

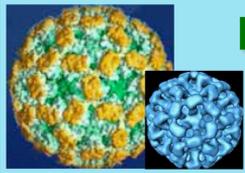
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**PREVALENCE AND SYMPTOMS
OF ROTAVIRUS AND CALICIVIRUS
INFECTIONS FROM PORCINE
POPULATION IN THE PROVINCE
OF ZARAGOZA, SPAIN**

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Prevalence and symptoms of Rotavirus and Calicivirus Infections from porcine population in the province of Zaragoza, Spain.

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Abstract

Objectives: determine the prevalence of rotavirus and Calicivirus, and their respective association to diarrhea, in the porcine population in Zaragoza province, Spain.

Material and Methods: Two hundred twenty two samples were collected at random from different farms in the province and from the main slaughter house facility in the city of Zaragoza. Rotavirus and Calicivirus detection were realized by RT-PCR. Fecal samples were scored as diarrheic or normal and grouped into five groups to match general farm management and age criteria: GrI- (suckling = 0-4 weeks) GrII-(weaning= 4-8 wks) GrIII- (transition = 8-16wks), GrIV- (fattening = 16-24 wks) and group 5(adults >24wks).

Results: Rotavirus and Calicivirus were detected in the samples with an overall prevalence of 16.7% and 12.2 %, respectively. While Rotavirus detection in feces was associated with Both age and Feces consistency, being more frequent in piglets less than 8 weeks old with OddRatios equal to 4.3 and 5.2, respectively. Calicivirus shedding in feces revealed to be homogeneously distributed among all ages, showing no significant association to feces consistency (OR=0.8).

Rotavirus from some suckling piglets belong to a (G9, P8) genotype. It is to be mentioned that the G9 genotype is the major G genotype circulating this year in Spain.

We have had troubles to sequence the PCR product from Norovirus. The primers used are primarily to detect Human Norovirus (JV12, JV13) and (Nvp290, Nvp10), and it is possible their specificity to detect Porcine Norovirus is not optimal. Indeed, JV12, JV13 pair were actually unable to detect positive samples detected by Nvp290, Nvp10 primers.

Conclusion: Norovirus and rotavirus are circulating in the porcine population in the province of Zaragoza, Spain. Norovirus infection contrary to rotavirus infection is asymptomatic. Specific primers to detect Porcine Norovirus are needed.

Material and Methods

Sampling: In this study we processed 221 fecal sample for Norovirus and Rotavirus detection. From September 2005 till December 2006, 93 fecal samples from different porcine production units in the province of Zaragoza, North of Spain, were sent by field veterinarian to our Laboratory. Another 88 samples were collected from the main slaughter house in Zaragoza city. Finally in March/April 2007, 40 samples from suckling piglets and their corresponding sows were obtained. For statistical convenience, we divided the samples in 5 groups and scored their appearance as diarrheic or non-diarrheic. Age groups roughly match the management changes these animal undergo: GrI-(suckling = 0-4 weeks) GrII-(weaning= 4-8 wks) GrIII- (transition = 8-16wks), GrIV- (fattening = 16-24 wks) and group 5(adults >24wks).

Rotavirus detection : a) For a rapid screening we used the immuno-chromatography ROTA-STRIP test. The test's specificity comes from a monoclonal antibody directed against Group A VP6 proteins of human Rotavirus that is conjugated to colloidal gold particles.

b) We also performed RT-PCR for VP4 a VP7 detection of Rotavirus group A. The RT was performed using Random Primers. Then for genotyping we used the nested multiplex PCR for the obtained product size (pb= pair of bases) would determine the corresponding G(1,2,3,4, 8,9 or 10) or P(4, 6, 8, 10 or 11) genotypes.

Calicivirus Detection : RT-PCR was also used for Norovirus detection. The RT reaction was initiated with Nvp10 primer. Conventional PCR were performed using the two pair of primer JV12-JV13 and Nvp10-p290.

We also applied a real time technique using 2 pairs of primers and their corresponding TaqMan probes designed by H. Vennema and ordered from Applied biosystems for GGI and GIIG of human Norovirus.

Statistical Analysis: Data analysis were performed using the following softwares: Microsoft excel 2000, Winepi (<http://www.winepi.net>), and SPSS software version 1.3 package (SPSS Inc. Chicago II).

Results and Discussion

ROTAVIRUS: 37 out of 221 samples were positive to rotavirus. Giving the level of prevalence in the suckling and weaning piglets (groups I and II) 26% and 47%, respectively, with a clear decreasing tendency in finishing piglet (group III and IV) 14.5 and 6.3%, respectively, to reach 0% in adults (see table 1).

