

ENFERMEDAD DEL OJO AZUL



Dr. José Ivan Sánchez Betancourt

ETIOLOGÍA

Esférico

Tamaño: 100 a 360 nm

Mononegavirales

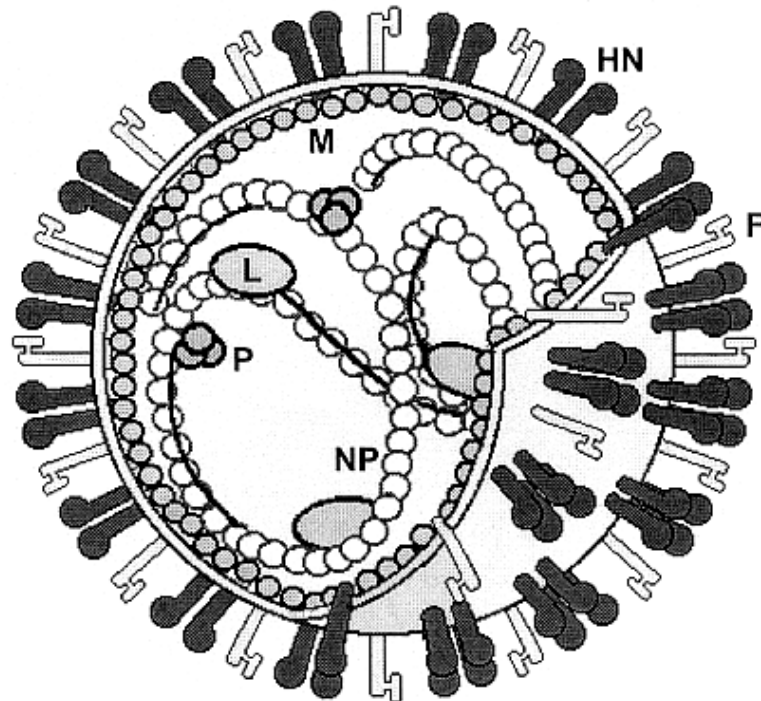
Paramixoviridae

Paramixovirinae

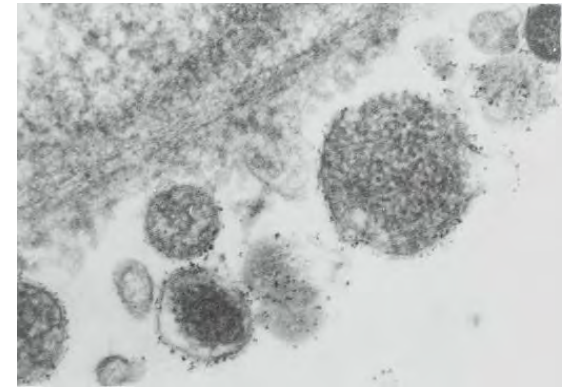
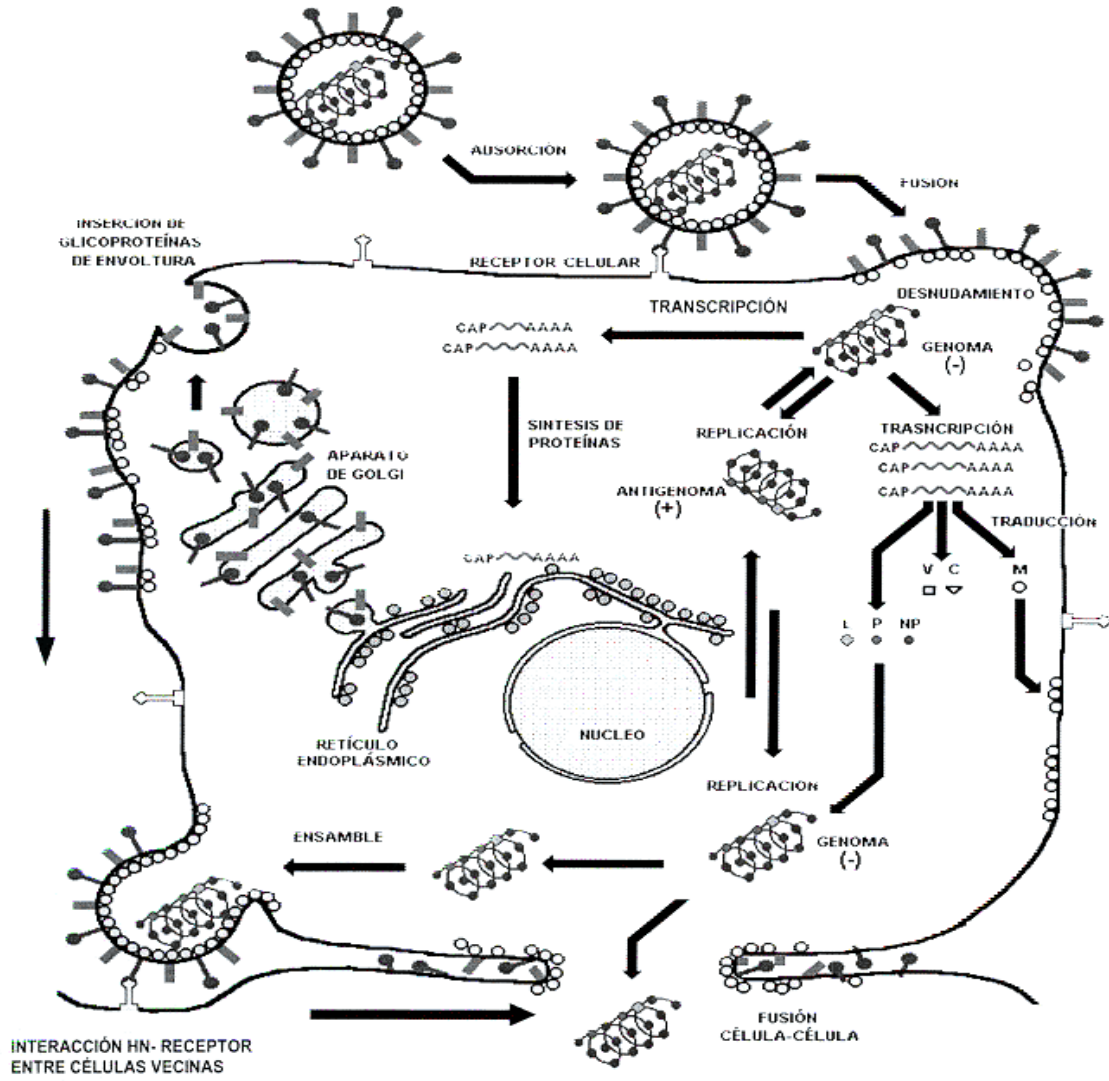
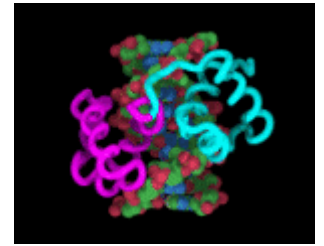
Rubulavirus

