.10    | 29 ± 1.1/6.3 ± 0.21 | FSGSGGTDFLTR | 374 ± 248             |
| G        | Immunoglobulin λ-1 light chain (Canis lupus familiaris) | 34      | 25.2/6.88    | 29 ± 0.9/7.1 ± 0.5 | KGTHTVVLGQP | 644 ± 327             |
| G1       | Immunoglobulin λ-1 light chain (Felis catus) | 39      | 27.8/8.17    | 29 ± 1.2/7.6 ± 0.6 | QSNKXYAASSYL | 555 ± 204             |
| G2       | Immunoglobulin λ-light chain VLJ region (Homo sapiens) | 42      | 29.0/8.14    | 29 ± 1.0/6.6 ± 0.3 | EFGETTKLTVLQPK | 642 ± 439             |
| G3       | Immunoglobulin λ-light chain VLJ region (Homo sapiens) | 30      | 29.0/8.14    | 27 ± 1.2/8.4 ± 0.5 | EFGETTKLTVLQPK | 393 ± 85              |
| G4       | Immunoglobulin λ-light chain VLJ region (Homo sapiens) | 40      | 29.0/8.14    | 27.7/8.9    | QSNKXYAASSYL | 285 ± 17              |
| F1       | Nuclear pore membrane glycoprotein 210 (Canis lupus familiaris) | 29      | 192.4/6.30   | 19.6 ± 1.5/5.8 ± 0.14 | TALLVTASISGHAPR | 227 ± 69              |
| F2       | Cytosol aminopeptidase (Canis lupus familiaris) | 29      | 56.2/8.03    | 21.0 ± 1.3/5.7 ± 0.07 | EILNISGPPLK | 125 ± 32              |

Abbreviations: Mr, molecular mass; pI, isoelectric point. Score number reflects the combined scores of all observed mass spectra that can be matched to amino acid sequences within that protein.

*Assigned spot ID as indicated in Figure 1.
†MASCOT results (SwissProt and NCBI databases).
‡MASCOT score reported.
§From SwissProt and NCBI databases.
|||
TABLE 2 Identification of faecal proteins from healthy cats by LC-MS/MS followed by MASCOT\textsuperscript{10} and SONAR\textsuperscript{11} software analysis

