Manuscript Details

Manuscript number	VETMIC_2017_82
Title	Seroprevalence for norovirus genogroup II, IV and VI in dogs
Article type	Short Communication

Abstract

Molecular and serological data suggest that noroviruses (NoVs) might be transmitted between humans and domestic carnivores. In this study we screened an age-stratified collection of canine sera (n=516) by using an ELISA assay based on virus-like particles (VLPs) of human NoVs GII.4 and GIV.1 and carnivore NoVs GIV.2 and GVI.2. Antibodies against GII.4 and GIV.1 human NoVs and GIV.2 and GVI.2 NoVs from carnivores were identified in dog sera (13.0%, 67/516) suggesting their exposure to homologous and heterologous NoVs. Analysis of the trends of age-class prevalence showed a gradual increase in the positive rate from 9.0% and 7.0%, in young dogs < 1 year of age to 15.0% in dogs older than 12 years, for GII.4 and GVI.2 NoVs, respectively. A significant difference in the IgG distribution by age classes was observed for GIV.1 NoVs, with the highest rate of antibodies (7.0%) in the age group < 1 year and the lowest (1.0%) in the age-classes 7-9 (P = 0.049). High correlation between the reactivity to GII.4 and GVI.2 NoVs was observed, likely due to conserved epitopes in the capsid structure.

Keywords	noroviruses (NoVs); genotypes GII.4, GIV.1, GIV.2 and GVI.2; dogs; antibodies.
Manuscript category	Viruses

Corresponding Author	Barbara Di Martino
Corresponding Author's Institution	University of Teramo
Order of Authors	Barbara Di Martino, Federica Di Profio, Irene Melegari, Vittorio Sarchese, Ivano Massirio, Giuseppe Palermo, Giovanni Romito, Eleonora Lorusso, Gianvito Lanave, Livia Bodnar, Canio Buonavoglia, Fulvio Marsilio, Kim Green, VIto Martella
Suggested reviewers	Jan Vinje, Linda Saif, João Mesquita, Tibor Farkas

Submission Files Included in this PDF

File Name [File Type]

Di Martino et al_cover letter.doc [Cover Letter]

Di Martino et al_manuscript.docx [Manuscript File]

Di Martino et al_Fig_1.tif [Figure]

Di Martino et al_Fig_2.tif [Figure]

Di Martino et al_Fig_3.tif [Figure]

Di Martino et al_Fig_S1.tif [Figure]

Di Martino et al_Table 1.docx [Table]

Di Martino et al_Highlights.docx [Highlights]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

UNIVERSITÁ DEGLI STUDI DI TERAMO

FACOLTÀ MEDICINA VETERINARIA TERAMO

To the Editor of
Veterinary Microbiology

Teramo, 1-25-2017

Dear Editor,
several data suggest that circulation of noroviruses (NoVs) may occur between pets and humans raising concerns of potential cross-species transmission. In this study, antibodies against GII.4 and GIV.1 human NoVs and GIV.2 and GVI.2 NoVs from carnivores were identified in dog sera (13.0%, 67/516) suggesting their exposure to homologous and heterologous NoVs. I hope you will find the manuscript "Seroprevalence for norovirus genogroup II, IV and VI in dogs" by Di Martino et al., written in a format corresponding to a short communication of interest for the journal.

With the best regards,

Barbara Di Martino

Prof Barbara Di Martino
Faculty of Veterinary Medicine of Teramo-
Università degli Studi di Teramo
64100 - Teramo- Italy
Tel/Fax: 0039 0861 266885
E-mail: bdimartino@unite.it

Seroprevalence for norovirus genogroup II, IV and VI in dogs

Barbara Di Martino [a*], Federica Di Profio [a], Irene Melegari [a], Vittorio Sarchese [a], Ivano Massirio [b], Giuseppe Palermo [c], Giovanni Romito [d], Eleonora Lorusso [e], Gianvito Lanave [e], Livia Bodnar [e], Canio Buonavoglia [e], Fulvio Marsilio [a], Kim Y. Green [f], Vito Martella [e]

[a] Faculty of Veterinary Medicine, Università degli Studi di Teramo, Italy,

[b] Azienda USL di Reggio Emilia, Reggio Emilia, Italy,

[c] CTO Veterinario, Arenzano, Genova, Italy,

[d] University Alma Mater Studiorum of Bologna, Ozzano Emilia, Italy

[e] Department of Veterinary Medicine, Università Aldo Moro di Bari, Valenzano, Italy,

[f] National Institutes of Health, Bethesda, MD, US

*** Corresponding author:**
Barbara Di Martino,
Faculty of Veterinary Medicine,
Università degli Studi di Teramo, Italy,
phone: +39-0861266845,
email: bdimartino@unite.it