For Odds ratio estimation, we further regrouped the data into 2 categories: samples from animals less than 8 weeks or older than 8 weeks old. Applying the Chi-squared test in an observational study revealed an OR of 4.3 (2.06-8.97) associating the age to the presence of rotavirus in a given sample, meaning that animals of less than 8 weeks of age are at higher risk than those aged more than 8 weeks to excrete rotavirus (table 2). These results are in agreement with most published data where rotavirus is found in higher percentages in younger piglets. When we considered rotavirus presence and feces consistency, the Odd Ratio revealed a clear association between Diarrheic samples and rotavirus presence in the sample OR= 5.21. Meaning that the probability to detect rotavirus from diarrheic sample are 5 times higher than non-diarrheic samples (table 3). Age being a possible confusing factor, since diarrhea is more frequent in younger animals, we applied the Mantel-Haenszel (OR MH) to account for this possible effect. In fact the Ponderate value for OR becomes 3.5(1.2-9.7) meaning that the weighing of the age influence, makes the real risk to associate diarrhea to rotavirus shedding is around 3.5 times instead of 5 times (table 4). A G9, P8 rotavirus isolate was found circulating in piglets. The G9 serotype is the most frequent genotype circulating nowadays in Spain. We were unable to genotype all the positive samples we obtained. Our set of primers do not cover all genotypes, and were mainly designed from human strains.

Calicivirus: 27 out of 221 samples were positive to Calicivirus. Similar prevalence rates were found in different age groups (12%) (table 1). Linda Saif group have not detected Norovirus in piglets. In our study we found a positive saw with two of its suckling piglets positive to Norovirus indicating a possible transmission from a Norovirus shedding saw to its progeny.

There were no difference in Calicivirus detection between diarrheic feces or non-diarrheic feces, meaning that at least diarrhea is not associated with calicivirus shedding in any age group (table 2 and 3). Most authors do observe the same, since most Norovirus positive animals are asymptomatic.

Introduction

The role of rotavirus in animal gastroenteritis is well documented in children and young animals. It is estimated that 30% of piglet diarrhea is associated with rotavirus infection. Resistance to rotavirus infection increases with age in human as well as in farm animals. Gene reassortment and recombination between animal rotavirus and human rotavirus are described in several research reports. Rotavirus A being the most prevailing group (80% of outbreaks). It is very well characterized and genotyping for VP4 and VP7 genes a standard procedure nowadays. Human Norovirus is getting more and more studied despite the lack of animal or cell culture models to replicate it. Norovirus has been classified in five genogroup (GGI, GGII, GGIII, GGIV and GGV). Recently, some researchers are suggesting up to eight genogroups. Some authors working on animal Norovirus, suspect that these animals may play a role of reservoir for emerging new viral species and had proved some genomic resemblance between animal species and human ones. However, others found significant differences in the ORFs sequences and the capsid between animal and human Norovirus that they believe it is hard to believe it is they may cause a zoonosis. Linda Saif group has been able to infect gnotobiotic piglets with human Norovirus strain causing to some of them a transient diarrhea.

Objectives

We wanted to determine the resemblance -if any- between rotavirus and Calicivurus in intensive porcine animal production units in Zaragoza province, North of Spain, as for their respective prevalence and association to diarrhea,

We also wanted to check if there might be any genetical similarity between human and porcine rotavirus and / or Calicivirus (Norovirus and Sapovirus) circulating nowadays in Spain.

Table 1. Prevalence (%) of rotavirus & Calicivirus

Group	N	Rotavirus Prevalence	Norovirus Prevalence
I (0-4 wks)	45	26,7%	13,3%
II (4-8 wks)	19	47,0%	10,5%
III (8-16)	83	14,5%	12,0%
IV (16-24)	63	6,3%	12,7%
V (>24 wks)	11	0,0%	9,1%
Total	221	16,7%	12,2%
Chi ²		P<0.001	P=0.892

Table 3. Prevalence of rotavirus & Calicivirus in relation to feces consistency

Diarrhea	N	Prevalence Rotavirus	Prevalence Norovirus
No	108	6,5%	13%
Yes	113	26,5%	11,5%
Total	221	16,7%	12,2
Chi-square		p<0,001	P=0,741
OR		5.21 (2.18-12.47)	.873 (.39-1.95)

•JV12 / JV13 pair of primers to detect Norovirus were much less sensitive than NVP10/P290

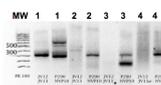


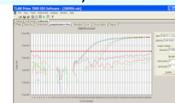
Table 2. Prevalence of rotavirus & Calicivirus in relation to age group.

Group	N	Prevalencia Rotavirus	Prevalencia Norovirus
<8 semanas	64	30,8%	12,9%
>8 semanas	157	10,2%	11,9%
Total	221	16,7%	12,2%
Chi-square test		P=0,001	P=0,953
Odds Ratio		5.21(2.1-12.4)	0.984

Table 4. stratified Odd Ratio using as variable the diarrhea

	OR	IC 95%
<8 weeks	22,12	1,8-119,2
>8 weeks	2,83	1,3-8,6
Total	5,21	2,1 - 12,4
Weighed Total (MH)	3,50	1,2 - 9,7

•Human primers for GGI and GGII were not useful to detect any Norovirus positive samples in a real time assay. Human GGI and GGII were successfully detected.



Conclusions

- Rotavirus and Calicivirus are circulating in the swine production units in Zaragoza despite the new technical advances in management.
- Rotavirus is shed and associated with diarrhea onset, mainly in piglets (suckling and weaning).
- Calicivirus is homogeneously distributed among different age groups of swine population.
- Calicivirus is not associated with diarrhea,
- PCR primers designed specially from swine isolated Noroviruses are needed and are to be optimized for Norovirus detection.