Genoma:

L	200K	(6782)
NP	68K	(2105)
P	52K	(1374)
M	40K	(1376)
HN	66K	(1906)
F	59K	(1845)



CICLO BIOLÓGICO



ANTECEDENTES

1980 La Piedad, Michoacán (Stephano,1981).

1982 Jalisco y Guanajuato (Stephano,1984).



1983 Encefalitis y signos respiratorios en cerdos de 15-45 Kg de peso Repeticiones, mortinatos, fetos momificados y abortos (Stephano, 1985).



1988 Orquitis, epididimitis y atrofia testicular con formación de granulomas.
(Campos, 1989).



1992 (Fuentes, 1992).

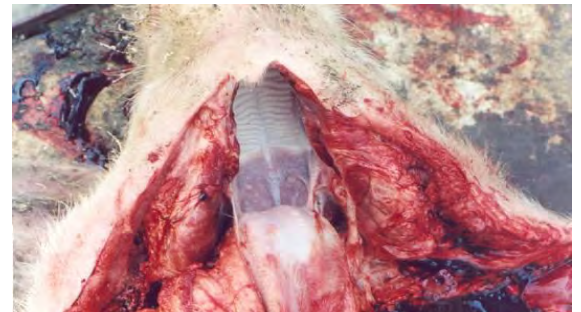
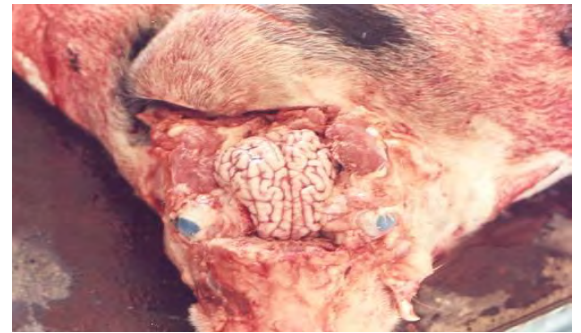


2004 Prevalencia del 20-30% en, Campeche, Q. Roo, Tabasco y Colima. Jalisco y Edo. Mex con prevalencia del 10-19%. (Milián *et al.* 2004).