| Spot ID\textsuperscript{a} | Protein name\textsuperscript{b} | Score\textsuperscript{c} | Mr (kDa)/pI\textsuperscript{d} | Mr (kDa)/pI\textsuperscript{e} | Sequence | Normalized quantity\textsuperscript{f} (x10\textsuperscript{3}) |
|--------------------------|-------------------------------|------------------------|-------------------------------|-------------------------------|---------|------------------------|
| A                        | Albumin (Felis catus)         | 54                     | 68.5/5.92                     | 68.2 ± 0.9/5.8 ± 0.15         | LYNEVTTEFAK | 88 ± 26                |
| B                        | Caspase recruitment domain-containing protein 10 (H. sapiens) | n.d.                   | 116/5.7                      | 61 ± 0.5/5.3 ± 0.15           | LAQLSEEK | 26 ± 14                |
| C                        | IgGc-binding protein (Felis catus) | 43                     | 318/5.28                     | 48.4 ± 0.3/5.8 ± 0.15         | LDSLVAQQLQSK | 33 ± 7                |
| E                        | Transthyretin precursor (Felis catus) | 48                     | 15.5/5.52                    | 38.8 ± 0.4/6.4 ± 0.21        | VLDAVGSPAVNVAVK | 52 ± 22              |
| G                        | IgA constant region, partial (Felis catus) | 37                     | 42.0/5.73                    | 34.0 ± 0.46/6.0 ± 0.15       | WQLGSQELSR | 16 ± 9                |
| I                        | IgA constant region, partial (Felis catus) | 37                     | 42.0/5.73                    | 31.5 ± 0.38/6.2 ± 0.15       | WQLGSQELSR | 47 ± 41               |
| L1                       | Immunoglobulin kappa light chain (Felis catus) | 48                     | 26.7/6.1                     | 28 ± 0.5/7.5 ± 0.07          | GIQESTTEQNSK | n.d.                   |
| L2                       | Immunoglobulin kappa light chain (Felis catus) | 41                     | 26.7/6.1                     | 28 ± 0.5/7.9                | GIQESTTEQNSK | n.d.                   |
| L3                       | Immunoglobulin heavy chain variable region 3 (Felis catus) | 42                     | 21.9/4.8                     | 27.2/8.4                    | DVQLVESGGDLVKPGGSLR | n.d.                  |
| L4                       | Immunoglobulin heavy chain variable region 3 (Felis catus) | 42                     | 21.9/4.8                     | 28.1/8.8                    | DVQLVESGGDLVKPGGSLR | n.d.                  |
| L5                       | Immunoglobulin G heavy chain variable region (Homo sapiens) | 22                     | 13.4/6.4                     | n.d.                        | DVQLVESGGGLVQGGLR | n.d.                   |
| M                        | Kallikrein-1 (Felis catus)     | 46                     | 28.8/5.5                     | 29.0 ± 0.35/5.9 ± 0.15       | LAEPAQITDAVR | 9 ± 6                  |
| N                        | Hypothetical protein LOC107375 (Mus musculus) | n.d.                   | 31.7/9.7                     | 28.0 ± 0.75/5.4 ± 0.15       | YGGMLDCMASSFR | 31 ± 25              |
| O2                       | Chymotrypsin-like elastase family member 3B (Felis catus) | 39                     | 28.8/5.53                    | 28.3 ± 0.83/5.6 ± 0.15       | VSAFNDWIEEV | 11 ± 6                |
| P                        | Interstitial collagenase-like (MMP-1) (Felis catus) | 43                     | 62.5/6.74                    | 19.4 ± 1.7/4.2 ± 0.25        | IENYTPDLPR | 36 ± 28                |
| Q                        | Kallikrein-1 (Canis familiaris) | 58                     | 28.8/4.9                     | 13.8 ± 1.13/4.5 ± 0.25       | LAEPAQITDAVR | n.d.                   |
| R                        | Deleted in malignant brain tumours 1 protein-like (Felis catus) | 45                     | 26.0/5.2                     | 14.6 ± 1.9/5.1 ± 0.28        | FEGGSPIVLDVIR | 9 ± 1                 |
| S                        | Deleted in malignant brain tumours 1 protein-like (Felis catus) | 51                     | 26.0/5.2                     | 14.6 ± 1.9/5.1 ± 0.28        | FEGGSPIVLDVIR | 9 ± 1                 |
| T                        | Superoxide dismutase (Cu-Zn) (Felis catus) | 54                     | 15.8/6.28                    | 15.3 ± 1.1/7.3 ± 0.17        | HVGDILGNVTAGK | 55 ± 48               |

Abbreviations: Mr, molecular mass; pl, isoelectric point.
Score number reflects the combined scores of all observed mass spectra that can be matched to amino acid sequences within that protein.
\textsuperscript{a}Assigned spot ID as indicated in Figure 2.
\textsuperscript{b}MASCOT results (SwissProt and NCBInr databases).
\textsuperscript{c}MASCOT score reported.
\textsuperscript{d}From SwissProt and NCBInr databases.
\textsuperscript{e}Experimental values were calculated from the 2DE maps by the PDQuest software (± standard deviation).

is also easy to explain the presence of immunoglobulins (spots H3, G, G1, G2, G3 and G4) as the result of a physiological activation of the immune system. The presence of multiple forms of the immunoglobulin λ−1 light chain, VL region (spots G2, G3, G4), may be a consequence of the rearrangements that the variable region of the immunoglobulin heavy and light chains undergoes in order to generate antibody diversity in response to and antigen stimulus. On the other hand, the importance of identification of the nuclear pore membrane glycoprotein 210 (spot F1), a transmembrane protein component of metazoan nuclear pores with unknown function, is challenging to ascertain.\textsuperscript{20}

