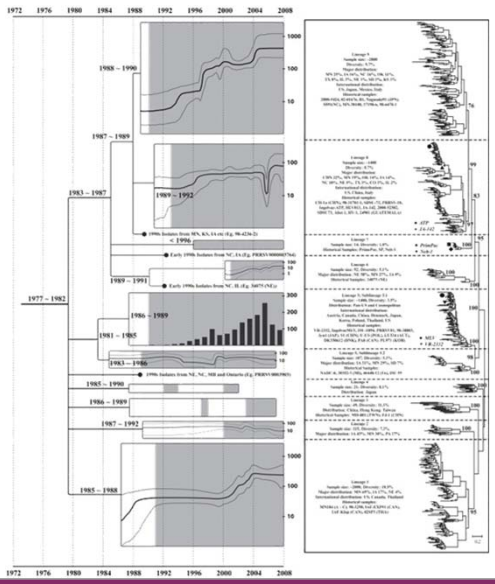
Cepas de alta virulencia del PRRSV: ¿cuándo han aparecido y qué sabemos de ellas?

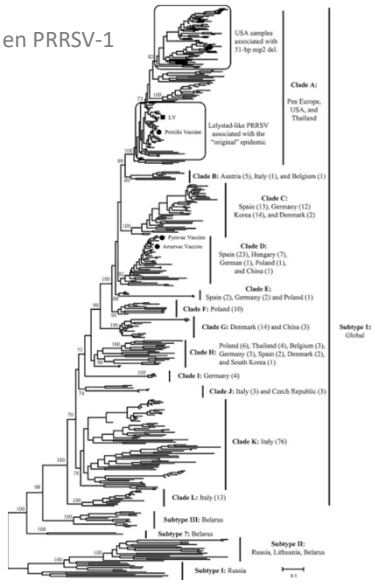
CINTA PRIETO
DPTO. DE SANIDAD ANIMAL
FACULTAD DE VETERINARIA
UNIVERSIDAD COMPLUTENSE DE MADRID

Variabilidad del PRRSV

9 linajes en PRRSV-2



Hasta 4 subtipos en PRRSV-1



Shi *et al.*, 2010, Virus Res., 154: 7-17

Variabilidad del PRRSV

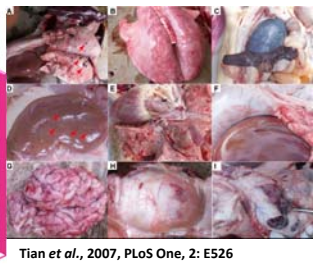
Consecuencias de la variabilidad genómica

- **Variabilidad biológica** (crecimiento *in vitro e in vivo*)
- **Variabilidad antigénica** (reactividad con anticuerpos poli- y monoclonales)
- **Variabilidad patogénica** (Diferencias en virulencia)

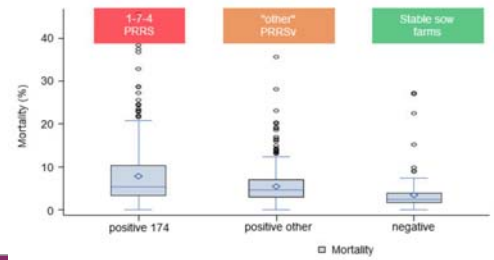
Diferencias en virulencia: cepas de alta virulencia

PRRSV-2

- 1996 • *PRRS atípico* (Estados Unidos)
- 2001 • Cepas 1-8-4 (Estados Unidos)
- 2007 • *Porcine High Fever Syndrome* (China)
- 2013 • Cepas 1-7-4 (Estados Unidos)
- 2020 • Cepas 1-4-4 (Estados Unidos)



Tian *et al.*, 2007, PLoS One, 2: E526



https://www.pig333.com/articles/assessing-impact-of-174-prrsv-on-wean-to-finish-pig-health_11239/

New PRRS 1-4-4 L1C variant presents dramatic symptoms, quick spread

by Pig Health Today
31 March 2021, at 9:12am

Nearly everyone in pork production is familiar with the clinical signs of porcine reproductive and respiratory syndrome (PRRS), but not everyone has experienced a new variant within the PRRS virus 1-4-4 lineage 1C (PRRSV 1-4-4 L1C). For herds in the upper Midwest that challenge surfaced late in 2020. Reports started with farms finding 10 or so sows off feed, and within a few days the tally would exceed 100 and continue to climb. About that same time, aborted litters would reach record numbers, and sow mortality rates spiked.

In the nursery and grow-finish phases, pigs would dramatically reduce water and feed intake, show signs of respiratory distress and mortality rates would climb beyond 25%, even in vaccinated pigs. As one client told Stephanie Rossow, DVM, swine disease pathologist at the University of Minnesota, "It's the worst PRRS I've seen in 33 years."

Diferencias en virulencia: cepas de alta virulencia

PRRSV-1

Subtipo 3

Lena (2007)

