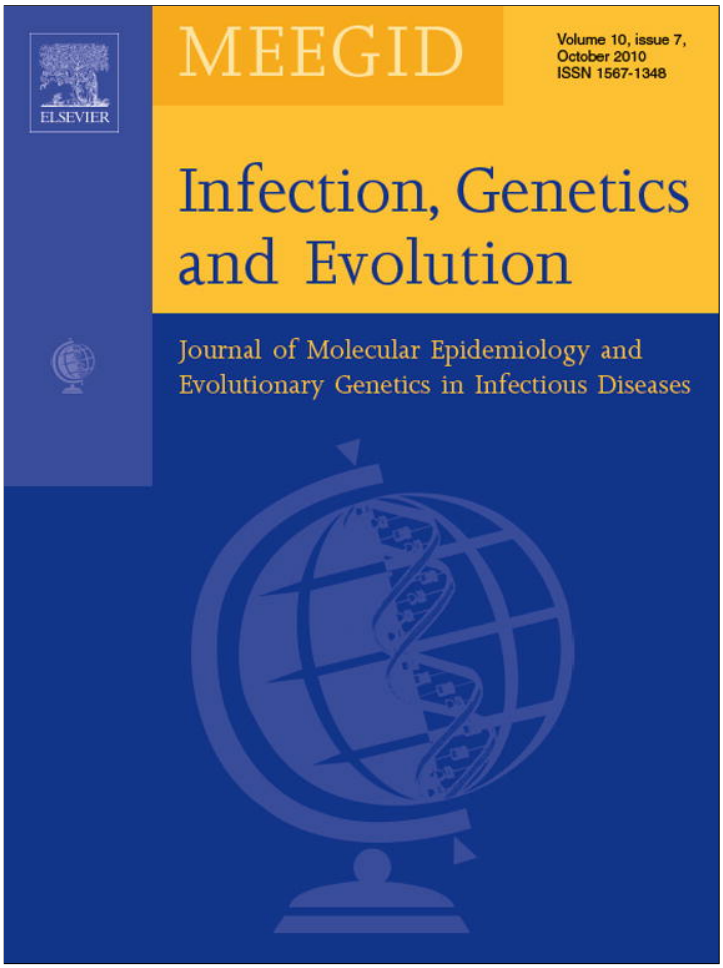

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Picobirnavirus causes persistent infection in pigs

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ABSTRACT

A study aimed to further understand the biology of porcine picobirnaviruses (PBV) was conducted between November 2003 and January 2008, on a farm located in the outskirts of Córdoba City, Argentina. PBV prevalence was examined by polyacrylamide gel electrophoresis and silver staining (PAGE S/S) on a total of 265 samples collected from pigs divided into four groups, according to age and physiological status. PBV detection rate was highest in the group of sows sampled within the lactogenic period (38.02%; $p < 0.05$), followed by pregnant sows (15.09%), piglets aged 2–5 months of age (18.42%) and adult (≥50 weeks) male pigs (0%). In addition, 103 samples collected in 3 follow-up studies were analyzed by PAGE S/S and reverse transcription followed by PCR (RT-PCR). Two of these studies followed female pigs from weaning up to slaughter and a third one from weaning up to 4 pregnancy periods. The results provide evidence that PBV establishes a persistent infection in the host with periods of silence intermingled with periods of low and high viral excretion. High PBV excretion levels were detected by PAGE S/S and were conditioned by age (primary infection) and host physiological status. Low PBV excretion levels were detected by RT-PCR throughout the entire study period. Sequence analysis of selected amplicons indicated that the virus excreted through the follow-up study was the same. These results suggest that porcine PBV is maintained in nature by transmission from infected asymptomatic individuals to susceptible ones.