No previous studies on the feline proteome have been performed for comparison. However, if we also look for the causes of protein presence in this group, the retrieval of albumin (spot A), enzymes (spots B, M, O2, P, Q and T) and immunoglobulins (spots C, G, I, L1, L2, L3, L4 and L5) can be explained as previously reported in the dog. The presence of superoxide dismutase [Cu-Zn] (spot T), an enzymatic antioxidant expressed by almost all mammalian cells and found in cytosol, mitochondria and extracellular surfaces can be explained as a result of physiological mucosal turnover.\textsuperscript{21} With regard to transthyretin precursor (spot E), it is a protein secreted from the liver and choroid plexus into the blood and the cerebrospinal fluid, respectively. It is a transporter for thyroid hormones and a retinol binding protein with very modest proteolytic activity towards very few substrates.\textsuperscript{22} In human medicine, a reduction in plasma transthyretin could be related to severe acute phase response to thyroid diseases, but in veterinary medicine its role is not completely elucidated.\textsuperscript{23} The few studies performed in animals have shown a lower concentration of transthyretin in 1 month diarrheic calves.
compared with healthy controls and reduced plasma concentrations in *Mycobacterium avium paratuberculosis* seropositive cows compared to healthy cattle. Further studies are needed to understand the possible reason(s) behind the presence of transthyretin precursor in the faeces of clinically healthy cats. Conversely, DM1T protein-like (spots R and S) is a member of the superfamily cysteine-rich scavenger receptor. This protein is involved in epithelial differentiation, innate immunity and inflammation, and it is produced also by mucosal cells of the GI tract, where it exerts protective effects on the epithelium; therefore its presence in faeces can be justified. Lastly, the presence of spot N (hypothetical protein LOC10737375) is impossible to interpret, due to the lack of a precise correspondence.

Limitations of the present pilot study include the enrolment of a small number of dogs and cats, the absence in inclusion criteria of complete clinicopathological testing or diagnostic imaging to exclude any possible underlying disease and the absence of diet standardisation. Unfortunately, considering the almost total absence of similar studies in the scientific literature, it is impossible to state if, and in case to what extent, individual variations, breed, diet, age, etc. could influence the faecal proteome. Although a sample pooling strategy was used to minimise biological variation, unfortunately this resulted in an inability to evaluate the effect of individual variation, or differences associated with age, breed or diet. Consequently, in the absence of studies to investigate the impact of these factors, it is possible that individual proteome would have suffered considerably between subjects. Furthermore, given the small sample size, the list of identified proteins may not be exhaustive. Even though such aspects need to be further addressed in future studies, we believe that the present data represent a first step towards the identification of the faecal proteome in clinically healthy dogs and, for the first time, cats.

**CONCLUSION**

The present pilot study demonstrates the presence of a range of proteins in the faeces of apparently healthy dogs and cats, as determined by 2-DE and LC-MS/MS. These findings may serve as a basis for larger, prospective studies to clarify the degree of normal individual variation, the effects of diet and other confounding factors and potentially to establish reference proteomic data against which diseased populations can be compared.

**AUTHOR CONTRIBUTIONS**

Cerquetella Matteo and Vincenzetti Silvia conceived and designed the study. Cerquetella Matteo, Mangiaterra Sara, Ricciutelli Massimo, Pucciarelli Stefania and Vincenzetti Silvia via performed the fieldwork, laboratory and statistical analysis. Cerquetella Matteo, Marchegiani Andrea, Ricciutelli Massimo and Vincenzetti Silvia wrote the paper. Rossi Giacomo, Gavazza Alessandra, Tesei Beniamino, Spaterna Andrea, Sagratini Gianni and Vincenzetti Silvia advised on study design and help in results interpretation. All authors contributed to the revision of the article and to the writing in its current form and approved the final version.

**ORCID**

Matteo Cerquetella [https://orcid.org/0000-0001-9471-0756](https://orcid.org/0000-0001-9471-0756)

Andrea Marchegiani [https://orcid.org/0000-0002-3629-0391](https://orcid.org/0000-0002-3629-0391)