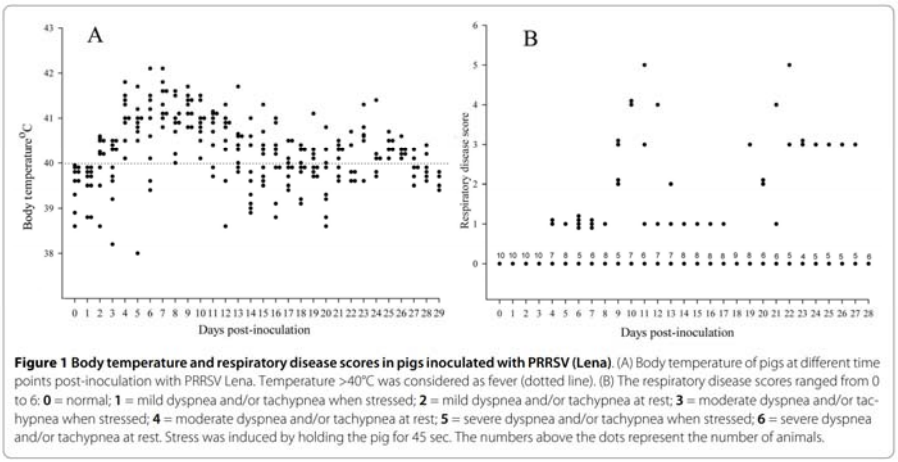


Figure 1 Body temperature and respiratory disease scores in pigs inoculated with PRRSV (Lena). (A) Body temperature of pigs at different time points post-inoculation with PRRSV Lena. Temperature >40°C was considered as fever (dotted line). (B) The respiratory disease scores ranged from 0 to 6: **0** = normal; **1** = mild dyspnea and/or tachypnea when stressed; **2** = mild dyspnea and/or tachypnea at rest; **3** = moderate dyspnea and/or tachypnea when stressed; **4** = moderate dyspnea and/or tachypnea at rest; **5** = severe dyspnea and/or tachypnea when stressed; **6** = severe dyspnea and/or tachypnea at rest. Stress was induced by holding the pig for 45 sec. The numbers above the dots represent the number of animals.

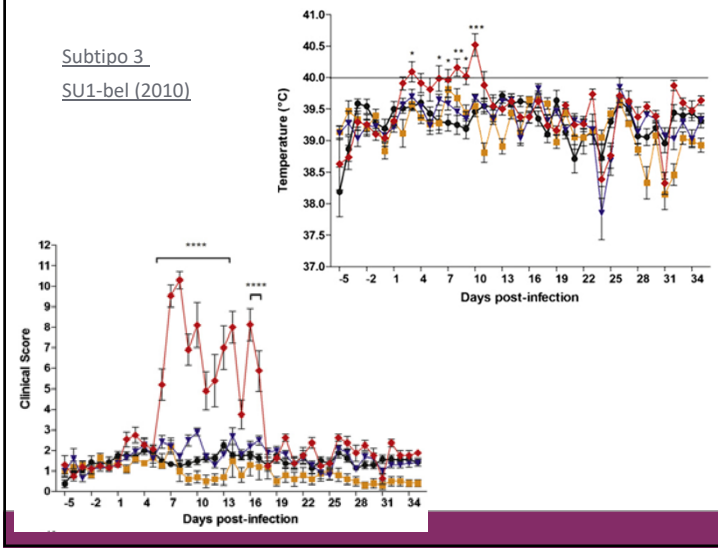
Karniychuk et al. / BMC Veterinary Research 6 (2010):30

Diferencias en virulencia: cepas de alta virulencia

PRRSV-1

Subtipo 3

SU1-bel (2010)



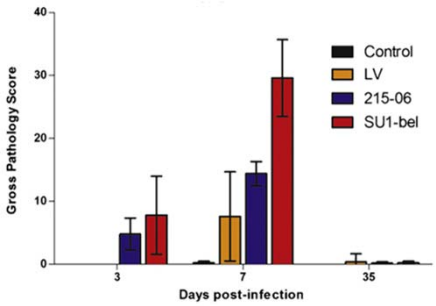


Fig. 1. Infection of pigs with PRRSV SU1-bel induced clinical disease and greater lung gross pathology. Animals infected with three strains of PRRSV, LV ( ), 215-06 ( ) and SU1-bel ( ), and mock-infected controls (*) had rectal temperatures (A) and clinical scores (B) monitored daily. Temperature above 408 was considered febrile. Animals were sacrificed at 3, 7 and 35 dpi when lungs were scored for gross pathology (C). Data shows the mean temperature and clinical score with error bars representing SEM for n = 20 (infected groups) and n = 16 (controls) between 5 and 3 dpi, n = 15 (infected groups) and n = 12 (controls) between 4 and 7 dpi (12 controls) and n = 10 (infected groups) and n = 8 (controls) between 8 and 35 dpi. Gross pathology data shows the mean gross pathology score with error bars representing SD for n = 5 (infected groups) and n = 4 (controls) at 3 and 7 dpi and n = 10 (LV and 215-06 groups) and n = 8 (SU1-bel and control groups) at 35 dpi. Significance is indicated by: ****p < 0.0001, ***p < 0.001, **p < 0.01 and *p < 0.05.

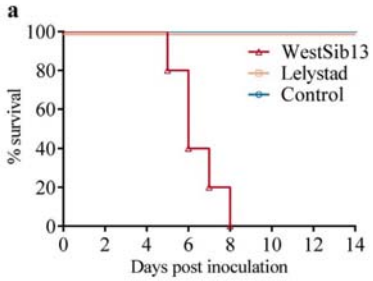
Morgan et al. / Veterinary Microbiology 163 (2013) 13–22

Diferencias en virulencia: cepas de alta virulencia

PRRSV-1

Subtipo 2

WestSib13 (2013)



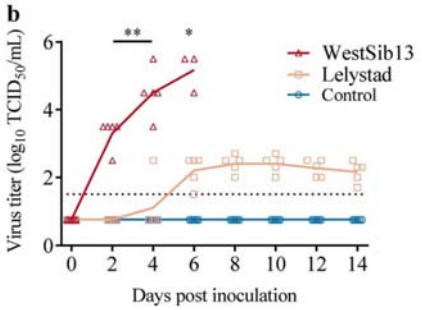


Fig. 1. Survival rate (a) and viremia (b) upon inoculation with the LV and WestSib13 strains.
Lines represent the mean titer in each group. The dotted line gives the detection limit for virus titration. Asterisks represent statistically significant differences with the LV group (*: $p < 0.05$, **: $p < 0.01$).

