



HAL
open science

Molecular characterization of bovine rotavirus strains circulating in Northern Italy, 2003–2005

M. Monini, F. Cappuccini, P. Battista, E. Falcone, A. Lavazza, F.M. Ruggeri

► **To cite this version:**

M. Monini, F. Cappuccini, P. Battista, E. Falcone, A. Lavazza, et al.. Molecular characterization of bovine rotavirus strains circulating in Northern Italy, 2003–2005. *Veterinary Microbiology*, 2008, 129 (3-4), pp.384. <10.1016/j.vetmic.2007.11.036>. <hal-00532368>

HAL Id: hal-00532368

<https://hal.science/hal-00532368v1>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Accepted Manuscript

Title: Molecular characterization of bovine rotavirus strains circulating in Northern Italy, 2003–2005

Authors: M. Monini, F. Cappuccini, P. Battista, E. Falcone, A. Lavazza, F.M. Ruggeri



PII: S0378-1135(07)00608-6
DOI: doi:10.1016/j.vetmic.2007.11.036
Reference: VETMIC 3910

To appear in: *VETMIC*

Received date: 22-5-2007
Revised date: 22-11-2007
Accepted date: 30-11-2007

Please cite this article as: Monini, M., Cappuccini, F., Battista, P., Falcone, E., Lavazza, A., Ruggeri, F.M., Molecular characterization of bovine rotavirus strains circulating in Northern Italy, 2003–2005, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.11.036

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Abstract**

2 A total of 232 stools collected from calves with rotavirus infection in herds located in Northern Italy
3 from 2003 to 2005 was investigated. Determination of the rotavirus G- and P-types was carried out
4 using nested RT-PCR. G6 was the most prevalent genotype, accounting for 78.5% of samples, G10
5 accounted for 9.9% of samples and viruses of G8 type were found in 4.7% of samples. In 3% of
6 samples, viruses were not classified due to concomitant infection with more G type strains, whereas
7 viruses in 3.9% of samples could not be characterized with any of the G-specific primers used in
8 this study. Most common P-types were P[11] and P[5], accounting for 65.1% and 25%,
9 respectively. In 2.6% of cases, samples reacted with multiple P-specific primers; no P[1] serotype
10 was identified. The G6P[11] combination was predominant throughout the study period, i.e. 52.5%
11 in 2003, 50% in 2004 and 40% in 2005. The incidence of G6P[5] increased from 13.1% in 2003 to
12 27% in 2004 and 25.5% in 2005. The G10P[11] combination decreased markedly from 18% in
13 2003 to 2.6% in 2004, rising again to 7.3% in 2005. G8P[11] viruses were similarly present in 2003
14 (5%) and 2004 (4.3%), declining slightly in 2005 (1.8%).

15

16 Keywords: Rotavirus; Genotyping; Bovine; Italy

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

1 **Introduction**

2 Group A rotaviruses are a major cause of acute gastroenteritis in the young of humans and many
3 animal species (Glass et al., 1997; Estes et al., 2007). Rotaviruses possess a genome of 11 segments
4 of double-stranded RNA inside a triple-layer protein capsid (Estes et al., 2007). The middle layer
5 protein, VP6, defines the serogroup (A-G), while the outer capsid proteins, VP4 and VP7, define
6 the G (VP7) and P (VP4) serotypes, elicit serotype specific neutralizing antibodies and are involved
7 in immunity (Estes et al., 2007). Currently, rotavirus genotyping by amplification of VP7 (Gouvea
8 et al., 1990, 1994b) and VP4 (Gouvea et al., 1994a; Gentsch et al., 1992) gene fragments using
9 serotype-specific primers is largely used in epidemiological studies (Estes et al., 2007). Based on
10 genetic characterization and phylogenetic analyses, at least 15 VP7 and 27 VP4 genotypes are
11 recognized (Martella et al., 2005, 2007), several of which infect both humans and animals (Estes et
12 al., 2007). Rotavirus strains with serotypes G6, G8, and G10, in association with VP4 types P6[1],
13 P7[5], and P8[11] are common in cattle, although G1, G3, and G11 strains are detected
14 sporadically. Unusual strains with G5, G7 and P[14], P[17] and P[21] genotypes have also been
15 described (El-Attar et al., 2002; Rao et al., 2000; Fukai et al., 2002; Estes et al., 2007).