1. Introduction

In 1988 in Brazil, a new group of viral agents was detected and described in the course of rotavirus research. Based on their characteristics, the viral agents were named "picobirnavirus": *pico* refers to the diminutive size of the virus particles (approximately 35 nm) and *birna* refers to their genome composed by 2 segments of double-stranded RNA, ranging in size from 2.4 to 2.6 kbp and 1.5 to 1.8 kbp for the slow and fast migrating segment, respectively (Pereira et al., 1988a). Several epidemiological studies have successively reported the presence of PBV in fecal specimens collected from not only domestic (Gatti et al., 1989; Ludert et al., 1991; Leite et al., 1990; Pereira et al., 1989; Gallimore et al., 1993) and wild animals (Pereira et al., 1988b; Haga et al., 1999) but also from humans with and without diarrheic syndrome (Pereira et al., 1994; Ludert and Liprandi, 1993; Cascio et al., 1996; Gallimore et al., 1995). These studies provide evidence of the wide circulation of these emerging viruses among several vertebrate species. However, the etiological association between diarrhea and the presence of the virus in animals as well as in humans remains to be established.

Prospective studies of patients infected with the human immunodeficiency virus (HIV) carried out in the United States (Grohmann et al., 1993) and in our laboratory in Argentina (Giordano et al., 1999) established a potential association between PBV infection and the diarrheic syndrome affecting severe immunosuppressed adults. In contrast, a study carried out in Venezuela (González et al., 1998) with HIV infected patients detected PBV in low frequency and only in samples from patients without diarrhea. More recently, the virus was also detected in immunosuppressed kidney transplant recipients (Valle et al., 2001).

Attempts to propagate PBV in tissue culture have proved unsuccessful (Pereira et al., 1989). Moreover, up to now there is no animal model for PBV. As a result, the technique used for the detection and identification of the virus until the year 2000 was restricted to the detection of the viral genome by polyacrylamide gel electrophoresis and silver staining (PAGE S/S). This technique although very useful has limited sensitivity. Thus, a reverse

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transcription-polymerase chain reaction (RT-PCR) assay was developed for the detection of human PBV by Rosen et al. (2000) and latter for porcine PBV strains by Carruyo et al. (2008). Both assays are based on the amplification of a conserved region of genome segment 2 of PBV, containing motifs 1 and 2 of the viral RNA-dependent RNA polymerase. These assays allowed the partial molecular and phylogenetic characterization of human strains isolated in China, the United States, Argentina, Hungary, India and Thailand (Rosen et al., 2000; Martínez et al., 2003; Bányai et al., 2003; Bhattacharya et al., 2006; Wakuda et al., 2005). In addition, the primers designed to detect human PBV strains (Rosen et al., 2000), were also found useful for the detection of several animal PBV strains (Bányai et al., 2008; Fregolente et al., 2009). However, the primer sets designed for the detection of human as well as porcine strains have shown limitations in their capacity to recognize all PBV strains circulating in both species, and it is now clear that there are emerging PBV strains that escape detection by these primers (Martínez et al., 2003; Carruyo et al., 2008; Bányai et al., 2008).

The ecological pattern of PBV circulation in nature is still largely unknown. In this work, we present preliminary results gained from studying PBV circulation among the pig population of a farm in Córdoba, Argentina. Our research aimed at increasing current knowledge about PBV circulation and its perpetuation patterns in nature, its mode of transmission and its possible association with diarrhea, as well as to explain the higher virus frequency reported among immunosuppressed humans compared with the general population.

2. Materials and methods

2.1. Study populations

A total of 368 pig fecal samples were collected from a farm situated in the outskirts of Córdoba City, Argentina.

The 265 specimens collected for the frequency study were obtained from November 3 to July 25, 2005, and were divided into four study groups: Group A: feces of pregnant sows ($n = 53$); group B: feces of sows with piglets within the lactation period ($n = 71$); group C: feces of juvenile pigs (8–20 weeks of age) at the moment of slaughter ($n = 114$) and Group D: feces of adult ($\geq$50 weeks) male pigs ($n = 27$). For all groups, just one sample per individual was collected. The specimens were classified as diarrheic or normal on the basis of their consistency.

The remaining 103 fecal samples were collected from three follow-up studies: two follow-up PBV excretion studies of two female pigs were carried out since weaning (approximately 30 days after birth) and up to animal death. Follow-up study 1 was carried out during a period of 2 months (from February 10, 2005 to March 30, 2005), and a total of 9 stool samples were collected at approximately weekly intervals. Follow-up study 2 was carried out during a period of 4 months (from April 1, 2005 to July 20, 2005), and a total of 16 stool samples were collected at approximately weekly intervals.