Lesiones a la necropsia

- Congestión meníngea
- Aumento de LCR
- Opacidad corneal
- Orquitis a los 14 días (edematización)
- Orquitis a los 30 días
(granuloma epidídimo)
- Atrofia testicular



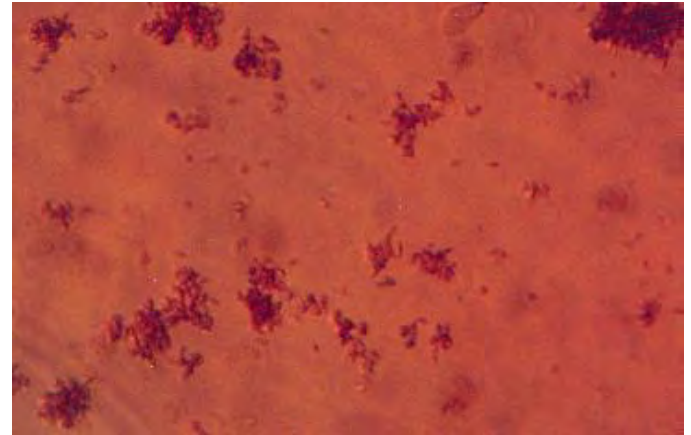
Diagnóstico

Antígeno

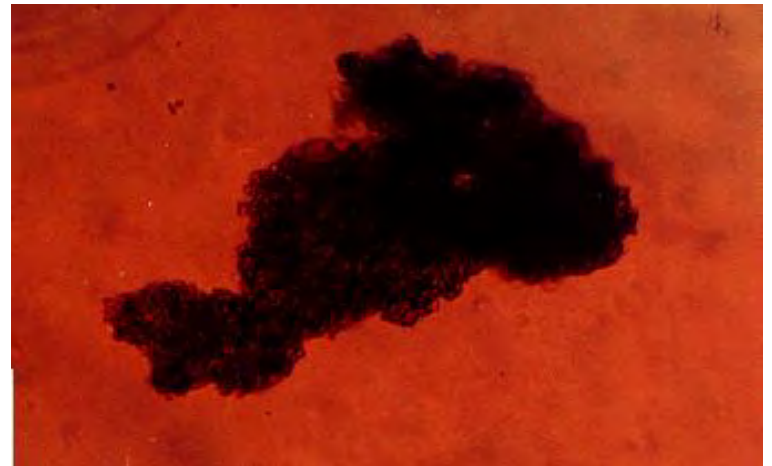
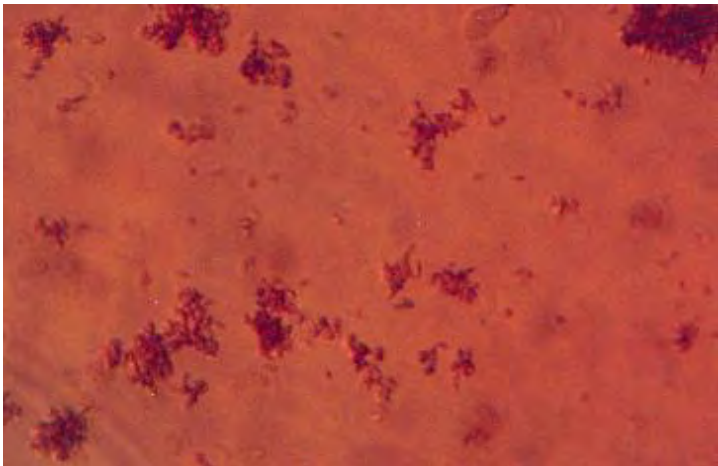
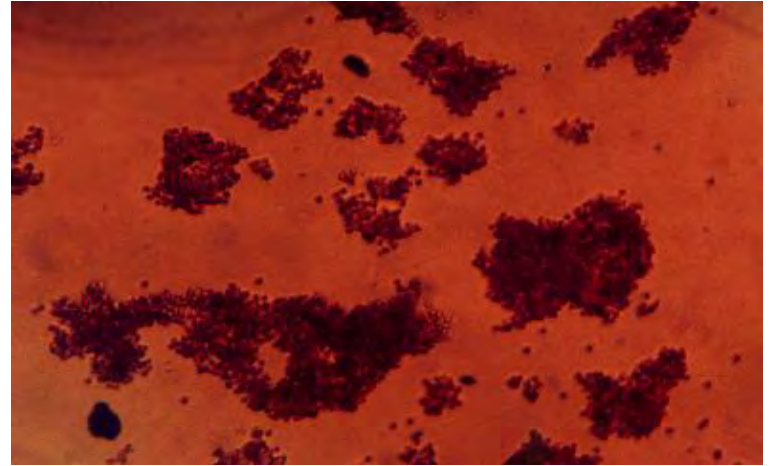
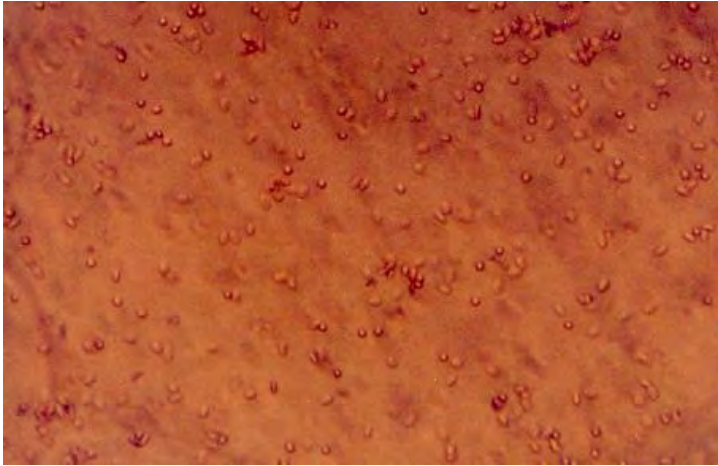
- Aislamiento viral (encéfalo, pulmón)
- Inmunofluorescencia
- PCR