16 Typical rotavirus types in humans are G1-4, and G9, combined to P[8] or P[4]. Strains with
17 common bovine G types, such as G8 and G10, have been identified in children with diarrhoea,
18 suggesting a zoonotic transmission (Iturriza-Gomara et al., 2004; Cook et al., 2004). Therefore,
19 surveillance of rotavirus G and P types is important to understand rotavirus ecology, interspecies
20 transmission and adaptation to new hosts. Furthermore, the recent introduction of two live human
21 vaccines in many countries, including Europe and North America, might exert a selective pressure
22 against circulating rotaviruses particularly those sharing the same G1-4 and P[8] specificities as the
23 vaccine strains (Desselberger et al., 2006).

24 We previously reported the genetic characterization of bovine rotaviruses collected in 1994-1998 in
25 Northern Italy (Falcone et al., 1999), showing that G6P[5] and G10P[11] accounted for most
26 strains. In the present study, we investigated bovine rotavirus isolated in 2003-2005 in the same
27 area to verify possible changes on the circulation of G and P genotypes.

28

29 **Materials and methods**

30 **Samples**

31 A total of 232 stools from calves with diarrhoea collected in herds in Northern Italy were studied.
32 Rotavirus diagnosis was performed by ELISA (Cordioli and Lavazza 1992), in some samples was
33 also confirmed by direct electron microscopy (EM).

34 **RNA extraction**

1 Samples were suspended in ddH₂O (10% vol/vol), and clarified by centrifugation at 6000 x g for 5
2 min. Rotavirus RNA was purified from 100 µl of suspension with the QIAamp RNA Mini kit
3 (Qiagen), and eluted in RNase-free water containing 200U/ml of RNase inhibitor. As control for
4 genotyping a set of well-characterized cell culture-adapted RV strains were used: Wa (G1P[8]),
5 NCDV(G6P[1]), UK (G6P[5]), B223 (G10P[11]), and 678 (G8P[5]), OSU (G5P[7]).

6 **RT-PCR of VP7 and VP4 genes**

7 For G- and P-typing, the protocols described by Gouvea et al. (1990; 1994a,b) and Gentsch et al.,
8 (1992) were used with some modifications. Reverse transcription (RT) and first PCR amplification
9 were performed in a one-tube format with the Access RT-PCR System (Promega). For
10 amplification of full-length VP7 (1,062 bp) gene (Gouvea et al., 1990), 5 µl viral dsRNA were
11 denatured (97°C, 5 min) with 0.5µM of Beg9-End9 primers; primers Con2-Con3 were used for
12 amplification of a 876 bp VP4-specific region (Gentsch et al., 1992). The denatured RNA was
13 added to a RT-PCR mixture containing the AMV/Tfl PCR buffer 5x, 1mM MgSO₄, 0.2mM dNTPs,
14 and 0.1 U/µl each of AMV Reverse Transcriptase and Tfl DNA Polymerase. Synthesis of cDNA
15 was achieved at 45°C for 45 min, 94°C for 2 min. The product was then subjected to 30 cycles at
16 94°C for 30 sec, 51°C for 1 min, and 68°C for 1 min 15 sec.