The third follow-up study was carried out during a 29-month period from one female pig (from August 1, 2005 to January 30, 2008; however, no samples were collected for 8 months from September 2006 to April 2007). The study period spanned from weaning (26 days after birth) up to its fourth reproductive cycle (898 days old). A total of 78 stool samples were collected approximately once a week, immediately after defecation, and the animal was isolated during the study.

The fecal specimens were stored at −20 °C until analysis. Samples from groups A to D were analyzed by PAGE S/S, while samples from follow-up studies 1, 2 and 3 were analyzed by PAGE S/S and RT-PCR.

2.2. Viral RNA extraction

Stool extracts (20%) were prepared in 0.02 M Tris–HCl, pH 7.2, and clarified by low speed centrifugation at 5000 × *g* for 10 min. Nucleic acid extraction was carried out according to Boom et al. (1990).

2.3. PBV dsRNA genome detection

The Laemmli (1970) system for electrophoresis was used. Polyacrylamide gel electrophoresis (PAGE) was carried out in a 10% polyacrylamide gel, 10 cm × 10 cm × 1 mm thick, in a vertical device (Bio-Rad, Hercules, CA) at 60 mA constant current for 3 h. Nucleic acid bands were visualized by silver staining according to Herring et al. (1982).

2.4. RT-PCR assay

The RNA was denatured by heating at 97 °C for 5 min and complementary DNA (cDNA) was obtained by reverse transcription using hexanucleotide random primers. PCR was carried out with primers PBV2-19/PBV2-281 according to Carruyo et al. (2008) in a 50 μl reaction mixture containing 10× PCR buffer 5 μl, $MgCl_2$ (50 μM) 2 μl, 0.4 mM dNTP's, 0.5 μM of each primer and 5 μl of cDNA. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of amplification (denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min) and a final extension of 72 °C for 7 min. The RT-PCR products (262 bp) were visualized by PAGE S/S. This method has a higher resolution and sensitivity than agarose gel electrophoresis and ethidium bromide. (Barril et al., 2006). The obtained amplicons were submitted for sequencing to Macrogen Laboratory Services (Seoul, Korea). The sequences were aligned using the BioEdit (http://www.mbio.ncsu.edu/BioEdit/page2.html) software, and similarities with other sequences were compared by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The nucleotide sequence determined in this study was deposited in the GenBank under the accession number GU176621.

3. Results

Picobirnavirus frequency in fecal specimens collected from the study groups A, B, C and D was determined by PAGE S/S. The virus was identified in 56 (21.13%) of the 265 fecal specimens (Table 1). The frequency of virus excretion was similar between groups A and C (15.09% vs. 18.42%, $p > 0.05$). However, the frequency of PBV detection in group B (38.02%) was significantly higher than those detected in groups A and C ($p < 0.05$). No PBV excretion was detected among pigs in group D. Most of the samples collected (98%) corresponded to healthy non-diarrheic animals.

One hundred and three fecal samples from 3 female pigs bred in individual boxes were collected weekly and analyzed for the

Table 1
Frequency of PBV detection in pig fecal specimens collected in a farm outside Cordoba City, Argentina, between November 3 and July 25, 2005.

Study group[a]	No. of specimens	PBV (+) by PAGE	Prevalence rate (%)
A	53	8	15.09
B	71	27	38.02[b]
C	114	21	18.42
D	27	0	0
Total	265	56	21.13

[a] Group A: pregnant sows; group B: sows during the lactogenic period; group C: pigs from 8 to 20 weeks of age; group D: adult ($\geq$50 weeks of age) male pigs.
[b] $p < 0.05$ (group B vs. all other study groups).

Table 2
Follow-up studies of PBV excretion on 2 female pigs followed from the weaning period until slaughter.