Yuzhakov *et al.* / Veterinary Microbiology 211 (2017) 22–28

Diferencias en virulencia: cepas de alta virulencia

PRRSV-1

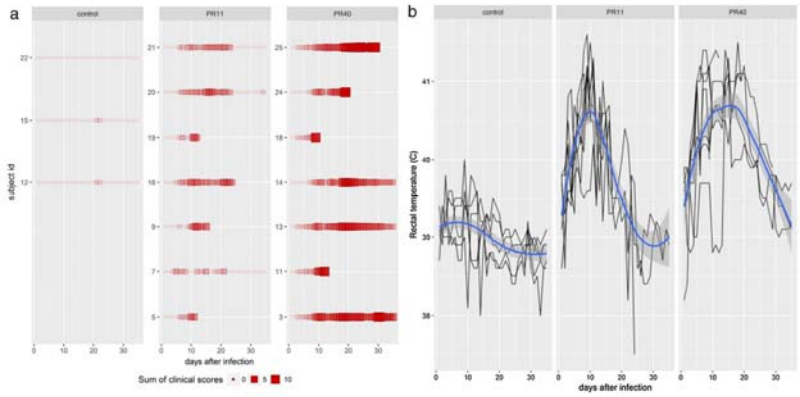
Subtipo 1

Cepa convencional (PR-11) vs. alta virulencia (PR-40)

- Flanders
- ACRO
- PR-40
- Rosalía

Signos clínicos

Fiebre



Canelli et al., *Vet. Microb. 210 (2017) 124–133*

Diferencias en virulencia: modelo respiratorio

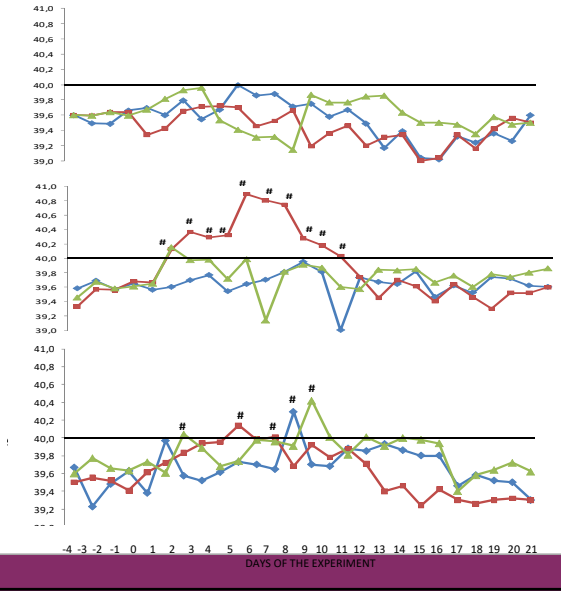
PRRSV-1

Subtipo 1

- Estudio comparativo de 26 cepas de PRRSV-1 de subtipo 1

Modelo respiratorio de la enfermedad

Signos clínicos: fiebre



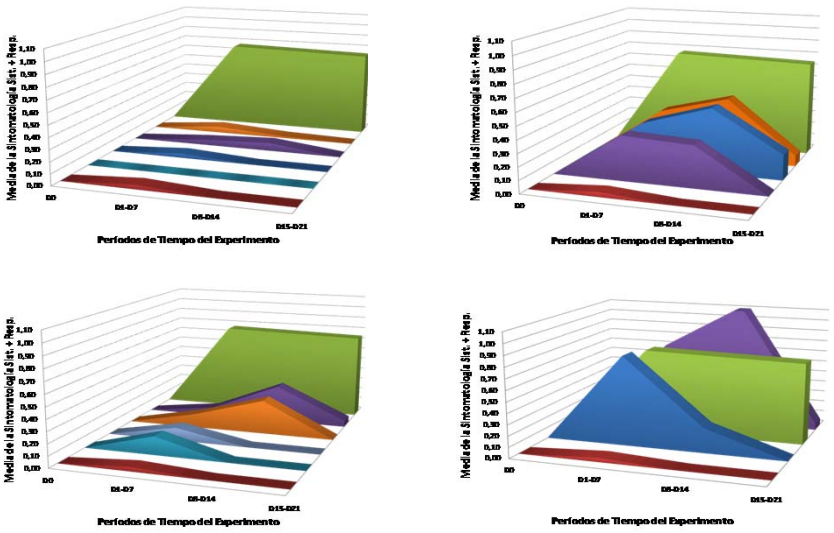
Martínez Lobo (2010) Tesis Doctoral.

Diferencias en virulencia: modelo respiratorio

PRRSV-1

Subtipo 1

Signos clínicos sistémicos y respiratorios



Martínez Lobo (2010) Tesis Doctoral.

Diferencias en virulencia: modelo respiratorio

PRRSV-1

Subtipo 1

Conclusiones del estudio → La virulencia es una variable continua

Martínez Lobo (2010) Tesis Doctoral.