Anticuerpo

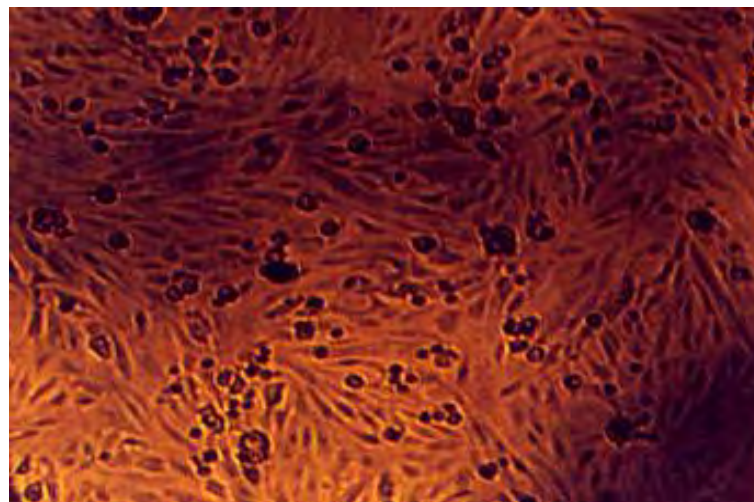
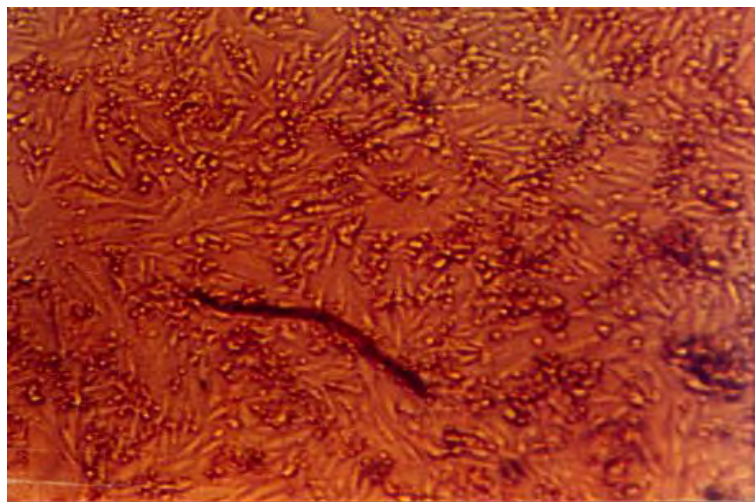
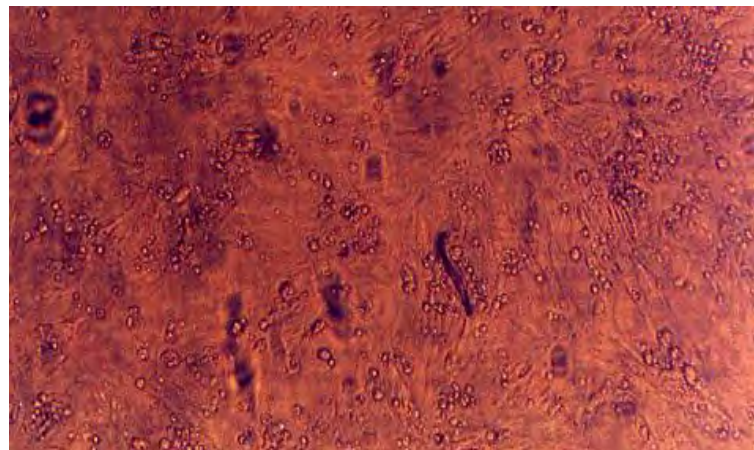
- IH (+) 1:16
- SN
- ELISA