Follow-up Study 1	Weaning	February 2005			March 2005					
	Age in days	28	33	37	39		45			60
	Sample	1	2	3	4	5	6	7	8	9
	PAGE S/S[a]	-	-	-	-	-	+	+	+	+
	RT-PCR[b]	-	-	-	-	-	-	-	-	-

Follow-up Study 2	Weaning	April 2005		May 2005					June 2005						July 2005		
	Age in days	29	33	40	47			59	63			70		80	112		
	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	PAGE S/S[a]	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	RT-PCR[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a]Polyacrylamide gel electrophoresis and silver staining.
[b]Reverse transcription-polymerase chain reaction.

presence of PBV by PAGE S/S and RT-PCR. For the 2 pigs enrolled in follow-up studies 1 and 2, it was determined by PAGE that they were born PBV negative and started to excrete PBV approximately 45 and 80 days after weaning, respectively. Both animals excreted the virus until the moment of slaughter. All the samples collected in follow-up studies 1 and 2 were negative by RT-PCR. Results for follow-up studies 1 and 2 are shown in Table 2.

The detection of an individual positive for PBV by RT-PCR utilizing primers PBV2-19/PBV2-281 allowed a detailed study of the viral excretion pattern in the follow-up study 3. The results are shown in Table 3 and can be summarized as follows: A sample negative by PAGE S/S but positive by RT-PCR was detected early during the first week after weaning (sample 1), and 2 months later the virus could also be detected by PAGE S/S (sample 9). Thereafter, PBV excretion was detected frequently by RT-PCR and sporadically by PAGE S/S. This period was followed by almost 6 months without virus detection by PAGE S/S and only occasional detection by RT-PCR (samples 17–36). The next period of active virus excretion, when the virus could be detected not only by RT-PCR (samples 37, 42, 45, 51) but also by both PAGE S/S and RT-PCR techniques (samples 38, 39, 44, 46–50) was determined by the first gestation and farrowing cycle. During this period, between April 2006 and August 2006, a continuous PBV excretion pattern was identified. A similar continuous PBV excretion pattern was again identified during the third and fourth reproductive cycles, albeit positive samples could only be detected by RT-PCR (samples 52–54 and 65, 76–78, respectively), suggesting shedding of lower viral loads than in the first reproductive cycle. No samples were collected during the sow second reproductive cycle. Finally, no symptoms of diarrhea were observed in the animal through the study period.

Eleven of 33 RT-PCR products obtained in this study were sequenced. The amplicons showed 100% homology in their

Table 3
Follow-up study 3 of PBV excretion on a female pig followed from the weaning period until four reproductive cycles.

Weaning (20 days old)	Sample	Age (in days)	PAGE S/S[a]	RT-PCR[b]
August 2005	1	26	-	+[c]
	2		-	-
	3		-	-
	4		-	-
September 2005	5	58	-	+
	6		-	+
	7		-	-
	8		-	-
	9		+	+[c]
October 2005	10	87	-	+
	11		-	-
	12		+	+
	13		-	+
	14		-	+
	15		+	+[c]
November 2005	16	117	-	+
	17		-	-
	18		-	-
	19		-	-
December 2005	20	147	-	-
	21		-	+
	22		-	+[c]
	23		-	-
January 2006	24	178	-	+
	25		-	-
	26		-	-
	27		-	-
	28		-	-
February 2006	29	208	-	-
	30		-	-
March 2006	31	237	-	-
	32		-	-
	33		-	-
April 2006 mating	34	268	-	-
	35		-	-
	36		-	-
	37		-	+[c]
	38		+	+
	39		+	+
May 2006	40	297	-	-
	41		-	-
	42		-	+
June 2006	43	327	-	-
	44		+	+[c]
	45		-	+
	46		+	+
July 2006 August 2006 first farrowing and lactation	47	356	+	+
	48		+	+
	49		+	+[c]
	50	388	+	+
	51		-	+
May 2007 Third farrowing and lactation	52	662	-	+[c]
	53		-	+
	54		-	+
	55		-	-
June 2007	56	694	-	-
	57		-	-
	58		-	-
	59		-	-
	60		-	+[c]
July 2007	61	724	-	-
	62		-	-
	63		-	-
	64		-	-
Sepetember 2007 mating	65	755	+	+[c]
	66		-	-
	67		-	-
	68		-	-
October 2007	69	786	-	-
	70		-	-
	71		-	-
	72		-	-
November 2007 December 2007 Fourth farrowing and lactation	73	816	-	-
	74		-	-
	75	847	-	-
	76		-	+[c]
Jan 2008	77	877	-	+
	78	898	-	+