Consecuencias de las diferencias en virulencia: capacidad de transmisión

Influencia del aislado en la frecuencia de excreción del PRRSV

Table II. Proportions of pigs shedding PRRSV by aerosol on each collection day after PRRSV inoculation

Agent(s) inoculated; animal age (mo)	Collection day; number of pigs shedding/number of pigs in the group											
	1	3	5	7	9	11	13	15	17	19	21	
PRRSV MN-30100												
2	0/5	1/5	2/5	1/5	0/5	0/5	0/5	1/5	0/5	1/5	0/5	6 positivos
6	0/4	1/4	4/4	2/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	8 positivos
PRRSV MN-30100 + *M. hyopneumoniae*												
2	0/4	0/4	0/4	1/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4	3 positivos
6	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	1 positivo
PRRSV MN-184												
2	0/6	0/6	3/6	0/6	1/6	1/6	2/6	2/6	1/6	1/6	3/6	14 positivos
6	0/5	3/5	2/5	0/5	0/5	1/5	1/5	1/4	1/4	1/4	0/4	10 positivos
PRRSV MN-184 + *M. hyopneumoniae*												
2	1/6	0/6	4/5	4/5	2/5	NC	4/5	2/5	5/5	NC	1/5	23 positivos
6	0/5	3/5	5/5	1/5	1/4	NC	0/4	0/4	1/4	0/4	0/4	11 positivos

NC — no collection because of severe respiratory illness

→ Mejor transmisión por vía aerógena (Cho *et al., Can J Vet Res. 2007 Jan;71(1):23-7)*

No se observan diferencias en la carga vírica en las secreciones

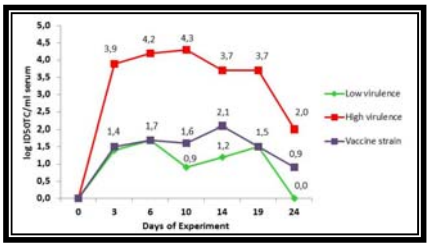
Cho et al. (2006) Can J Vet Res, 70:297–301

Consecuencias de las diferencias en virulencia: capacidad de transmisión

Grupos experimentales

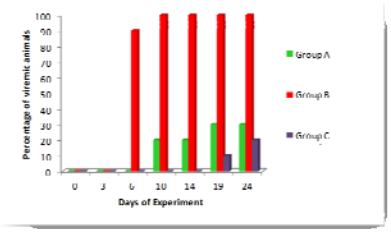
- Grupo A (aislado de baja virulencia)
- Grupo B (aislado de alta virulencia)
- Grupo C (cepa vacunal)

Viremia en los animales inoculados

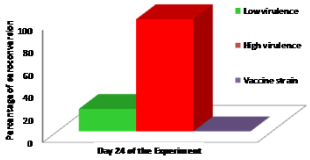


Tasa de transmisión

Viremia en los centinelas



Seroconversión en los centinelas



Aislados de alta virulencia >>> Aislado de baja virulencia ≅ Cepa vacunal

Consecuencias de las diferencias en virulencia: protección cruzada

Diferencias en protección cruzada entre cepas

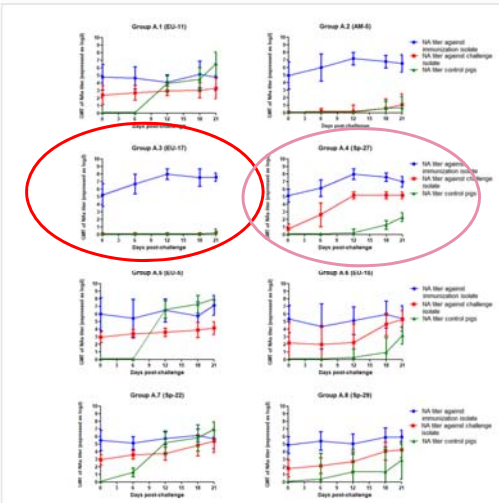


TABLE 3.1 | Virus isolation from serum samples collected in Experiment A (pigs immunized with Sp-3a).

Days post-challenge	Group A.1 (EU-11)		Group A.2 (AM-5)		Group A.3 (EU-17)		Group A.4 (Sp-27)	
	Immunized	Controls	Immunized	Controls	Immunized	Controls	Immunized	Controls
D0	0/5 (-)[a]	0/3 (-)	0/5 (-)	0/3 (-)	0/5 (-)	0/3 (-)	0/5 (-)	0/3 (-)
D3	0/5[a] (-)	3/3 (1.89 ± 0.54)	1/5 (1.66)	3/3 (1.67 ± 0.29)	4/5 (1.70 ± 0.09)[b]	3/3 (3.29 ± 0.06)	4/5 (1.67 ± 0.24)	3/3 (2.28 ± 0.63)
D6	0/5[a] (-)	3/3 (2.11 ± 0.54)	0/5[a] (-)	3/3 (1.67 ± 0.29)	1/5 (3.23)	2/2[c] (3.05 ± 0.55)	2/5 (2.00 ± 0.71)	3/3 (3.05 ± 0.42)
D9	0/5[a] (-)	3/3 (1.55 ± 0.00)	0/5[a] (-)	3/3 (1.55 ± 0.00)	0/5[a] (-)	3/3 (2.70 ± 0.67)	0/5[a] (-)	3/3 (2.72 ± 0.68)
D12	0/5[a] (-)	3/3 (1.50 ± 0.00)	0/5[a] (-)	3/3 (1.83 ± 0.58)	1/5 (1.66)	3/3 (1.89 ± 0.54	0/5[a] (-)	3/3 (2.28 ± 0.25
D15	0/5[a] (-)	3/3 (1.50 ± 0.00)	0/5[a] (-)	3/3 (2.00 ± 0.50)	1/5 (1.50)	3/3 (2.27 ± 1.20)	0/5[a] (-)	3/3 (2.22 ± 0.48)
D18	0/5[a] (-)	3/3 (1.55 ± 0.09)	0/5[a] (-)	3/3 (1.55 ± 0.09)	1/5 (1.66)	3/3 (2.72 ± 0.85)	0/5[a] (-)	3/3 (1.67 ± 0.29)
D21	0/5[a] (-)	3/3 (1.50 ± 0.00)	1/5 (1.50)	3/3 (1.78 ± 0.48)	1/5 (1.66)	3/3 (1.80 ± 0.38)	0/5[a] (-)	3/3 (1.67 ± 0.29)

[a]Frequency of isolation of PRRSV from serum samples. Mean titer and standard deviation are indicated in parentheses.
[b]Indicates statistically significant differences in the frequency of isolation between the immunized and control pigs.
[c]Indicates statistically significant differences in the viral titers in serum samples between the immunized and control pigs.
[d]The serum sample of one of the control pigs was not available on day 6 post-challenge.