17 For G-typing, 2 µl of RT-PCR product, diluted 1:100 in H₂O, were used as the template for the
18 second PCR amplification in 10x PCR buffer II (KCl, 50mM; Tris-HCl, 10mM [pH 8.3]),
19 containing MgCl₂ (2mM), dNTPs (200µM), 0.02 U/µl of AmpliTaq Gold DNA polymerase (Perkin-
20 Elmer), and primers specific for bovine rotavirus (50nM each). These included primers for G5, G6,
21 G8, G10, and G11 types and sBeg9 (Gouvea et al 1994b; Martella, personal communication).
22 Samples resulting negative were further analyzed with primers specific for human genotypes G1,
23 G2, G12 and End9 (Gouvea et al., 1990, Gray, personal communication). The mixtures were
24 subjected to 30 cycles at 94°C for 1 min, 45°C for 2 min, and 72°C for 1 min. PCR products were
25 analyzed on a 1.5% agarose gel in TAE (40mM Tris-acetate, 1mM EDTA [pH 8]), stained with
26 ethidium bromide.

27 For P-typing, the second PCR (94°C, 1 min; 55°C, 2 min; 72°C, 1 min; 30 cycles) was performed
28 with primer Con2 or Con3 and primers specific for genotypes P[6], P[7], P[1], P[5], and P[11]
29 (Gentsch et al., 1992; Gouvea et al., 1994a, Martella et al. 2006).

30 **DNA sequencing**

31 A subset (37) of rotavirus strains were characterized by nucleotide sequencing of PCR products.
32 Amplified DNA was purified by using the Wizard SV Gel and PCR Clean-Up System (Promega),
33 and sequenced using an ABI sequencer apparatus (model ABI3730XL; Applied Biosystems).

1 Multiple alignments were performed using DNASIS MAX v.2. Sequences were compared against
2 the GenBank database using the BLAST program.

4 **Results**

5 Samples were collected from cattle farms located in Northern Italy regions Lombardia and Emilia-
6 Romagna. Farms included both dairy and beef herds, and were sampled during 2003-2005. A total
7 of 232 field samples from cases of neonatal calf diarrhea, positive for rotavirus by group A ELISA
8 and/or EM, were also analyzed by VP4 and VP7 RT/PCR. Second round nested-PCR was
9 performed to characterize rotavirus G and P types of -positive samples. The results of typing are
10 shown in Table 1. The G type was determined in 223 (96.1%) samples. The incidence of G types
11 was 78.5% for G6, 4.7% for G8, 9.9% for G10; mixed types were found in 3% of cases. Nine
12 specimens (3.9%) remained untypable. In 2004, G10 was replaced as the second most common type
13 by other genotypes, decreasing from 21.3 % in 2003 to 3.4% in 2004, increasing again to 11% in
14 2005. Instead, G8 viruses remained constant throughout the study (3.6-5.2%). In 2004, the number
15 of mixed types increased to 4.3% and was 3.6% in 2005. The untyped specimens were 6.5% in
16 2003 and 9.1% in 2005, whereas all rotavirus strains in 2004 were G-genotyped.

17 The P genotype was determined in 215 (92.7%) cases. The incidence of P genotypes was 65.1% for
18 P[11], 25% for P[5], 2.6% mixed, and 7.3% untyped. The incidence of each P type varied less from
19 year to year than the incidences of G types. Overall, P[11] was the most frequent type throughout
20 the study, varying from 80.3% of strains in 2003 to 60.3% in 2004 and 58.2% in 2005. The decrease
21 of P[11] type was balanced by the increase of either P[5] viruses or viruses with a mixed genotype.
22 P[5] was 16.4% in 2003, 28.5% in 2004 and 27.3% in 2005, and mixed types occurred in 1.6% of
23 cases in 2003, 1.7% in 2004 and 5.4% in 2005. Untyped samples were 1.6% in 2003, 9.5% in 2004
24 and 9.1% in 2005.