Abstract

Molecular and serological data suggest that noroviruses (NoVs) might be transmitted between humans and domestic carnivores. In this study we screened an age-stratified collection of canine sera (n=516) by using an ELISA assay based on virus-like particles (VLPs) of human NoVs GII.4 and GIV.1 and carnivore NoVs GIV.2 and GVI.2. Antibodies against GII.4 and GIV.1 human NoVs and GIV.2 and GVI.2 NoVs from carnivores were identified in dog sera (13.0%, 67/516) suggesting their exposure to homologous and heterologous NoVs. Analysis of the trends of age-class prevalence showed a gradual increase in the positive rate from 9.0% and 7.0%, in young dogs < 1 year of age to 15.0% in dogs older than 12 years, for GII.4 and GVI.2 NoVs, respectively. A significant difference in the IgG distribution by age classes was observed for GIV.1 NoVs, with the highest rate of antibodies (7.0%) in the age group < 1 year and the lowest (1.0%) in the age-classes 7-9 (P = 0.049). High correlation between the reactivity to GII.4 and GVI.2 NoVs was observed, likely due to conserved epitopes in the capsid structure.

.

Keywords: noroviruses (NoVs); genotypes GII.4, GIV.1, GIV.2 and GVI.2; dogs; antibodies.

Introduction

Noroviruses (NoVs) are major human pathogens associated with acute gastroenteritis (Patel et al., 2008). NoVs belong to the genus *Norovirus* in the family *Caliciviridae*. The genome of NoVs consists of a single-stranded positive-sense RNA molecule of ~7.5kb that is organized into three open reading frames (ORFs). ORF1 encodes a polyprotein that is co-translationally cleaved into seven proteins required for replication, while ORF2 encodes the major capsid protein (VP1) and ORF3, a minor capsid protein (VP2) (Green, 2013). Based on the full-length VP1 capsid protein, the *Norovirus* genus comprises seven genogroups (G), which can be subdivided in at least 40 genotypes (Green, 2013; Vinje, 2015). Viruses belonging to GI, GII and GIV can infect humans, with GII.4 strains that are the most prevalent worldwide (Green, 2013). NoVs (GIV.2, GVI.1, GVI.2 and GVII) have been also identified in carnivores (Martella et al., 2007; Martella et al., 2008; Martella et al., 2009; Mesquita et al., 2010; Pinto et al., 2012; Tse et al., 2012; Di Martino et al., 2016). Some NoV strains from carnivores are genetically related to human GIV NoVs, suggesting common pathways in their evolution (Martella et al., 2007).

Molecular and serological studies suggest that circulation of NoVs may occur between pets and humans raising concerns of potential cross-species transmission. Human sera may contain specific IgG antibodies against carnivore GIV.2 and GVI.2 NoVs (Mesquita et al., 2013; Di Martino et al., 2014) and in turn, dog sera may contain antibodies against human NoVs of genogroup I and II (Caddy et al., 2015). In a study in Mexico, having dogs in or near home was recognised as a risk factor for acquisition of IgA antibodies specific for NoVs in infants (Peasey et al., 2004). Also, partial capsid sequences of human GII.4 and GII.12 NoVs have been detected in household dogs in contact with human patients affected by NoV gastroenteritis (Summa et al., 2012).

In order to draw a more complete picture of NoV epidemiology in dogs, we screened an age-stratified collection of canine sera by using an enzyme-linked immunosorbent assay (ELISA) based on virus-like particles (VLPs) generated from human NoVs of genotype GII.4 and GIV.1 and from carnivore NoVs of genotype GIV.2 and GVI.2.

Materials and methods

Serum sample collection

A total of 516 serum samples were collected between March 2013 and July 2015 by a convenience sampling of household dogs admitted to veterinary clinics from different Italian regions. The samples were divided on the basis of age groups: <1 year, 1–3 years, 3-year age groups from 4 to 12, and >12 years of age.