[a]Polyacrylamide gel electrophoresis and silver staining.
[b]Reverse transcription and polymerase chain reaction.
[c]Amplicons sequenced.

nucleotide sequence (262 bp). Blast analyses of the sequence obtained revealed a 96% homology with a porcine Venezuelan strain (GenBank accession number EU104360) (Carruyo et al., 2008). In addition, 62–72% homology was observed in an overlapping region of 95 nucleotides with 3 genogroup I Argentinean human PBV sequences (GenBank accession numbers AY805390.1; AY949206.1; AY949205.1) (Martínez et al., 2003).

4. Discussion

The present study was carried out in a pig population on a breeding farm located in the outskirts of the city of Córdoba, Argentina. PBV was detected at dissimilar frequencies in fecal samples of pigs of different ages and also with different physiological characteristics. The survey data from the farm studied suggest that PBV infection, as detected by PAGE S/S, is a frequent event given that the virus was excreted by approximately 21% of the porcine population, and up to 38.02% of those animals sampled during the lactation period (Table 1). Previous studies carried out in animal populations, using PAGE S/S, detected PBV with higher frequencies in stool samples obtained from juvenile than from adult individuals (Pereira et al., 1989; Ludert et al., 1991). In agreement, our results show a higher PBV detection rate in the juvenile pigs (Group C) than in the adults (group D). Also in agreement with previous results (Ludert et al., 1991; Carruyo et al., 2008), in this study PBV was identified with similar frequency among diarrheic and non-diarrheic animals (data not shown) and none of the animals infected showed any sign of diarrhea or any other illness. Thus, the importance of PBV as an etiological agent of diarrhea remains elusive up to date.

In order to establish a deeper understanding of the significance of PBV detection in the samples, the results should be analyzed considering the limitations of the detection methods. To date, the only method available to detect all strains of PBV in human and animal fecal samples is the visualization of the genome profile by PAGE. Although very useful, the PAGE S/S technique is limited by its relative insensitivity. Thus, a positive PBV sample detected by PAGE S/S reveals a high viral load in the sample and suggests a period of active viral replication in the host. According to the data obtained from the follow-up studies 1, 2 and 3, the primary PBV infection (as detected by PAGE S/S) takes place between 10 and 12 weeks of age and is characterized by prolonged viral shedding. Of note, individuals included in Group C are in the age group when most primary infections take place. This fact could explain the higher PBV detection rates observed in juvenile animals in this and other studies (Pereira et al., 1989; Ludert et al., 1991).

Interestingly, in this study, the highest frequency of virus excretion as detected by PAGE was observed in sows during the lactation period (group B). A similar observation was made during the follow-up study 3. In that study, PBV excretion was observed by PAGE at the final stage of gestation and during lactation time, about five and a half months after silent viral activity. These observations clearly suggest that a particular physiological status, such as the farrowing and lactation, might establish the necessary conditions for PBV excretion at levels high enough as to be detected by PAGE S/S (Brenner and Gürtler, 1977; Le Cozler et al., 1999; Sorrells et al., 2007; Malmkvist et al., 2009). Stress conditions generated by pig farming practices during the farrowing and lactation periods may be at the base for this observation (Pavičić et al., 2003; Tuchscherer et al., 2002). It has been demonstrated that cortisol is one of the hormones secreted during the stress syndrome and serum cortisol levels are an indicator of animal welfare (Chacón Pérez et al., 2004). Cortisol is known to affect lymphoid cells, to slow down lymphocyte proliferation, to decrease antibody quantities and lymph node size thus decreasing the capacity of pigs to resist infection (Kelley, 1988). On note, the results obtained in this study in pigs and those previously reported in immunocompromised human populations, where frequent and active virus excretion have been described for patients infected with HIV or after kidney transplant (Pereira et al., 1994; Ludert and Liprandi, 1993; Cascio et al., 1996; Grohmann et al., 1993; Giordano et al., 1999; González et al., 1998; Valle et al., 2001), suggest that an association between virus excretion levels and immunosuppression or a particular physiological status might exist. Further studies are needed to determine if the asymptomatic excretion of PBV could revert to diarrhea in association with host factors such as immune status (Grohmann et al., 1993).