25 The combinations of G and P types are reported in Table 1. G6P[11] combination remained the
26 predominant one although it decreased along years. Conversely, the incidence of G6P[5] increased
27 from 13.1% of 2003 to 27% in 2004 and 25.5% in 2005. G10P[11] strains sharply decreased from
28 18% in 2003 to 2.6% in 2004, being 7.3% in 2005. G8P[11] viruses were constant in 2003 (5%) and
29 2004 (4.3%) declining in 2005 (1.8%). As previously shown by others (Falcone et al., 1999, Fukai
30 et al., 2002), rotaviruses with a P[5] type exhibited most frequently a G6 type, whereas
31 combinations of P[5] with either G10 or G8 occurred less frequently. To confirm results of
32 genotyping RT-PCR, 37 samples from all G- (20 G6, 11 G8 and 6 G10) and P- (10 P[11] and 10
33 P[5]) types identified were further analyzed by nucleotide sequencing of the DNA obtained from G-
34 and/or P-typing PCR. Sequence comparisons revealed a close relationship between the VP7 and

1 VP4 genes of strains found in our study and bovine strains reported in GenBank (95% to 99%
2 sequence similarity, data not shown).

3 The samples which resulted negatives at both G and P genotyping (0.8%) did not yield any visible
4 band after first round amplification, and could not be examined further. In 20 cases where only the
5 G or P type was determined by PCR, the amount of DNA after the first amplification was not
6 sufficient to allow sequencing, and the complete genotype could not be determined. These DNAs
7 were then subjected to a second round of genotyping PCR with primers for the most prevalent
8 human rotavirus types, but none yielded a positive result.

9

10 **Discussion**

11 In this study we determined the P and G types of rotaviruses circulating in bovine farms in the
12 North of Italy, an area encompassing 30% of the whole cattle population of the country. The RT-
13 PCR based genotyping method used was further confirmed to be a useful epidemiological tool for
14 examining strain diversity (Desselberger, et al., 2001), although it has gaps in encompassing novel
15 or uncommon rotavirus genotypes and mixed infections. Previous reports (Fukai et al., 2002; Rao et
16 al., 2000) have shown that most bovine rotavirus strains belong to G6, G10, [P5], and [P11]
17 genotypes. Also, a study on bovine strains conducted in Italy in the late 90s revealed that G6P[5]
18 was very common (38.3%), followed by G10P[11] (31.5%) (Falcone et al., 1999). The results of the
19 present study confirm that bovine rotavirus diversity in Northern Italy is limited to a narrow range
20 of bovine-specific genotypes. Compared to human rotavirus studies indicating a great variation of
21 predominant strains between years in a same area (Desselberger et al., 2006), the molecular
22 epidemiology of bovine rotavirus in Italy seems not to change remarkably with time. In fact, our
23 data altogether confirm that most bovine strains in each of the three years investigated presented a
24 G6 VP7 in combination with either a P[11] or P[5] VP4. A G8 VP7 in association with P[5] or
25 P[11] seems to be rare in bovines as recently reported in Southern Italy (Pisanelli et al., 2005).
26 Compared to our previous findings in the 1990's, the relative frequencies of different VP4 and VP7
27 types appear to have changed remarkably. In fact, in 1994-8 the predominant combination was
28 G6P[5] (Falcone et al., 1999) while in the present study G6P[11] became predominant. Another
29 major finding is the decrease of the G10P[11] combination from 2003 to 2004. This type was
30 frequent in 1994-8 representing approximately 30% of viruses (Falcone et al., 1999) whilst it
31 accounted for less than 3% of strains in 2004. These observations confirm previous reports (Fukai et
32 al., 2002; Pisanelli et al., 2005) that the incidence of G and P types may change significantly with
33 time. Whether this reflects changes in serotype-specific herd immunity is unclear. In fact, it has
34 been proposed that despite differences in serotypes, other cross-reactive antigens or antigenic sites

1 might ensure broad spectrum immunity in animals infected with a single rotavirus type (Blutt et al.,
2 2006).

3 In the present study, a limited number of strains could not be G-typed. Three of them might be
4 ELISA false-positive since they were also P-untypable, and no DNA was visible after the first
5 round RT-PCR. Concerning P-typing, a higher number (17) of samples remained untyped; this
6 finding may either reflect sequence variation within gene 4 (Martella et al., 2006) or suggest the
7 emergence of novel rotavirus genotypes (Fukai et al., 2007).