Virus-like Particles (VLPs)

The recombinant baculoviruses carrying the genes for the viral capsid proteins of the Hu/NoV/GII.4/MD14512/1987/US, Hu/NoV/GIV.1/SaintCloud/624/1998/US, Lion/NoV/GIV.2/Pistoia/387/06/ITA and Dog/NoV/GVI.2/FD53/2007/ITA were obtained as previously described (Bok et al., 2009; Di Martino et al., 2010; Di Martino et al., 2015). For large-scale production of VLPs, 100 ml of *Spodoptera frugiperda* (*Sf9*) cells ($1x10^6$ cell/ml) suspension culture were infected with the recombinant baculovirus at a multiplicity of infection of three plaque forming units/cell. The recombinant capsid proteins were concentrated by ultracentrifugation through a 17% sucrose cushion in TEN-buffer (100 mM NaCl; 50 mM Tris-HCl, pH 7.5; 1 mM EDTA) and purified on a discontinuous 20 to 60% (wt/vol) sucrose gradient. The collected fractions were dialyzed against PBS, and the protein concentration of VLP preparations was determined by measuring the optical density at 280 nm (OD_{280}) and visually by running aliquots on SDS-10% PAGE containing bovine serum albumin (BSA) standards. The presence of VLPs was confirmed by western blotting (WB) and electron microscopy, as previously described (Bok et al., 2009; Di Martino et al., 2010; Di Martino et al., 2014).

Enzyme-linked immunoassay (ELISA)

For the development of the antibody-detection ELISA, the supernatant of mock infected cells, GII.4, GIV.1, GIV.2 and GVI.2 VLPs were diluted to a final concentration of 4 µg/ml in carbonate-

105 bicarbonate buffer (0.05 M, pH 9.6) and 100 µl of each antigen were added to the well of a 96-well
106 EIA plate (Costar, Italy). The wells were washed five times with 0.1% Tween-PBS (PBS-T) and
107 then blocked with 200 µl of PBS containing 2% BSA at room temperature for two hours. Each
108 serum sample was tested at the initial dilution of 1:100 and the plates were incubated at 37°C for 1
109 hour. After incubation with horseradish peroxidase-conjugated goat anti-dog immunoglobulin G
110 (IgG) (Sigma-Aldrich, Italy) at dilution of 1:5,000 for 30 min at 37°C, the reaction was developed
111 with the addition of 100 µl per well of 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) (ABTS)
112 substrate. The cut-off point of the ELISA test was established as the mean of the OD_{405} readings of
113 50 dog sera negative in WB for each NoV antigen plus 2 standard deviations. For each tested
114 sample a positive/negative ratio (OD_{405} of VLPs/OD_{405} of mock infected cells) $\geq$ 2.0 was used to
115 evaluate the background binding. All the sera with an OD_{405} values $\geq$ 0.5 at the initial dilution of
116 1:100 were considered positive and titrated in twofold dilutions. Mean ELISA antibody titres were
117 calculated and expressed as the reciprocal of the highest serum dilution with a positive absorbance
118 ($OD_{405} \geq 0.5$) for each NoV antigen.
119
120 *Statistical analysis*
121 The data were analysed using Prism Graphpad Software. Fisher's exact test was used to determine
122 the differences in seroprevalence among the age groups. Pearson's chi-squared test was applied to
123 assess the correlation among NoV genotype serum titers detected in the tested dogs. A P value of
124 <0.05 was considered statistically significant.
125
126 *Evaluation of serological cross-reactivity between GII.4 and GVI.2 NoVs*
127 In order to assess the antigenic relationships between GII.4 and GVI.2 VLPs, we performed
128 blocking assay experiments. Briefly, 6 dog sera positive for both the antigens with titres >1:800
129 were pre-incubated with optimized concentrations of GII.4 and GVI.2 VLPs (2, 4, 8, 16 µg/ml) for
130 1 h at 37 °C. After incubation, the sera were tested in ELISA for the presence of GII.4 and GVI.2

antibodies, starting at dilution of 1:100. The possible inter-genogroup serological cross reactivity between GII.4 and GVI.2 VLPs was also investigated in WB analysis, by assessing the reactivity of five human sera positive for GII.4 with titres of 1:200 (Di Martino et al., 2014) against GVI.2 VLPs.

157 **Results and discussion**

158 Out of 516 dog sera, 13.0% (67/516) reacted with at least one NoV antigen. In detail, 52 sera
159 reacted with the GII.4 VLPs with a prevalence rate of 10.1%, at dilutions ranging from 1:100 to
160 1:3,200. A total of 23 (4.5%) samples reacted with GIV antigens, with titres ranging from 1:100 to
161 1:800. Of these, 17 (3.3%) resulted positive for both GIV.1 and GIV.2, while 3 (0.58%) sera reacted
162 only with GIV.1 at dilutions from 1:200 to 1:800 and an additional 3 (0.58%) samples reacted only
163 with GIV.2, at final dilution of 1:100. Forty-six (8.9%) sera reacted with GVI.2 VLPs, at dilutions
164 ranging from 1:100 to 1:3,200 (Table 1).