TABLE 3.2 | Virus isolation from serum samples collected in Experiment A (pigs immunized with Sp-3a).

Days post-challenge	Group A.5. (EU-5)		Group A.6. (EU-15)		Group A.7. (Sp-22)		Group A.8. (Sp-29)	
	Immunized	Controls	Immunized	Controls	Immunized	Controls	Immunized	Controls
D0	0/5 (-)[a]	0/3 (-)	0/5 (-)	0/3 (-)	0/5 (-)	0/3 (-)	0/5 (-)	0/3 (-)
D3	0/5[a] (-)	3/3 (2.33 ± 0.33)	0/5[a] (-)	3/3 (2.24 ± 0.37)	0/5[a] (-)	3/3 (3.72 ± 0.26)	0/5[a] (-)	3/3 (3.39 ± 0.79)
D6	0/5[a] (-)	3/3 (1.83 ± 0.44)	0/5[a] (-)	3/3 (2.78 ± 0.63)	0/5[a] (-)	3/3 (2.22 ± 0.38)	0/5[a] (-)	3/3 (2.61 ± 0.09)
D9	0/5[a] (-)	3/3 (2.22 ± 0.48)	0/5[a] (-)	3/3 (1.78 ± 0.48)	0/5[a] (-)	3/3 (2.16 ± 0.44)	0/5[a] (-)	3/3 (2.22 ± 0.35)
D12	0/5[a] (-)	3/3 (1.67 ± 0.29)	0/5[a] (-)	3/3 (2.16 ± 0.01)	0/5[a] (-)	3/3 (1.50 ± 0.00)	0/5[a] (-)	3/3 (2.00 ± 0.50)
D15	0/5[a] (-)	3/3 (1.77 ± 0.20)	0/5[a] (-)	3/3 (1.83 ± 0.29)	0/5[a] (-)	3/3 (1.50 ± 0.00)	0/5[a] (-)	2/3 (2.00 ± 0.71)
D18	0/5[a] (-)	3/3 (1.94 ± 0.63)	0/5[a] (-)	3/3 (1.55 ± 0.09)	0/5[a] (-)	3/3 (1.55 ± 0.09)	0/5[a] (-)	3/3 (1.83 ± 0.29)
D21	0/5[a] (-)	3/3 (1.50 ± 0.00)	0/5[a] (-)	3/3 (1.50 ± 0.00)	0/5[a] (-)	3/3 (1.74 ± 0.42)	0/5[a] (-)	3/3 (1.83 ± 0.58)

[a]Frequency of isolation of PRRSV from serum samples. Mean titer and standard deviation are indicated in parentheses.
[b]Indicates statistically significant differences in the frequency of isolation between the immunized and control pigs.

FIGURE 2 | GMT of NAs against the immunization and challenge PRRSV isolates in immunized pigs and against challenge PRRSV isolate in control pigs groups included in the study from the day of challenge to the end of the experiment in Experiment A. The graph illustrates the GMT ± standard deviation.

Martínez-Lobo et al. / Front Immunol. 2021 Jul 26;12:691145.

Diferencias en virulencia: posibles causas

Correlación entre gravedad de los signos clínicos y la intensidad de la viremia

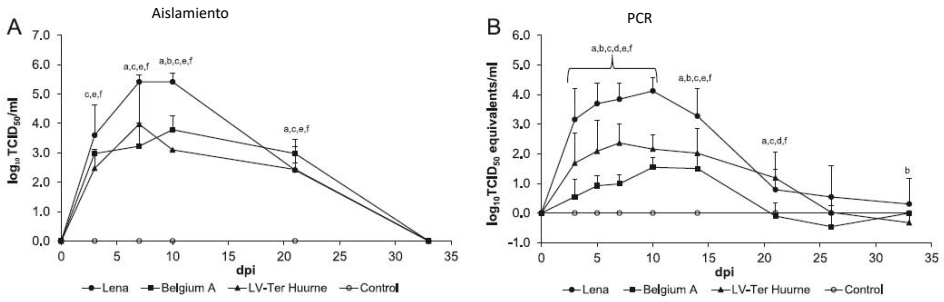


Fig. 2. PRRSV levels in serum of pigs infected with different strains of PRRSV. (A) Infectious PRSV isolated from PRRSV inoculated pigs not immunized with an inactivated Aujeszky's disease (ADV) vaccine. Each data point represents the average of three or four pigs ±S.D. (B) PRRSV RNA levels in serum as determined by qRT-PCR. Data points from d.p.i. 0 to 3 represents the average of sixteen pigs ±S.D., from d.p.i. 4 to 7 of twelve pigs and from d.p.i. 8 to 33 of seven or eight pigs. "a" denotes a significant difference ($p < 0.05$) between Lena and Belgium A-infected pigs; "b" between Lena and LV-infected pigs; "c" between Lena and control pigs; "d" between Belgium A and LV-infected pigs; "e" between Belgium A and control pigs; and "f" between LV and control pigs.