8 Sequential replacement of rotavirus antigenic types has been described during a long-term survey in
9 a bovine herd in Italy (Martella et al., 2003), and a periodic shift on the VP4 and VP7 specificities
10 has been observed for bovine rotavirus in Japan (Fukai et al., 2002) and human rotaviruses in
11 Southern America (Timenetsky Mdo et al., 1994), possibly related to mechanisms of antigenic
12 escape.

13 Presently, three killed or live modified vaccines are used in Italy to prevent neonatal rotavirus
14 diarrhoea of ruminants, belonging to G5P[7], G6P[1] or G6P[5], respectively (Pisanelli et al.,
15 2005). Rotavirus genotype surveillance in cattle to detect emerging strains is important to monitor
16 circulating strains in the farming population and optimize current animal vaccines. In addition, data
17 might help identify animal rotavirus strains with a possible zoonotic potential (Cook et al., 2004),
18 although such an event has not been reported in Italy. Most molecular epidemiology studies on
19 human rotavirus in Italy have shown conventional human G and P types, similarly to the rest of
20 Europe (Van Damme et al., 2007). However, atypical genotypes such as G8, G10 and G12 have
21 been recently reported in children with diarrhoea in Central Italy (Ansaldi et al., 2007) even if their
22 possible animal origin was not addressed. Differently than in the Netherlands (van der Heide,
23 2005), the occurrence of interspecies transmission of rotaviruses between cattle and humans has
24 been reported in Slovenia (Steyer et al., 2007), confirming earlier reports of zoonotic bovine
25 rotaviruses from India (Kang et al., 2005).

26

27 **Acknowledgements**

28 This work was partially supported by grants from the Italian Ministry of Health (Progetto
29 Finalizzato 2003, “Diversità genetica ed antigenica dei rotavirus, studio dei meccanismi evolutivi ed
30 implicazioni ai fini diagnostici e vaccinali”), from ISS/NIH (“Molecular and antigenic evolution of
31 rotavirus strains of human and animal origin”), and from the European Commission, DG Research
32 Quality of Life Program 6th Framework (EVENT, SP22-CT-2004-502571).

33

34 **References**

- 1 Ansaldo F., Pastorino B., Valle L., Durando P., Sticchi L., Tucci P., Biasci P., Lai P., Gasparini R.,
2 Icardi G., 2007. Paediatric Leghorn Group. Molecular characterization of a new variant of rotavirus
3 P[8]G9 predominant in a sentinel-based survey in central Italy. *J Clin. Microbiol.* 45(3), 1011-1015
4 Blutt S.E., Warfield K.L., O'Neal C.M., Estes M.K., Conner M.E., 2006. Host, viral, and vaccine
5 factors that determine protective efficacy induced by rotavirus and virus-like particles (VLPs).
6 *Vaccine* 24(8), 1170-1179.
- 7 Cook, N., Bridger, J., Kendall, K., Gomara, M.I., El-Attar, L., Gray, J., 2004. The zoonotic
8 potential of rotavirus. *J. Infect.* 48(4), 289-302.
- 9 Cordioli, P., Lavazza A., 1992. Comparison between ELISA test and immunoelectronmicroscopic
10 methods for the detection of rota and coronavirus in faeces of calves. *Atti Soc. Ital. Buiatria XXIV*,
11 521–526.
- 12 Desselberger U., Iturriza-Gomara M., Gray J., 2001. Rotavirus epidemiology and surveillance.
13 *Novartis Found. Symp.* 238, 125–147.
- 14 Desselberger U, Wolleswinkel-van den Bosch J, Mrukowicz J, Rodrigo C, Giaquinto C, Vesikari
15 T., 2006. Rotavirus types in Europe and their significance for vaccination. *Pediatr Infect Dis J.* 25(1
16 Suppl), S30-41.
- 17 El-Attar, L., W. Dhaliwal, M. Iturriza-Gomara, and J. C. Bridger. 2002. Identification and
18 molecular characterization of a bovine G3 rotavirus which causes age-independent diarrhea in
19 cattle. *J. Clin. Microbiol.* 40:937–942.
- 20 Estes, M.K, and Kapikian, A.Z., 2007. Rotaviruses. In: Knipe, D.M., Howley, P.M., (Eds.), *Fields*
21 *Virology*, 5th ed., vol. 2 Lippincott Williams and Wilkins, Philadelphia, Pennsylvania, pp. 1917–
22 1958.
- 23 Falcone, E., Tarantino, M., Trani, L.D., Cordioli, P., Lavazza, A., Tollis, M., 1999. Determination
24 of bovine rotavirus G and P serotypes in Italy by PCR. *J. Clin. Microbiol.* 37, 3879–3882.
- 25 Fukai K., Maeda Y, Fujimoto K, Itou T, Sakai T., 2002. Changes in the prevalence of rotavirus G
26 and P types in diarrheic calves from the Kagoshima prefecture in Japan. *Vet Microbiol.* 86(4), 343-
27 349.
- 28 Fukai K., Takahashi T., Tajima K., Koike S., Iwane K., Inoue K., 2007. Molecular characterization
29 of a novel bovine group A rotavirus. *Vet Microbiol.* 123(1-3), 217-224.
- 30 Gentsch, J.R., Glass R.I., Woods P., Gouvea V., Gorziglia M., Flores J., Das B.K., Bhan M.K.,
31 1992. Identification of group rotavirus gene 4 types by polymerase chain reaction. *J. Clin.*
32 *Microbiol.* 30, 1365–1373.
- 33 Glass, R.I., Breesee, J.S., Parashar, U., Miller, M., and Gentsch, J.R., 1997. Rotavirus vaccines at
34 the threshold. *Nature Med.* 3, 1324-1325.