165 Analysis of the trends of age-class prevalence by Fisher's exact test showed a gradual increase in
166 the positive rate from 9.0% and 7.0% in young dogs <1 year of age to 15.0% in dogs older than 12
167 years for GII.4 and GVI.2 NoVs, respectively. A significant difference in the IgG distribution by
168 age-classes was observed for GIV.1, with the highest rate of antibodies (7.0%) in the group <1 year
169 and the lowest (1.0%) in the age-class 7-9 ($P = 0.049$). A similar age-related pattern was also found
170 for GIV.2 (Fig. 1).

171 The results obtained are consistent with the hypothesis that dogs may be exposed to both human
172 and carnivore NoVs throughout their life. The magnitude of GII.4 (10.1%) circulation in household
173 dogs was higher in our serological survey than in a previous investigation conducted in UK, where
174 4.9% and 8.8% of kennel and household dogs, respectively, possessed antibodies for NoVs using a
175 pool of GII.3, GII.4, GII.6 and GII.12 VLPs (Caddy et al., 2015). By converse, the overall
176 prevalence of antibodies against carnivore GIV.2 (3.9%) was lower than that reported (56.6%) in
177 UK (Caddy et al., 2013), but similar to the rate (4.8%) previously reported in an Italian dog
178 population (Di Martino et al., 2010). Furthermore, in our study IgG antibodies reacting only against
179 human GIV.1 VLPs were found in dog sera, suggesting exposure to human GIV NoVs. In general,
180 the seroprevalence for carnivore GVI.2 NoVs revealed in our investigation was lower than the
181 prevalences reported in previous studies (Caddy et al., 2013; Mesquita et al., 2014). In UK (Caddy

et al., 2013) the seropositivity for GVI.2 VLPs was 15.9%, while in a multi-centric study conducted in 14 European countries the overall rate was 36.0% (Mesquita et al., 2014).

In our analysis, the majority of the NoV-seropositive dogs showed reactivity for multiple genogroup antigens. Pearson's rank analysis revealed a high correlation between the levels of antibodies to GIV.1 and GIV.2 antigens (Fig. 2a), while a weak correlation was found when comparing the antibody titers to GIV.1 and to GIV.2 antigens with the antibody titers to GII.4 and to GVI.2 VLPs, respectively (Fig. 2b, 2c, 2d, 2e). Of interest, we found a high correlation between the level of antibodies to GII.4 and GVI.2 VLPs (Fig. 2f), in particular in the dog sera that strongly reacted against both antigens (from >1:800 to 1:3,200). Previous evidence indicates that GII.4 VLPs are antigenically unrelated to GIV NoVs, whilst antigenic cross-reactivity has been observed between GIV.1 and GIV.2 genotypes (Di Martino et al., 2014).

The antigenic relationships between GII.4 and GVI.2 VLPs was investigated by blocking assay experiments. In our analysis, we found for all the six sera examined that at concentrations higher than 8 μg of each antigen per ml, binding of GVI.2 antibodies was blocked (~50% reduction of OD_{405} values) by GII.4 VLPs (Fig. 3a), and in turn binding of GII.4 antibodies was blocked by GVI.2 VLPs, although with a ~25% reduction of the OD_{405} values (Fig. 3b). Also, by testing in WB analysis five human sera positive for GII.4 with titres of 1:200, we found reactivity against GVI.2 at the initial dilution of 1:100 (Fig. S1). Overall, these findings suggest a strong serologic cross-reactivity between human GII.4 and carnivores GVI.2 NoVs, likely due to the existence of conserved epitopes in the capsid VP1 protein (Parra et al., 2012).

In conclusion, screening of canine sera with multiple NoV antigens demonstrated that dogs are exposed to both human (heterologous) and animal (homologous) NoV strains. Different age-related patterns were observed between the antibody prevalence to GII/GVI and to GIV antigens. Understanding the ecology and dynamics of transmission of NoVs in carnivores will be helpful to assess more precisely if and to which extent pets may pose a risk of infection by homologous and heterologous NoV strains for humans.

Acknowledgements

This study was supported by grants from the University of Teramo, Italy, and by the Italian Ministry of University and Research.

References

Bok, K., Abente, E.J., Realpe-Quintero, M., Mitra, T., Sosnovtsev, S.V., Kapikian, A.Z., Green, K.Y., 2009. Evolutionary dynamics of GII.4 noroviruses over a 34-year period. J. Virol. 83, 11890-11901.

Caddy, S., Emmott, E., El-Attar, L., Mitchell, J., de Rougemont, A., Brownlie, J., Goodfellow, I., 2013. Serological evidence for multiple strains of canine norovirus in the UK dog population. PLoS One 5: e81596.