The natural history of PBV infection may start to be defined based on the results obtained in the follow-up study 3. PBV infection was characterized by periods of high and low active viral excretion (detected by PAGE and RT-PCR, respectively) interspaced by silent periods. Remarkably, the sequence analysis suggested that the sow excreted virus with the same sequence during its lifetime. It is likely that the sampling period of the follow-up study 3 (29 months) was not long enough to allow for the detection of genetic changes or environmental circumstances did not permit the emergence of genetic variants (Domingo et al., 1996). Alternatively, the amplicon obtained may correspond to a highly conserved and stable region of the RNA-dependent RNA polymerase gene.

The negative results obtained by RT-PCR in samples that were positive by PAGE S/S in follow-up studies 1 and 2 are not an unusual finding. PBV strains isolated from different individuals exhibit a high genetic diversity (Martínez et al., 2003; Bányai et al., 2003; Bhattacharya et al., 2006). Thus, these may be strains that escape detection by primers PBV2-19/PBV2-281 (Carruyo et al., 2008).

In summary, the results herein presented suggest that PBV is acquired early in life and establishes a persistent infection, with periods of high viral activity intermingled with periods of silence. Thus, PBV infected animals could remain life-long asymptomatic carriers and serve as reservoirs of infection, as have been observed for other bisegmented double-stranded RNA viruses (Reno, 1999). In support, evidence for PBV persistent and asymptomatic infection in humans was published recently (Zhang et al., 2006; Victoria et al., 2009). As the PBV has a wide spectrum of hosts, future studies will be necessary to further understand the dynamics of virus circulation.

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References

Bányai, K., Jakab, F., Reuter, G., Bene, J., Melegh, B., Szücs, G., 2003. Sequence heterogeneity among human picobirnaviruses detected in a gastroenteritis outbreak. Arch. Virol. 148, 2281–2291.

Bányai, K., Martella, V., Bogdán, Á., Forgách, P., Jakab, F., Meleg, E., Bíró, H., Melegh, B., Szücs, G., 2008. Genogroup I picobirnaviruses in pigs: evidence for genetic diversity and relatedness to human strains. J. Gen. Virol. 89, 534–539.

Barril, P.A., Martinez, L.C., Giordano, M.O., Castello, A.A., Rota, R.P., Isa, M.B., Masachessi, G., Ferreyra, L.J., Glikmann, G., Nates, S.V., 2006. Detection of group a human rotavirus G9 genotype circulating in Córdoba, Argentina, as early as 1980. J. Med. Virol. 78 (8), 1113–1118.

Bhattacharya, R., Sahoo, G., Nayak, M., Saha, D., Sur, D., Naik, T., Bhattacharya, S., Krishnan, T., 2006. Molecular epidemiology of human picobirnaviruses among children of a slum community in Kolkata, India. Infect. Genet. Evol. 6 (6), 453–458.

Boom, R., Sol, C.J., Salimans, M.M., Jansen, C.L., Wertheim-van Dillen, P.M., van der Noordaa, J., 1990. Rapid and simple methods for purification of nucleic acids. J. Clin. Microbiol. 28, 495–503.

Brenner, K.V., Gürtler, H., 1977. Concentrations of cortisol, glucose and free fatty acids in the blood plasma of swine in relation to age and in sows close to farrowing. Arch. Exp. Veterinarmed. 31 (5), 741–747.

Carruyo, G.M., Mateu, G., Martínez, L.C., Pujol, F.H., Nates, S.V., Liprandi, F., Ludert, J.E., 2008. Molecular characterization of porcine picobirnaviruses and development of a specific reverse transcription-PCR assay. J. Clin. Microbiol. 46 (7), 2402–2405.