- 1 Gouvea, V., Glass R.I., Woods P., Taniguchi K., Clark H.F., Forrester B., Fang Z.Y., 1990.
2 Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens.
3 *J. Clin. Microbiol.* 28, 276–282
- 4 Gouvea, V., Santos N., Timenetsky M.C., 1994a. VP4 typing of bovine and porcine group A
5 rotaviruses by PCR. *J. Clin. Microbiol.* 32, 1333–1337.
- 6 Gouvea, V. Santos N., Timenetsky M.C., 1994b. Identification of bovine and porcine rotavirus G
7 types by PCR. *J. Clin. Microbiol.* 32, 1338–1340.
- 8 Iturriza-Gomara, M., Kang, G., Mammen, A., Jana, A.K., Abraham, M., Desselberger, U., Brown,
9 D., Gray, J., 2004. Characterization of G10P[11] rotaviruses causing acute gastroenteritis in
10 neonates and infants in Vellore, India. *J. Clin. Microbiol.* 42(6), 2541-2547.
- 11 Kang G., Kelkar S.D., Chitambar S.D., Ray P., Naik T., 2005. Epidemiological profile of rotaviral
12 infection in India: challenges for the 21st century. *J Infect Dis.* 192 Suppl 1, S120-126.
- 13 Martella, V., Decaro, N., Pratelli, A., Tempesta, M. Buonavoglia, C., 2003. Variation of rotavirus
14 antigenic specificity observed in a dairy herd over a long-term survey. In: Kobayashi, N. (Ed.),
15 *Molecular Epidemiology of Rotavirus*. Research Signpost, Trivandrum, India, pp. 17-23.
- 16 Martella V, Ciarlet M, Baselga R, Arista S, Elia G, Lorusso E, Banyai K, Terio V, Madio A,
17 Ruggeri FM, Falcone E, Camero M, Decaro N, Buonavoglia C., 2005. Sequence analysis of the
18 VP7 and VP4 genes identifies a novel VP7 gene allele of porcine rotaviruses, sharing a common
19 evolutionary origin with human G2 rotaviruses. *Virology* 337(1), 111-123.
- 20 Martella V., Banyai K., Ciarlet M., Iturriza-Gomara M., Lorusso E., De Grazia S., Arista S., Decaro
21 N., Elia G., Cavalli A., Corrente M., Lavazza A., Baselga R., Buonavoglia C., 2006. Relationships
22 among porcine and human P[6] rotaviruses: evidence that the different human P[6] lineages have
23 originated from multiple interspecies transmission events. *Virology.* 344(2), 509-519.
- 24 Martella V., Ciarlet M., Banyai K., Lorusso E., Arista S., Lavazza A., Pezzotti G., Decaro N.,
25 Cavalli A., Lucente M. S., Corrente M., Elia G., Camero M., Tempesta M., Buonavoglia C., 2007.
26 Identification of group A porcine rotavirus strains bearing a novel VP4 (P) genotype in Italian swine
27 herds. *J. Clin. Microbiol.* 45(2), 577-580
- 28 Pisanelli G, Martella V, Pagnini U, De Martino L, Lorusso E, Iovane G, Buonavoglia C., 2005.
29 Distribution of G (VP7) and P (VP4) genotypes in buffalo group A rotaviruses isolated in Southern
30 Italy. *Vet Microbiol.* 110(1-2), 1-6.
- 31 Rao, C.D., Gowda, K., Reddy, B.S., 2000. Sequence analysis of VP4 and VP7 genes of nontypeable
32 strains identifies a new pair of outer capsid proteins representing novel P and G genotypes in bovine
33 rotaviruses. *Virology* 276 (1), 104–113.