Caddy, S.L., de Rougemont, A., Emmott, E., El-Attar, L., Mitchell, J.A., Hollinshead, M., Belliot, G., Brownlie, J., Le Pendu, J., Goodfellow, I., 2015. Evidence for human norovirus infection of dogs in the United Kingdom. J. Clin. Microbiol. 53, 1873–1883.

Di Martino, B., Di Profio, F., Melegari, I., Sarchese, V., Cafiero, M.A., Robetto, S., Aste, G., Lanave, G., Marsilio, F., Martella, V., 2016. A novel feline norovirus in diarrheic cats. Infect. Genet. Evol. 38, 132-137.

Di Martino, B., Di Profio, F., Ceci, C., Di Felice, E., Green, K.Y., Bok, K., De Grazia, S., Giammanco, G.M., Massirio, I., Lorusso, E., Buonavoglia, C., Marsilio, F., Martella, V., 2014. Seroprevalence of norovirus genogroup IV antibodies among humans, Italy, 2010–2011. Emerg. Infect. Dis. 20, 1828–1832.

Di Martino, B., Marsilio, F., Di Profio, F., Lorusso, E., Friedrich, K.G., Buonavoglia, C., Martella V., 2010. Detection of antibodies against norovirus genogroup GIV incarnivores. Clin. Vaccine Immunol. 17, 180–182.

260 Green K., 2013. Caliciviridae: the Noroviruses, In: Knipe DM, Howley PM, Cohen JI, Griffin DE,
261 Lamb RA, Martin MA, et al.(ed), Fields virology, 6th ed. Lippincott Williams & Wilkins,
262 Philadelphia, pp 583–609.
263
264 Martella, V., Campolo, M., Lorusso, E., Cavicchio, P., Camero, M., Bellacicco, A.L., Elia, G.,
265 Greco, G., Corrente, M., Desario, C., Arista, C., Banyaj, K., Koopmans, M., Buonavoglia, C., 2007.
266 Norovirus in captive lion cub (Panthera leo). Emerg. Infect. Dis. 13, 1071–1073.
267
268 Martella, V., Decaro, N., Lorusso, E., Radogna, A., Moschidou, P., Amorisco, F., Lucente, M.S.,
269 Desario, C., Elia, G., Banyai, K., Carmichael, L.E., Buonavoglia, C., 2009. Genetic heterogeneity
270 and recombination in canine noroviruses. J. Virol. 83, 11391–11396.
271
272 Martella, V., Lorusso, E., Decaro, N., Elia, G., Radogna, A., D'Abramo, M., Desario, C., Cavalli,
273 A., Corrente, M., Germinaro, C.A., Banyai, K., Di Martino, B., Marsilio, F., Carmichael, L.E.,
274 Buonavoglia, C., 2008. Detection and molecular characterization of a canine norovirus. Emerg.
275 Infect. Dis. 14, 1306–1308.
276
277 Mesquita, J.R., Barclay, L., Nascimento, M.S.J., Vinjé, J., 2010. Novel norovirus in dogs with
278 diarrhea. Emerg. Infect. Dis.16, 980–982.
279
280 Mesquita, J.R., Costantini, V.P., Cannon, J.L., Lin, S.C., Nascimento, M.S., Vinjé, J., 2013.
281 Presence of antibodies against genogroup VI norovirus in humans. Virol. J. 10, 176.
282
283 Mesquita, J.R., Delgado, I., Costantini, V., Heenemann, K., Vahlenkamp, T.W., Vinjé, J.,
284 Nascimento, M.S., 2014. Seroprevalence of canine norovirus in 14 European countries. Clin.
285 Vaccine Immunol. 21, 898-900.

286 Parra, G.I., Azure, J., Fischer, R., Bok, K., Sandoval-Jaime, C., Sosnovtsev, S.V., Sander, P.,
287 Green, K.Y., 2013 Identification of a Broadly Cross-Reactive Epitope in the Inner Shell of the
288 Norovirus Capsid. PLoS One 8, e67592.
289
290 Patel, M.M., Widdowson, M.A., Glass, R.I., Akazawa, K., Vinjé, J., Parashar, U.D., 2008.
291 Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg. Infect. Dis.
292 14, 1224 –1231.
293
294 Peasey, A.E., Ruiz-Palacios, G.M., Quigley, M., Newsholme, W., Martinez, J., Rosales, G., Jiang,
295 X., Blumenthal, U.J., 2004. Seroepidemiology and risk factors for sporadic norovirus/Mexico
296 strain. J. Infect. Dis. 189, 2027–2036.
297
298 Pinto, P., Wang, Q., Chen, N., Dubovi, E.J., Daniels, J.B., Millward, L.M., Buonavoglia, C.,
299 Martella, V., Saif, L.J., 2012. Discovery and genomic characterization of noroviruses from a
300 gastroenteritis outbreak in domestic cats in the US. PLoS One 7, e32739.
301
302 Summa, M., von Bonsdorff, C.H., Maunula, L., 2012. Pet dogs - A transmission route for human
303 noroviruses? J. Clin. Virol. 53, 244–247.
304
305 Tse, H., Lau, S.K., Chan, W.M., Choi, G.K., Woo, P.C., Yuen, K.Y., 2012. Complete genome
306 sequences of novel canine noroviruses in Hong Kong. J. Virol. 86, 9531–9532.
307
308 Vinjé, J., 2015. Advances in laboratory methods for detection and typing of norovirus. J. Clin.
309 Microbiol. 53, 373–381.
310