Weesendorp et al. / Veterinary Microbiology 163 (2013) 1–12

Diferencias en virulencia: posibles causas

Capacidad de replicación en un rango más amplio de tipos celulares

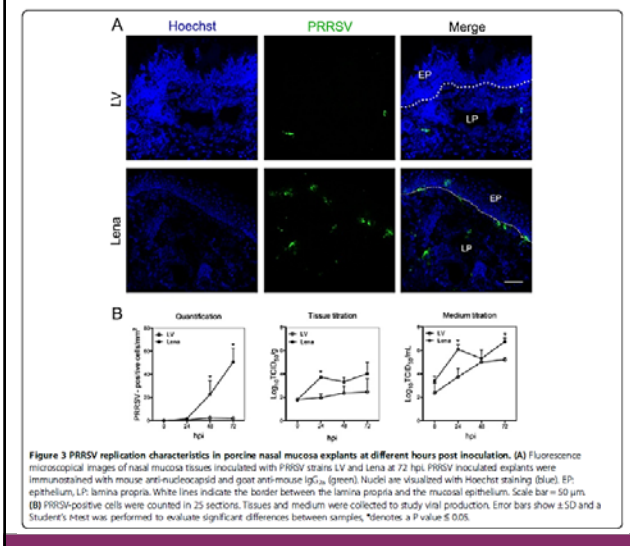


Figure 3 PRRSV replication characteristics in porcine nasal mucosa explants at different hours post inoculation. (A) Fluorescence microscopical images of nasal mucosa tissues inoculated with PRRSV strains LV and Lena at 72 hpi. PRRSV inoculated explants were immunostained with mouse anti-nucleocapsid and goat anti-mouse IgG_{2a} (green). Nuclei are visualized with Hoechst staining (blue). EP: epithelium, LP: lamina propria. White lines indicate the border between the lamina propria and the mucosal epithelium. Scale bar = 50 μm. **(B)** PRRSV-positive cells were counted in 25 sections. Tissues and medium were collected to study viral production. Error bars show ±SD and a Student's *t*-test was performed to evaluate significant differences between samples, *denotes a P value ≤ 0.05.

- LV: replicación en células CD163+ Sn+
- Lena replicación en células CD163+ Sn+, CD163+ Sn-

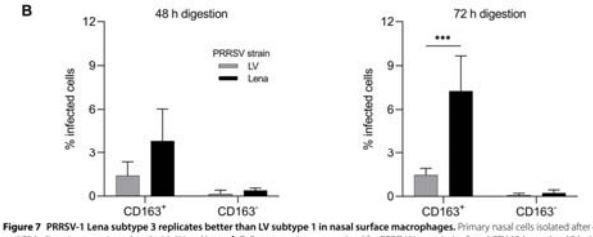
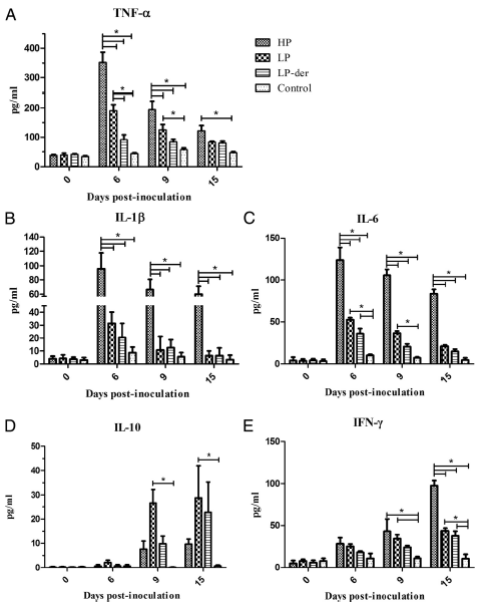


Figure 7 PRRSV-1 Lena subtype 3 replicates better than LV subtype 1 in nasal surface macrophages. Primary nasal cells isolated after 48 h and 72 h digestion were inoculated with LV and Lena. **A** Cells were co-immunostained for PRRSV N-protein (red) and CD163 (green) at 12 hpi. Scale bar: 25 μm. Small boxes in the IF pictures represent zoomed pictures of the infected CD163+ cells indicated by arrows. **B** Identification and quantification of PRRSV-1 LV and Lena-infected cells. Statistical significance was determined by two-way ANOVA followed by Tukey's post hoc test (***$p < 0.001$). All data are expressed as mean value of three experiments ± SD. All inoculated cells are from the same group used for cell characterization (Figure 5).

Frydas et al. Veterinary Research 2013, 44:73 Oh et al. Vet Res (2020) 51:21

Diferencias en virulencia: posibles causas



Correlación entre virulencia e inducción de citoquinas proinflamatorias

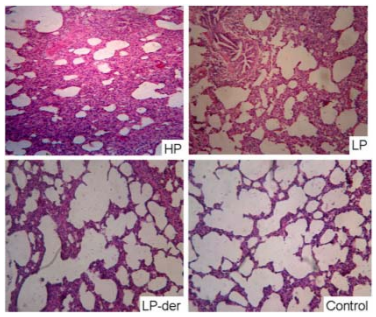


Figure 4 Serum levels of porcine TNF-α (A), IL-1β (B), IL-6 (C), IL-10 (D), and IFN-γ (E) were measured with commercial ELISA kits. Data are represented as mean (± SEM) generated from 6 pigs in all groups at 0 to 15 d.p.i. and 4 pigs in group HP at 9 to 15 d.p.i. (*) indicates a significant difference ($p < 0.05$) between groups.