Cascio, A., Bosco, M., Vizzi, E., Giamanco, A., Ferraro, D., Arista, S., 1996. Identification of picobirnavirus from faeces of Italian children suffering from acute diarrhea. Eur. J. Epidemiol. 12, 545–547.

Chacón Pérez, G., García-Belenguer Laita, S., Illera del Portal, J.C., Palacio Liesa, J., 2004. Validation o fan EIA technique for the determination of salivary cortisol in cattle. Span. J. Agric. Res. 2 (1), 45–51.

Domingo, E., Escarmís, C., Sevilla, N., Moya, A., Elena, S., Quer, J., Novella, I., Holland, J., 1996. Basic concepts in RNA virus evolution. FASEB J. 10, 859–8564.

Fregolente, M.C.D., Castro-Dias, E., Soares Martins, S., Rosado Spillki, F.R., Marques Allegretti, S., Viccari Gatti, M.S., 2009. Molecular characterization of picobirnavirus from new hosts. Virus Res. 143, 134–136.

Gallimore, C.I., Lewis, D., Brown, D.W.G., 1993. Detection and characterization of a novel bisegmented double-stranded RNA virus (picobirnavirus) from rabbit faeces. Arch. Virol. 133, 63–73.

Gallimore, C.I., Appleton, H., Lewis, D., Green, J., Brown, D.W., 1995. Detection and characterization of bisegmented double-stranded RNA viruses (Picobirnaviruses) in human faecal specimens. J. Med. Virol. 45, 135–140.

Gatti, M.S.V., De Castro, A.F.P., Feraz, M.M.G., 1989. Viruses with bisegmented double-stranded RNA in pig faeces. Res. Vet. Sci. 47, 97–398.

Giordano, M.O., Martínez, L.C., Rinaldi, D., Espul, C., Isa, M.B., Depetris, A., Medeot, S., Nates, S., 1999. Diarrhea and enteric emerging viruses in HIV-infected patients. J. AIDS Res. Hum. Retroviruses 15, 1427–1432.

González, G.G., Pujol, F.H., Liprandi, F., Deibis, L., Ludert, J.E., 1998. Prevalence of enteric viruses in human immunodeficiency virus seropositive patients in Venezuela. J. Med. Virol. 55, 288–292.

Grohmann, G.S., Glass, R.I., Pereira, H.G., Monroe, S.S., Hightower, A.W., Weber, R., Bryan, R.T., 1993. Enteric viruses and diarrhea in HIV-infected patients. N. Engl. J. Med. 329, 14–20.

Haga, I.R., Martins, S.S., Hosomi, S.T., Vicentini, F., Tanaka, H., Gatti, M.S., 1999. Identification of a bisegmented double-stranded RNA virus (picobirnavirus) in faeces of giant anteaters. Vet. J. 158, 234–236.

Herring, A.J., Inglis, N.F., Ojech, C.K., Snodgrass, D.R., Menzies, J.D., 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver stained polyacrylamide gels. J. Clin. Microbiol. 16, 473–477.

Kelley, K.W., 1988. Cross-talk between the immune and endocrine systems. J. Anim. Sci. 66, 2095–2108.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.) 227, 680–685.

Le Cozler, Y., Beaumal, V., Neil, M., David, C., Dourmad, J.Y., 1999. Changes in the concentrations of glucose, non-esterified fatty acids, insulin, cortisol and some mineral elements in the plasma of the primiparous sow before, during and after induced parturition. Reprod. Nutr. Dev. 39 (2), 161–169.

Leite, J.P.G., Monteiro, S.P., Fialho, A.M., Pereira, H.G., 1990. A novel avian virus with trisegmented double-stranded RNA and further observations on previously described similar viruses with bisegmented genome. Virus Res. 16, 119–126.

Ludert, J.E., Liprandi, F., 1993. Identification of viruses with bi and trisegmented double-stranded RNA genome in faeces of children with gastroenteritis. Res. Virol. 144, 219–224.

Ludert, J.E., Liprandi, F., et al., 1991. Identification in porcine faeces of a novel virus with a bisegmented double stranded RNA genome. Arch. Virol. 117, 97–107.