Figure captions

Fig. 1 - IgG antibodies to GII.4, GIV.1, GIV.2 and GVI.2 VLPs in dog sera of different age groups.

Fig. 2 - Pearson's rank analysis of the levels of antibodies among GII.4, GIV.1, GIV.2 and GVI.2 VLPs. Each scatterplot shows the correlation between genotype serum titers detected in the tested dogs (a: GIV.1/GIV.2; b: GII.4/GIV.1; c: GII.4/GIV.2; d: GVI.2/GIV.1; e: GVI.2/GIV.2; f: GII.4/GVI.2). All Pearson ranking values were statistically significant with a $p < 0.0001$.

Fig. 3 – Blocking assay experiment. A canine serum positive for GII.4 and GVI.2 antigens with titres ≥1:800 was pre-incubated with GII.4 **(a)** and GVI.2 **(b)** VLPs at concentrations of 2, 4, 8 and 16 μg/ml and tested in ELISA for the presence of antibodies against both VLPs.

Fig. S1 - WB analysis of GII.4 and GVI.2 VP1 with a human serum sample positive in ELISA for GII.4 NoVs. Line 1: Precision Plus protein Standards (Bio-Rad, Italy); line 2: GII.4 VP1; line 3: GVI.2 VP1. Serum dilution: 1:100.

Conflict of interest statement

All Authors declare that there are no financial or other relationships that might lead to a conflict of interest. All authors have seen and approved the manuscript and have contributed significantly to the work.

Fig. 1

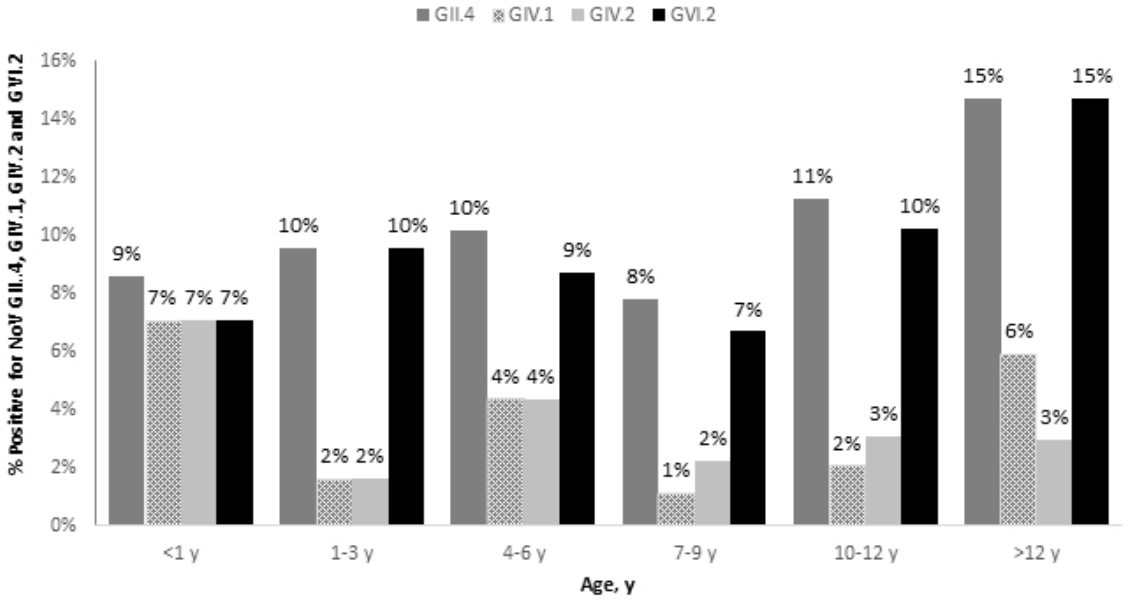
GII.4
GIV.1
GIV.2
GVI.2
% Positive for NoV GII.4, GIV.1, GIV.2 and GVI.2
16%
14%
12%
10%
8%
6%
4%
2%
0%
9%
7%
7%
7%
10%
2%
2%
10%
10%
4%
4%
9%
8%
1%
2%
7%
11%
2%
3%
10%
15%
6%
3%
15%
<1 y
1-3 y
4-6 y
7-9 y
10-12 y
>12 y
Age, y