- 1 Steyer, A, Poljsak-Prijatelj, M., Bufon, T.L., Marcun-Varda, N., Marin, J., 2007. Rotavirus
2 genotypes in Slovenia: unexpected detection of G8P[8] and G12P[8] genotypes. *J. Med. Virol.*
3 79(5), 626-32.
- 4 Timenetsky Mdo C, Santos N, Gouvea V. Survey of rotavirus G and P types associated with human
5 gastroenteritis in Sao Paulo, Brazil, from 1986 to 1992., 1994. *J Clin Microbiol.* 32(10), 2622-4.
- 6 Van Damme, P., Giaquinto, C., Maxwell, M., Todd, P., Van der Wielen, M.; REVEAL Study
7 Group, 2007. Distribution of rotavirus genotypes in Europe, 2004-2005: the REVEAL Study.*J.*
8 *Infect. Dis.* 195 Suppl 1:S17-25.
- 9 van der Heide R., Koopmans M.P., Shekary N., Houwers D.J., van Duynhoven Y.T., van der Poel
10 W.H., 2005. Molecular characterizations of human and animal group a rotaviruses in the
11 Netherlands. *J. Clin. Microbiol.* 43(2), 669-675
- 12
13
14
15

Table 1. Relative frequencies of various combinations of G and P genotypes of group A rotavirus detected in bovines, Italy 2003-2005

Year	No. of strains of genotype (%)										Total
	G6[P11]	G6[P5]	G10[P11]	G8[P11]	G10[P5]	G8[P5]	Mixed	G-untyped	P-untyped	G/P untyped	
1994-8*	23 (15.4)	57 (38.3)	47 (31.5)	5 (3.4)	4 (2.7)	2 (1.3)	5 (3.4)	6 (4%)	0	0	149
2003	32 (52.5)	8 (13.1)	11 (18)	3 (5)	2 (3.3)	0	1 (1.6)	3 (4.9)	0	1 (1.6)	61
2004	58 (50)	31 (26.7)	3 (2.6)	5 (4.3)	1 (0.9)	1 (0.9)	6 (5.1)	0	11 (9.5)	0	116
2005	22 (40)	14 (25.5)	4 (7.3)	1 (1.8)	1 (1.8)	0	5 (9)	3 (5.5)	3 (5.5)	2 (3.6)	55
Total (2003-5)	112 (48.3)	53 (22.8)	18 (7.8)	9 (3.9)	4 (1.7)	1 (0.4)	12 (5.2)	6 (2.6)	14 (6)	3 (1.3)	232

*Data for 1994-8 are "rearranged" from Falcone et al., 1999 and reported for comparison.