Hemoaglutinación

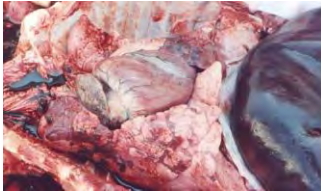


Células Vero



PATOGENIA

VIRUS



DESCARGAS
NASALES



INSPIRACIÓN



PULMONES



VIA SISTÉMICA



NASOFARINGE



TERMINACIONES
NERVIOSAS



LÓBULOS
OLFATORIOS



SNC



(Collier , 2000; Lorio, 1986; Bowden, 2001; Chua, 2000)

México

Enfermedad Endémica



Model 310
Version 3.4.1
ABI-CE1
Version 3.3.1

HNF3-6Sample19
Veterinaria (Ivan)
HNF3-6
Lane 19

Signal G:340 A:326 T:219 C:122
DT POP6(BD Set-Any Primer)
BD Matrix
Points 1215 to 10200 Pk 1 Loc: 1215

Page 1 of 2
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dom., 8 juni 2003 16:09
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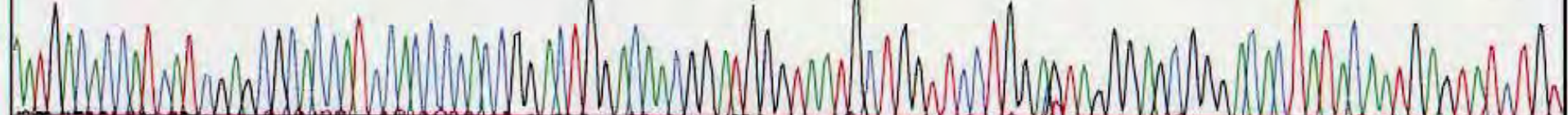
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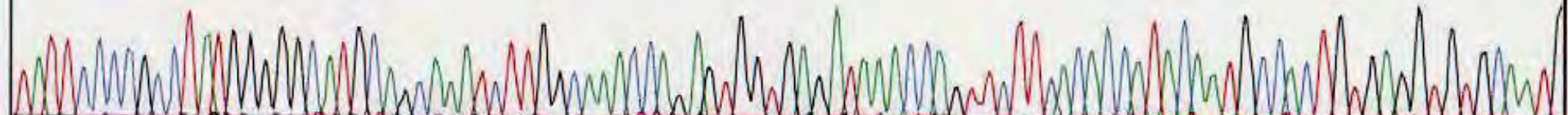
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240 250 260 270 280 290 300 310 320 330



G G T C A G C C T C A A T T T T C C C A A C G A G C A G C T G C C T C C T A T T A C C C C G T T A C T T T T C A A A C A G A T G G A T T C G C T C T G C A A T T G T T G C C T G C C C T T A C C G A C

340 350 360 370 380 390 400 410 420 430



G C T A T T T A T C A G A C T C A G T G C A C C T T G A T T C C C C T C C C G A A T A G A A T G T A A T G A T G G G A A G T G A A G G G C G A A T C T T C A C A C T T G G T G A C C G C T T G T T T T

440 450 460 470 480 490 500 510 520 530



Genetic and antigenic changes in porcine rubulavirus

José I. Sánchez-Betancourt, María E. Trujillo, Susana E. Mendoza, Julio Reyes-Leyva, Rogelio A. Alonso

Abstract

Blue eye disease, caused by a porcine rubulavirus (PoRV), is an emergent viral swine disease that has been endemic in Mexico since 1980. Atypical outbreaks were detected in 1990 and 2003. Growing and adult pigs presented neurological signs, mild neurological signs were observed in piglets, and severe reproductive problems were observed in adults. Amino acid sequence comparisons and phylogenetic analysis of the hemagglutinin-neuraminidase (HN) protein revealed genetically different lineages. We used cross-neutralization assays, with homologous and heterologous antisera, to determine the antigenic relatedness values for the PoRV isolates. We found antigenic changes among several strains and identified a highly divergent one, making up a new serogroup. It seems that genetically and antigenically different PoRV strains are circulating simultaneously in the swine population in the geographical region studied. The cross neutralization studies suggest that the HN is not the only antigenic determinant participating in the antigenic changes among the different PoRV strains.