Fig. 2

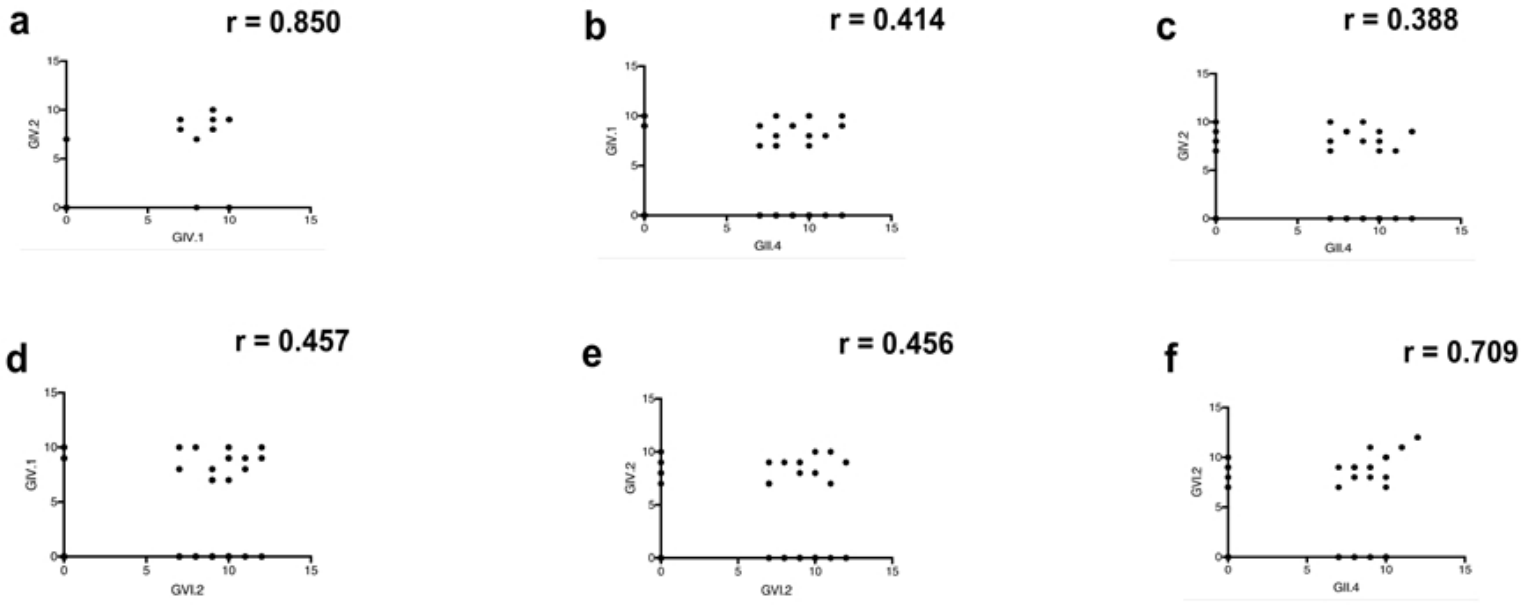
a
r = 0.850
GIV.2
GIV.1
b
r = 0.414
GIV.1
GII.4
c
r = 0.388
GIV.2
GII.4
d
r = 0.457
GIV.1
GVI.2
e
r = 0.456
GIV.2
GVI.2
f
r = 0.709
GVI.2
GII.4