Malmkvist, J., Damgaard, B.M., Pedersen, L.J., Jørgensen, E., Thodberg, K., Chaloupková, H., Bruckmaier, R.M., 2009. Effects of thermal environment on hypothalamic–pituitary–adrenal axis hormones, oxytocin, and behavioural activity in periparturient sows. J. Anim. Sci. 87 (9), 2796–2805.

Martínez, L.C., Isa, M.B., Paván, J., Alvarado, L., Giordano, M., Rinaldi, D., Nates, S.V., 2003. Molecular diversity of partial-length genomic segment 2 of human picobirnavirus. Intervirology 46 (4), 207–213.

Pavičić, Ž., Vučemilo, M., Tofant, A., Vijtiuk, N., Popovič, M., Balenovič, M., Balenovič, T., 2003. Effect of immunostimulator Baypamun® on plasma cortisol concentration in gilts regrouped during the late stage of pregnancy. Acta Vet. Brno 72, 509–514.

Pereira, H.G., Fíalho, A.M., Flewett, T.H., Teixeira, J.M., Andrade, Z.P., 1988a. Novel viruses in human faeces. Lancet 2, 103–104.

Pereira, H.G., Flewett, T.H., Candeias, J.A., Barth, O.M., 1988b. A virus with bisegmented double-stranded RNA genome in rat (*Oryzomys nigripes*) intestines. J. Gen. Virol. 69, 2749–2754.

Pereira, H.G., De Araujo, H.P., Fíalho, A.M., De Castro, L., Monteiro, S.P., 1989. A virus with bi-segmented double stranded RNA genome in guinea pig intestines. Mem. Inst. Oswaldo Cruz 84, 137–140.

Pereira, H.G., Linhares, A.C., Candeiras, J.A.N., Glass, R.I., 1994. Vigilancia de los virus de la gastroenteritis en los laboratorios nacionales del Brasil. Bol. Of. Sanit. Panam. 116, 27–36.

Reno, P.W., 1999. Infectious pancreatic necrosis and associated aquatic birnaviruses. In: Woo, P.T.K., Bruno, D.W. (Eds.), Fish Diseases and Disorders, Volume 3: Viral, Bacterial and Fungal Infections. pp. 1–55.

Rosen, B.I., Fang, Z.Y., Glass, R.I., Monroe, S.S., 2000. Cloning of human Picobirnavirus genomic segments and development of and RT-PCR detection assay. Virology 277 (2), 316–329.

Sorrells, A.D., Eicher, S.D., Harris, M.J., Pajor, E.A., Richert, B.T., 2007. Periparturient cortisol, acute phase cytokine, and acute phase protein profiles of gilts housed in groups or stalls during gestation. J. Anim. Sci. 85 (7), 1750–1757.

Tuchscherer, M., Kanitz, E., Otten, W., Tuchscherer, A., 2002. Effects of prenatal stress on cellular and humoral immune responses in neonatal pigs. Vet. Immunol. Immunopathol. 86 (3–4), 195–203.

Valle, M., Martínez, L.C., Ferreira, L.J., Giordano, M., Isa, M.B., Paván, J., Boccardo, G., Massari, P., Nates, S.V., 2001. Agentes virales asociados al síndrome diarreico en pacientes transplantados renales. Medicina (B. Aires) 61, 179–182.

Victoria, J.G., Kapoor, A., Li, L., Blinkova, O., Slikas, B., Wang, C., Naeem, A., Zaidi, S., Delwart, E., 2009. Metagenomic analyses of viruses in stool samples from children with acute flaccid paralysis. J. Virol. 83 (9), 4642–4651.

Wakuda, M., Pongsuwanna, Y., Taniguchi, K., 2005. Complete nucleotide sequences of two RNA segments of human picobirnavirus. J. Virol. Methods 126, 165–169.

Zhang, T., Breitbart, M., Lee, W.H., Run, J.Q., Wei, C.L., Soh, S.W., Hibberd, M.L., Liu, E.T., Rohwer, F., Ruan, Y., 2006. RNA viral community in human feces: prevalence of plant pathogenic viruses. PLoS Biol. 4 (1), 108–118.