Table 1. Amino acid substitutions present in the hemagglutinin-neuraminidase (HN) antigen of the porcine rubulavirus (PoRV) isolates in comparison to the LPM/1984 strain. The amino acid changes are indicated

Position	18	20	37	68	121	122	156	193	223	252	264	291	298	347	370	382	407	432	436	447	450	456	462	467	469	475	484	487	500	511	512	514	526	
LPM/1984	A	R	V	I	I	P	F	A	A	V	N	A	V	Q	S	Q	L	F	P	L	L	G	S	T	V	D	S	G	T	A	I	E	T	
PAC4/1993							L																											
PAC2/1990							L		T			D							L				N				I				I		T	
PAC3/1992							L		T			D							L				N				I				I		T	
CI/1991						R	L												L				N				I				I			
QI/1991							L												L	M			I	P			I				I			C
QII/1999				M			L								P				L		R		N				I				I			
CIV/1999	V				V		L							E			I	L	L				N		T	I	I	S	I					
PAC6/2001		K	I				L	T		I	T	I							L			E								I	S		K	
PAC7/2002		K	I				L	T		I	T	I							L			E								I	S		K	
PAC8/2002		K	I				L	T		I	T	I							L											I	S		K	
PAC9/2003			I				L	T		I	T	I			M				L											I	S		K	

Table II. Antigenic relatedness values among porcine rubulavirus (PoRV) strains

Virus strain	PAC2/1990	PAC3/1992	PAC4/1993	PAC6/2001	PAC7/2002	PAC8/2002	PAC9/2003
PAC2/1990	1	0.7	0.7	0.5	0.5	0.5	0.5
PAC3/1992		1	0.7	0.5	0.5	0.3	0.3
PAC4/1993			1	0.7	0.7	0.5	0.5
PAC6/2001				1	1	1	1
PAC7/2002					1	1	1
PAC8/2002						1	0.7
PAC9/2003							1

Molecular characterization of the hemagglutinin-neuraminidase gene of porcine rubulavirus isolates associated with neurological disorders in fattening and adult pigs

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Abstract

“Blue eye disease” is a viral infection of swine endemic in Mexico, which produces fatal encephalitis accompanied by respiratory signs and corneal opacity in suckling piglets. An atypical blue eye disease outbreak presented high rates of neurological signs in fattening and adult pigs from 2000 to 2003. In order to identify the basis of increased neurovirulence, the hemagglutinin-neuraminidase (HN) gene of several porcine rubulavirus isolates were sequenced and compared with that of La Piedad Michoacan virus and other isolates that did not produce neurological disorders in weaned pigs. Nine amino acid mutations distinguished the high neurovirulent PAC6–PAC9 viruses, whereas five mutations characterized the low neurovirulent PAC2 and PAC3 viruses. HN protein three-dimensional models showed that the main conformation and functional domains were preserved, although substitutions A₂₂₃T and A₂₉₁D occurred in PAC2 and PAC3 viruses, as well as A₅₁₁K and E₅₁₄K presented in PAC6–PAC9 viruses considerably modified the properties of the HN protein surface. The increased positive charge of the HN protein of PAC6–PAC9 viruses seems to be associated with their increased neurovirulence.

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Keywords: Porcine rubulaviruses; Hemagglutinin-neuraminidase; Sequencing; Protein structure; Structure–function analysis

Table 1
Neurovirulence of porcine rubulavirus isolated during several blue eye disease outbreaks

Virus name	Accession number	State	Year	Neurovirulence ^a			References
				Suckling	Fattening	Adults	
LPMV ^b	S77541	Michoacan	1984	20–30%	–	–	Moreno-López et al. (1986)
PAC2 ^c	EF413172	Jalisco	1990	<10%	–	–	Reyes-Leyva et al. (2002)
PAC3 ^c	EF413173	Jalisco	1992	<10%	–	–	Ramírez-Mendoza et al. (1999)
PAC4 ^d	EF413174	Michoacan	1993	>30%	ND	–	Reyes-Leyva et al. (2002)
CI ^b	AY463798	Michoacan	1991	>20%	–	–	Paniagua-Buelnas (2000)
CII ^b	AY487249	Michoacan	1991	>20%	–	–	Paniagua-Buelnas (2000)
CIII ^b	AY487251	Michoacan	1999	>20%	–	–	Paniagua-Buelnas (2000)
CIV ^b	AY487250	Michoacan	1999	>20%	–	–	Paniagua-Buelnas (2000)
PAC6 ^e	EF413175	Jalisco	2001	10%	20%	–	This study
PAC7 ^e	EF413176	Jalisco	2002	10%	20%	–	This study
PAC8 ^e	EF413177	Jalisco	2002	10–20%	20%	–	This study
PAC9 ^f	EF413178	Jalisco	2003	10–15%	ND	10%	This study

^a Indicates the percentage of neurological signs in infected pigs.

^b Neurological signs were just observed in suckling pigs.