Fig. 3

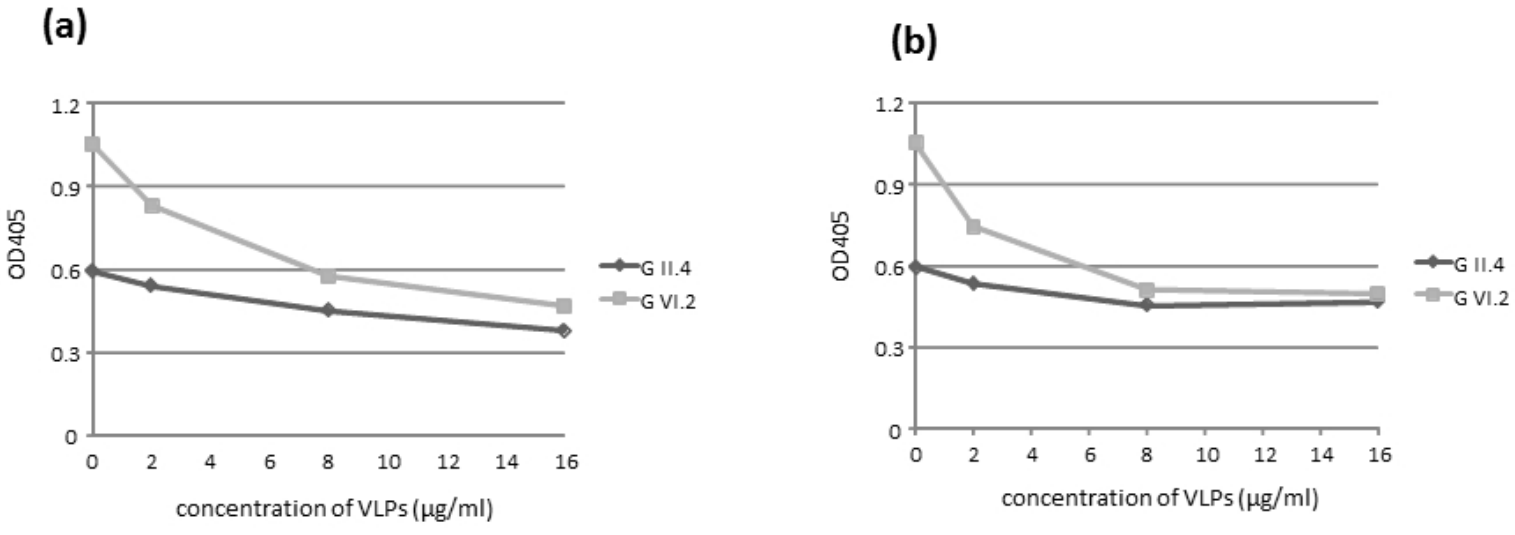
(a)
1.2
0.9
0.6
0.3
0
OD405
0
2
4
6
8
10
12
14
16
concentration of VLPs (μg/ml)
G II.4
G VI.2
(b)
1.2
0.9
0.6
0.3
0
OD405
0
2
4
6
8
10
12
14
16
concentration of VLPs (μg/ml)
G II.4
G VI.2

Fig. S1

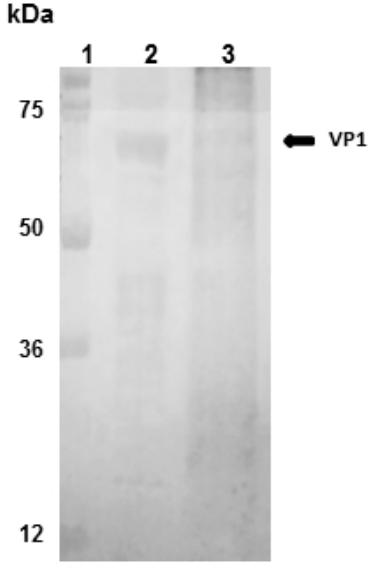

Table 1

IgG antibody titres to GII.4, GIV.1, GIV.2 and GVI.2 VLPs in dog sera. Mean ELISA antibody titres were calculated and expressed as the reciprocal of the highest serum dilution with a positive absorbance ($OD_{405} \geq .5$) for GII.4, GIV.1, GIV.2 and GVI.2 VLPs.

	Serum dilutions						
NoV† VLPs*	100 (%)	200 (%)	400 (%)	800 (%)	1,600 (%)	3,200 (%)	Total (%)
GII.4	7/516 (1.4%)	9/516 (1.7%)	7/516 (1.4%)	22/516 (4.3%)	3/516 (0.6%)	4/516 (0.8%)	52/516 (10.1%)
GIV.1	4/516 (0.8%)	3/516 (0.6%)	7/516 (1.4%)	6/516 (1.2%)	0/516 (0%)	0/516 (0%)	20/516 (3.9%)
GIV.2	5/516 (1.0%)	5/516 (1.0%)	7/516 (1.4%)	3/516 (0.6%)	0/516 (0%)	0/516 (0%)	20/516 (3.9%)
GVI.2	5/516 (1.0%)	5/516 (1.0%)	12/516 (2.3%)	16/516 (3.1%)	4/516 (0.8%)	4/516 (0.8%)	46/516 (8.9%)

†NoV, norovirus
*VLPs, virus-like particles

Highlights

- Noroviruses (NoVs) are a major cause of epidemic gastroenteritis in humans.
- We detected IgG antibodies against human and carnivore NoVs in household dog sera.
- This finding indicates that dogs are exposed to homologous and heterologous NoVs.