Zhang et al. Virology Journal 2013, 10:135

Determinantes genéticos de virulencia

Factores de virulencia no identificados

Posible implicación de las proteínas no estructurales

Posible implicación de las proteínas estructurales

Determinantes genéticos de virulencia

Deleciones en nsp2 y virulencia

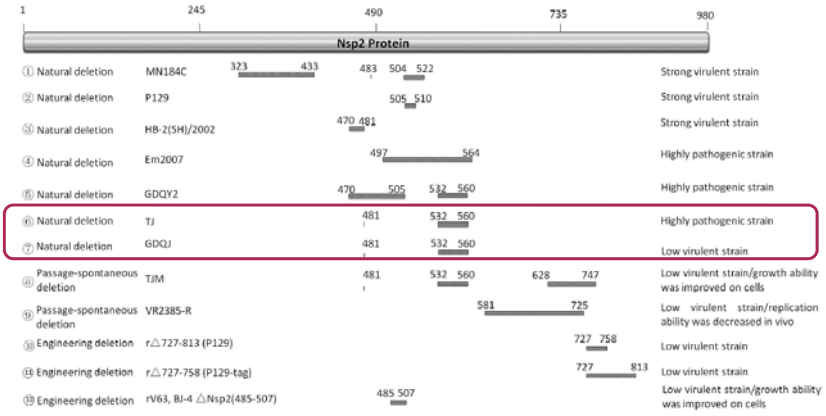


Fig. 1. Deletions to the Nsp2 proteins of several NA genotype PRRSV strains. The Nsp2 of PRRSV VR-2332 is 980 amino acids in length. The deletions to the Nsp2 gene of several typical strains are indicated by black boxes. The first seven strains are naturally deleted isolates, the next two are spontaneous passage deletions, and last three are engineering deletions

Wang et al. / Research in Veterinary Science 95 (2013) 1–7

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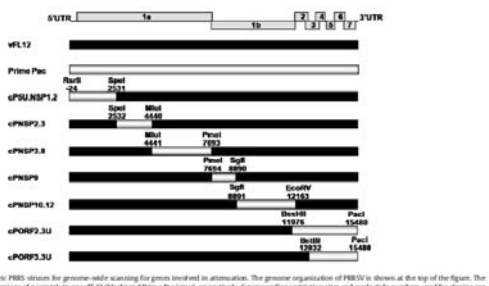
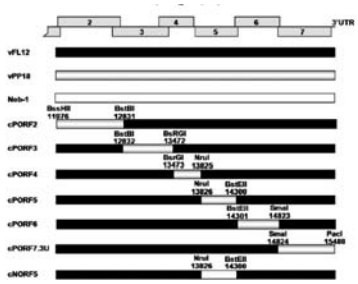
Construcción de quimeras





Proteínas no estructurales

Proteínas estructurales

Kwon et al. (2008). Virology 380: 371–378

Determinantes genéticos de virulencia

Construcción de quimeras

Papel nsp3-8/nsp2

Table 3
Viability scores of offsprings born from sows infected with parental or chimeric viruses – primary genome-wide scanning[a]

Viruses	Genetic background	Sow no.[a]	Viability at Birth Dead	Birth Live	15 days of age Live	Survival[b] (%)
vFL12	FL12	1	12	3	0	0
		2	13	1	0	
Prime Pac	Prime Pac	1	0	11	9	77*
		2	0	14	10	
cP5UNSP12	FL12	1	1	12	9	51*
		2	6	6	4	
cPNSP2.3	FL12	1	4	12	9	56*
		2	1	15	9	
cPNSP3.8	FL12	1	0	15	12	75*
		2	3	10	9	
cPNSP9	FL12	1	13	3	0	3
		2	9	11	1	
cPNSP10.12	FL12	1	5	11	10	56*
		2	5	5	5	
cPORF2.3U	FL12	1	0	15	9	69*
		2	2	7	7	
cPORF3.3U	FL12	1	0	10	10	71*
		2	3	9	5	

[a] Two sows each per group. Arbitrary numbers.
[b] Least squares means of each group. The asterisk indicates that the means are significantly different ($P \leq 0.05$) than the mean of vFL12 group.

Papel GP5/GP2

Table 4
Viability scores of offsprings born from sows infected with chimeric viruses representing single structural genes[a]

Viruses	Genetic background	Sow no.[a]	Viability at Birth Dead	Birth Live	15 days of age Live	Survival[b] (%)
vFL12	FL12	1	12	3	0	0
		2	13	1	0	
cPORF2	FL12	1	9	6	4	24
		2	7	2	2	
cPORF3	FL12	1	15	0	0	7
		2	9	5	2	
cPORF4	FL12	1	9	1	0	3
		2	12	3	1	
cPORF5	FL12	1	7	7	4	44*
		2	3	7	6	
cPORF6	FL12	1	8	4	0	2
		2	11	4	2	
cPORF7.3U	FL12	1	13	4	1	6
		2	13	2	1	
cNORF5	FL12	1	10	4	2	7
		2	10	5	0	

[a] Two sows each per group. Arbitrary numbers.
[b] Least squares means of each group. The asterisk indicates that the means are significantly different ($P \leq 0.05$) than the mean of vFL12 group.