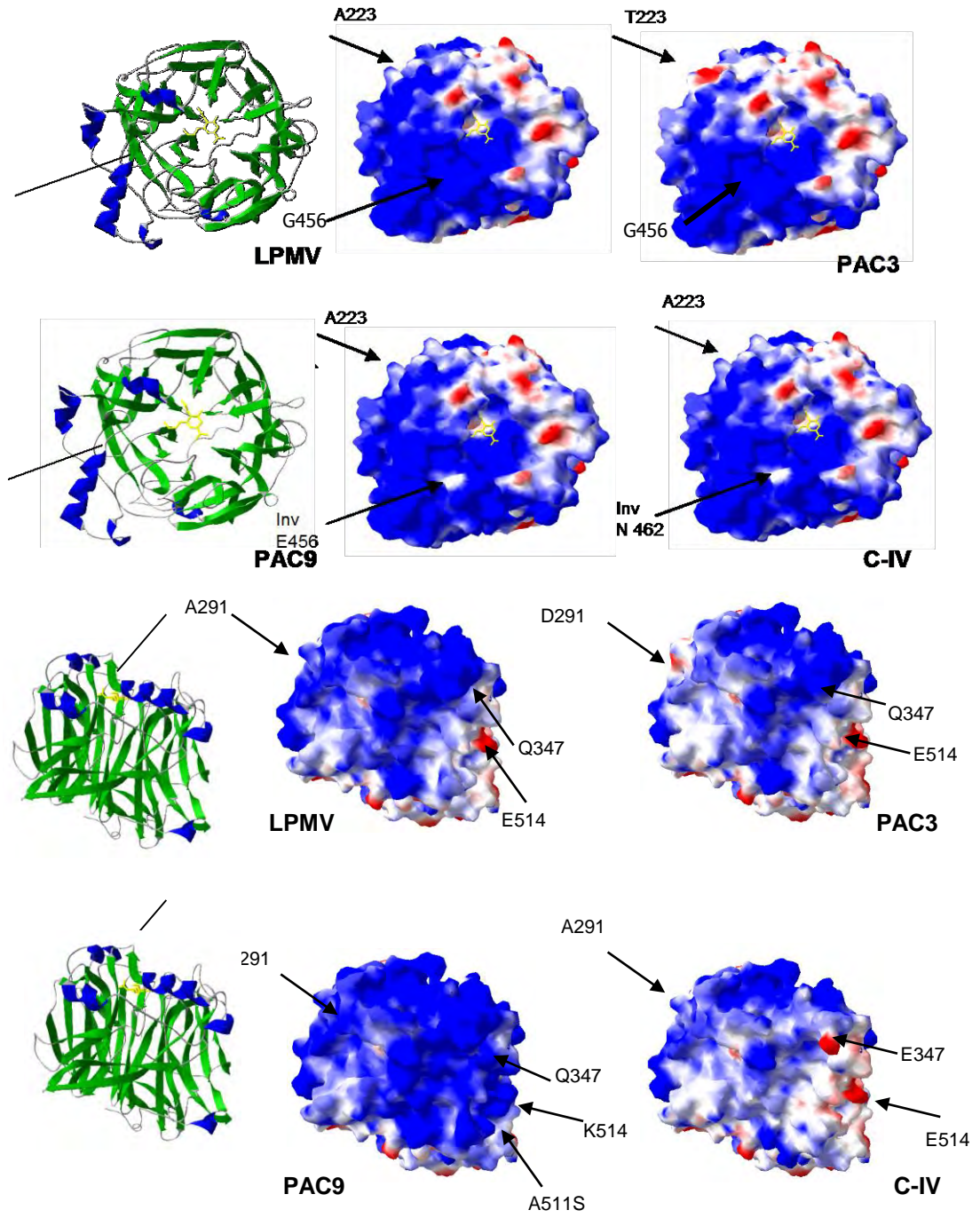
^c These viruses produced low neurovirulence in piglets.

^d Neurological signs were occasionally observed in weaned pigs.

^e These viruses produced neurovirulence in suckling and fattening pigs.

^f This outbreak occurred in a farrowing farm, thus there was not available data in fattening pigs. Means neurological signs were not observed. ND not determined.

Potencial Electroestático de la proteína HN



ORIGINAL ARTICLE

Identification of Antigenic Variants of the Porcine Rubulavirus in Sera of Field Swine and their Seroprevalence

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Keywords:

antigenic variation; blue eye disease; porcine rubulavirus

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Summary

We sampled sera from 1013 non-vaccinated swine from four states in Mexico, Guanajuato, Jalisco, Michoacán and the Estado de Mexico, to analyse anti-porcine rubulavirus antibody titres against three different porcine rubulavirus isolates (PAC-4/1993, PAC-6/2001, and PAC-9/2003) using a hemagglutination inhibition assay. The results revealed that there were antigenic differences among the isolates assessed. In particular, the estimated correlation between the PAC-4/1993 and PAC-6/2001 (0.50) isolates and between the PAC-4/1993 and PAC-9/2003 isolates (0.56) displayed a moderate positive correlation. In contrast, there was a strong positive correlation between the PAC-6/2001 and PAC-9/2003 isolates (0.73). We also found that in the state of Guanajuato, PAC-4/1993 was the isolate that was most frequently identified; in Jalisco, the isolate was PAC-6/2001; and in Michoacán, the isolate was PAC-9/2003. By contrast, in the Estado de Mexico, all three isolates appeared to circulate with a low seroprevalence. In general, the analysed sera from the four states displayed a porcine rubulavirus serological prevalence ranging from 9% to 23.7%. These data indicate that there is not complete antibody cross-antigenicity among the three isolates, and the antigenic variations in the antibody response found in this study implies that the use of a monovalent vaccine would not generate complete protection against the different antigenic subtypes.

Table 1. Average antibody titer for the PAC-4/1993, PAC-6/2001, and PAC-9/2003 isolates in the sera of swine from four federal states in Mexico

Isolate site	PAC-4/1993		PAC-6/2001		PAC-9/2003	
	Mean*	SEM	Mean	SEM	Mean	SEM
Edo. Mex.	4.72 ^a	0.13	4.63 ^a	0.11	4.56 ^a	0.11
Guanajuato	5.41 ^b	0.13	4.54 ^{a,b}	0.11	4.46 ^{a,b}	0.11
Jalisco	5.20 ^b	0.11	6.46 ^c	0.09	5.71 ^c	0.09
Michoacán	5.0 ^{a,b}	0.13	5.21 ^d	0.11	5.46 ^c	0.11

^{a,b,c,d}Mean values per column with different letters are significantly different ($P < 0.05$). *, Values expressed as Log_2 ; SEM, standard error of the mean; Edo. Mex., Estado de Mexico.

Table 2. Prevalence of the PAC-4/1993, PAC-6/2001 and PAC-9/2003 isolates

Site	Number of samples	PAC-4 (% prevalence)	PAC-6 (% prevalence)	PAC-9 (% prevalence)
Edo. Mex.	222	23 (10.3)	19 (8.5)	20 (9)
Guanajuato	232	55 (23.7)	21 (9)	15 (6.4)
Jalisco	320	53 (16.5)	117 (36.5)	78 (24.3)
Michoacán	239	32 (13.9)	32 (13.9)	48 (20.9)

Edo. Mex., Estado de Mexico.

Table 3. Sera samples positive for two or three different viral isolates

Site Positive PAC-6/PAC-4 (%)	Positive PAC-9/PAC-4 (%)	Positive PAC-9/PAC-6 (%)	Positive PAC-4/PAC-6/PAC-9/total (%)
Edo. Mex. 6/23 (26.1)	6/23 (26.1)	3/19 (15.8)	5/62 (8.1)
Guanajuato 7/55 (12.7)	3/55 (5.5)	0/21 (0)	12/91 (13.1)
Jalisco 8/53 (15.1)	0/53 (0)	31/117 (26.5)	38/248 (15.3)
Michoacán 1/32 (3.1)	7/32 (21.8)	13/32 (40.6)	13/112 (11.6)

Efficacy of quantitative RT-PCR for detection of the nucleoprotein gene from different porcine rubulavirus strains

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Table 2 Quantification by qRT-PCR and virus titration of various porcine rubulavirus strains

Strain	HA titration TCID ₅₀ /mL	CPE titration TCID ₅₀ /mL	IIF titration TCID ₅₀ /mL	RT-PCR quantification relative TCID ₅₀ /mL (RNA copies/mL)
PPMV	0	7.73	5.79	9.14 (10.18)
LPMV	2.80	3.80	6.37	10.27 (11.58)
PAC-1	3.30	7.06	6.80	10.06 (11.32)
PAC-2	0	9.06	5.04	9.89 (11.11)
PAC-3	0	7.53	5.96	10.37 (11.70)
PAC-4	5.30	8.30	9.80	10.08 (11.57)
PAC-5	2.80	4.80	8.30	10.23 (11.53)
PAC-6	2.80	4.80	8.27	9.90 (11.12)
PAC-9	0	7.53	9.06	9.47 (11.82)
Mean ^a	1.88*	6.73**	7.26**	9.94*** (11.32)
SEM	0.64	0.60	0.54	0.13

SEM, standard error of the mean; HA, hemagglutination; TCID₅₀, 50 % tissue culture infectious dose; CPE, cytopathic effect; IIF, indirect

CONCLUSIONES

- ✓ Se demuestran 2 diferentes linajes, dentro de los cuales hay diferentes “clusters”.
- ✓ Existen cambios genéticos que modifican la antigenicidad de los virus.
- ✓ Se deben realizar monitoreos constantes de las cepas que circulan actualmente en México y considerar la generación de vacunas multivalentes con amplio rango de protección.

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