**REFERENCES**

1. Cerquetella M, Rossi G, Spaterna A. Fecal proteomic analysis in healthy dogs and in dogs suffering from food responsive diarrhea. Sci World J. 2019;2019:1–7.
2. Cerquetella M, Rossi G, Spaterna A. Proteomics of canine feces from healthy Boxer dogs: a pilot study. Poster Abstract (ESCG-P-1). J Vet Intern Med. 2019;33(2):1067–8.
3. Dhondalay GK, Rael E, Acharya S. Food allergy and omics. J Allergy Clyn Immunol. 2018;141(1):20–9.
4. Debyser G, Mesuree B, Clement L. Faecal proteomics: a tool to investigate dysbiosis and inflammation in patients with cystic fibrosis. J Cyst Fibros. 2016;15(2):242–50.
5. Jin P, Wang K, Huang C. Mining the fecal proteome: from biomarkers to personalised medicine. Expert Rev Proteomics. 2017;14(5):445–59.
6. Karp NA, Lilley KS. Investigating sample pooling strategies for DGGE experiments to address biological variability. Proteomics. 2009;9(2):388–97.
7. Vincenzetti S, Pucciarelli S, Huang Y. Biomarkers mapping of neuropathic pain in a nerve chronic constriction injury mice model. Biochimie. 2019;158:172–9.
8. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 1976;72(1-2):248–54.
9. Vincenzetti S, Amici A, Pucciarelli S. A proteomic study on donkey milk. Biochem Anal Biochem. 2012:109.
10. Shevchenko A, Tomas H, Havel J’s. In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat Protoc. 2007;2(6):2856–60.
11. Mascot software. Matrix science. 2020. [www.matrixscience.com](http://www.matrixscience.com). Accessed 29 February 2020.
12. Sonar software. Genomic solutions. 2020. [http://hs2.proteome.ca/prowl/knexus.html](http://hs2.proteome.ca/prowl/knexus.html). Accessed 29 February 2020.
13. Magdeldin S, Enany Y, Yoshida B. Basics and recent advances of twodimensional- polyacrylamide gel electrophoresis. Clin Proteomics. 2014;11(1):16.
14. Christians U, McCrery S, Klawitter J. The role of proteomics in the study of kidney diseases and in the development of diagnostic tools. In: Edelstein C, editor. Biomarkers of kidney disease. New York: Academic Press; 2011. p. 101–76.
15. Cecilian F, Eckersall D, Burchmore R. Proteomics in veterinary medicine: applications and trends in disease pathogenesis and diagnostics. Vet Pathol. 2014;51(2):351–62.
16. Ghodasara P, Sadowski P, Satake N. Clinical veterinary proteomics: techniques and approaches to decipher the animal plasma proteome. Vet J. 2007;2009(2):386–403.
17. CanisOme database. CanisOme. 2020. [http://salivatec.viseu.ucp.pt/canisome/](http://salivatec.viseu.ucp.pt/canisome/). Accessed 29 February 2020.
18. Gutiérrez S, Pérez-Andrés J, Martínez-Blanco H. The human digestive tract has proteases capable of gluten hydrolysis. Mol Metab. 2017;6(7):693–702.
19. Ide K, Kato K, Sawa Y, Hayashi A. Comparison of the expression, activity, and fecal concentration of intestinal alkaline phosphatase between healthy dogs and dogs with chronic enteropathy. Am J Vet Res. 2016;77(7):721–9.
20. Olsson M, English MA, Mason J. Despite WT1 binding sites in the promoter region of human and mouse nucleoporin glycoprotein 210, WT1 does not influence expression of GP210. J Negat Results Biomed. 2004;3(7). [https://doi.org/10.1186/1477-5751-3-7](https://doi.org/10.1186/1477-5751-3-7).
21. McMichael MA. Oxidative stress, antioxidants, and assessment of oxidative stress in dogs and cats. J Am Vet Med Assoc. 2007;231(5):714–20.
22. Tangthavewattana S, Leelaswatwattana L, Prapunpoj P. The hydrophobic C-terminal sequence of transthyretin affects its catalytic kinetics towards amidated neuropeptide Y. FEBS Open Bio. 2019;9:594–604.
23. Tóthová C, Nagy O. Transthyretin in the evaluation of health and disease in human and veterinary medicine. In: Gaze DC, editor. Pathophysiology, altered physiological states. London: InTech Open; 2017.

24. Rosenstiel P, Sina C, End C. Regulation of DMBT1 via NOD2 and TLR4 in intestinal epithelial cells modulates bacterial recognition and invasion. J Immunol. 2007;178(12):8203–11.

25. Zhao Y, Tao Q, Wu J. DMBT1 has a protective effect on allergic rhinitis. Biomed Pharmacother. 2020;121:109675.

How to cite this article: Matteo C, Andrea M, Sara M, et al. Faecal proteome in clinically healthy dogs and cats: Findings in pooled faeces from 10 cats and 10 dogs. Vet Rec Open. 2021;8:e9. https://doi.org/10.1002/vro2.9