Kwon et al. (2008). Virology 380: 371–378

Determinantes genéticos de virulencia

Posible papel de nsp 9 y nsp 10

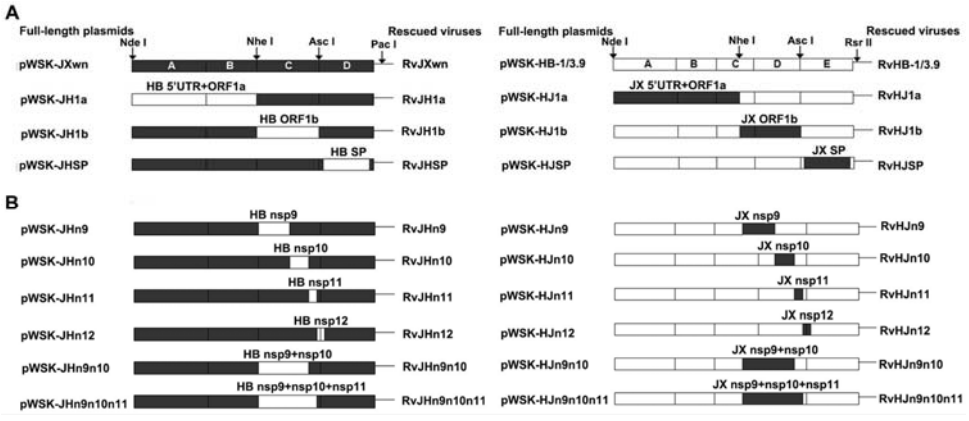
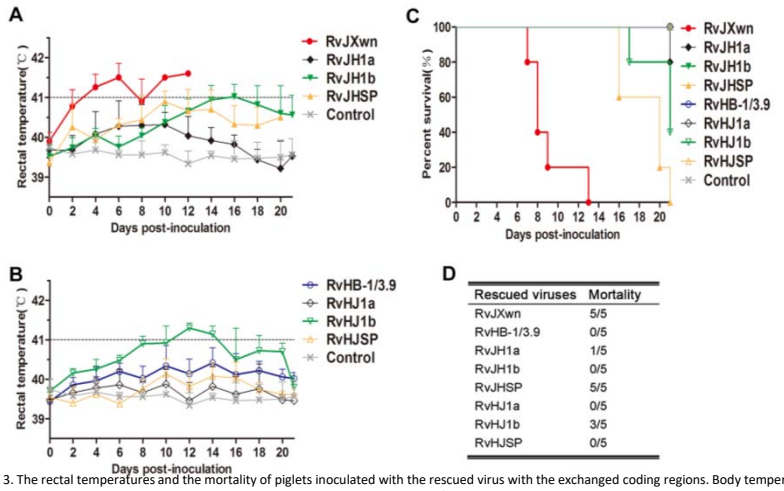


Figure 1. Construction strategy of the full-length cDNA clones. (A) Full-length infectious clones with exchanged 59UTR+ORF1a, ORF1b, and structural proteins (SPs)-coding regions. (B) Full-length infectious clones with exchanged nonstructural protein (Nsp)-coding region within ORF1b. The boxes represent the genomic fragments of parental backbone viruses RvJXwn (black) or RvHB-1/3.9 (white). Unique restriction enzyme sites used for cloning are shown above the bars. The designations of each full-length plasmid are shown on the left side and each rescued virus on the right side.

Li et al (2014) / PLoS Pathogens 10 (7): e1004216

Determinantes genéticos de virulencia

Posible papel de nsp 9 y nsp 10



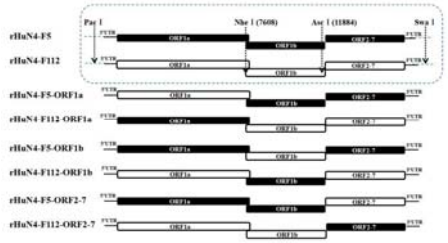
D

Rescued viruses	Mortality
RvJXwn	5/5
RvHB-1/3.9	0/5
RvJH1a	1/5
RvJH1b	0/5
RvJHSP	5/5
RvHJ1a	0/5
RvHJ1b	3/5
RvHJSP	0/5

Figure 3. The rectal temperatures and the mortality of piglets inoculated with the rescued virus with the exchanged coding regions. Body temperatures of piglets inoculated with rescued viruses with the RvJXwn backbone (A) and with the RvHB-1/3.9 backbone (B). Body temperatures are shown as the means 6 standard deviations (error bars), except the number of survival piglets in each group was less than two. The survival curves (C) and mortalities (D) of infected piglets in each group (n = 5) are shown.

Li et al (2014) / PLoS Pathogens 10 (7): e1004216

Determinantes genéticos de virulencia



Posible papel de distintos fragmentos del genoma

Quimeras entre cepa HP y su versión atenuada

e 1. Mean values (±SD) of macroscopic scores of lung tissues after infection.

Designation	Number	Macroscopic (Lung) Mean ± SD	Pathological Changes (Score) ≤30	30 to 50	≥50	
rHuN4-F5	5	65 ± 27.36	0	3	2	Virulenta
rHuN4-F5-ORF1a	5	30.8 ± 14.72	2	3	0	
rHuN4-F5-ORF1b	5	33.4 ± 17.08	2	3	0	
rHuN4-F5-ORF2-7	5	20 ± 15.03	5	0	0	
rHuN4-F112	5	17.6 ± 11.15	5	0	0	Atenuada
rHuN4-F112-ORF1a	5	36.8 ± 21.11	3	1	1	
rHuN4-F112-ORF1b	5	41.6 ± 19.76	3	1	1	
rHuN4-F112-ORF2-7	5	27.4 ± 14.19	4	0	1	
Control	5	15.2 ± 13.92	5	0	0	

Todos los cambios entre cepa virulenta y atenuada produce niveles de lesión intermedios

Jiang et al Viruses 2022, 14, 40

Resumen

- Las cepas del PRRSV difieren en virulencia tanto en el caso de PRRSV-1 como en el de PRRSV-2
- La virulencia se puede considerar una variable continua
- Las cepas de mayor virulencia parecen tener una mayor capacidad de transmisión
- Las cepas de mayor virulencia podrían tener mayor facilidad para eludir la respuesta inmunitaria previa
- La virulencia se ha asociado a una mayor capacidad de replicación, aunque también podría jugar un papel la capacidad para inducir citoquinas proinflamatorias
- Las bases genéticas de la virulencia no han podido ser determinadas con precisión hasta la fecha, aunque parece que podrían tener una naturaleza multigénica

¿Preguntas?