Instituto Nacional de Tecnología Agropecuaria
Centro de Investigación en Ciencias Veterinarias y Agronómicas
Instituto de Virología "Dr. Scholein Rivenson"

Swine Influenza in South and Central America

Ariel Pereda, DVM PhD
Institute of Virology - INTA

Swine Influenza
Technical Meeting
Minnesota, March 19th 2014

Last Technical Meeting (Rome, 2013)

Colombia (Universidad Nacional de Colombia)
Seroprevalence aprox. 50% Cauca Valley region and Antioquia
Isolates: pdm H1N1, probably cH1N1

Chile
Overall seropositivity of 48% to H1N1 strains and 22% to H3N2 strains
Producers have began vaccinating sows (during the gestation and maternity period)
Isolates: cH1N1, pdm H1N1, swH3N2

Guatemala
Overall seropositivity of 19% to NP Elisa
Isolates: pdm H1N1, huH3N2

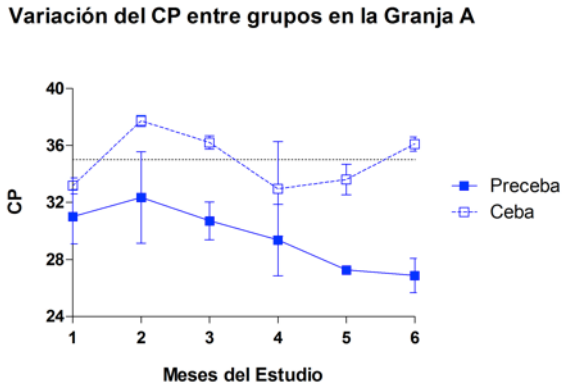
Argentina Viruses isolated till 2012
Differential seroprevalence between farms depending in the infection status
HIs: 90% pdmH1N1, 10% H3
Isolates: pdmH1N1, huH3N2 + pdm internal genes, δ2 H1N1 + pdm internal genes, δ1 H1N2 + pdm internal genes

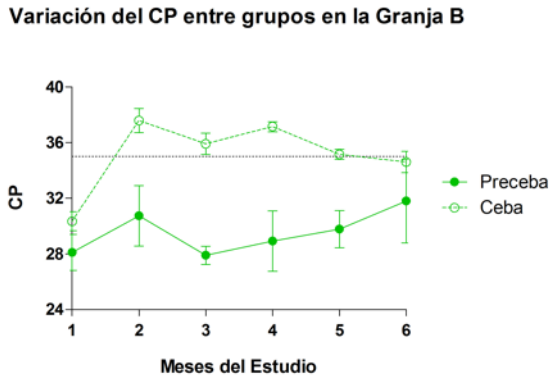
South and Central America
SIV Surveillance

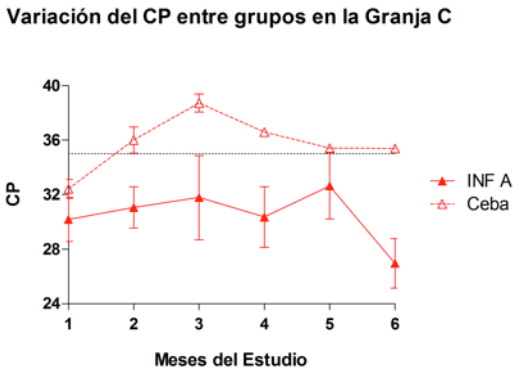
Colombia

- Started to implement Oral Fluid analysis
- SIV, PRRS and PCV2 by Real Time PCR

Post weaning vs fattening







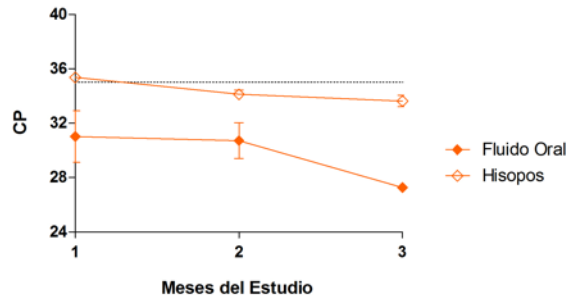
Post weaning vs fattening + Swabs vs OF

Post weaning

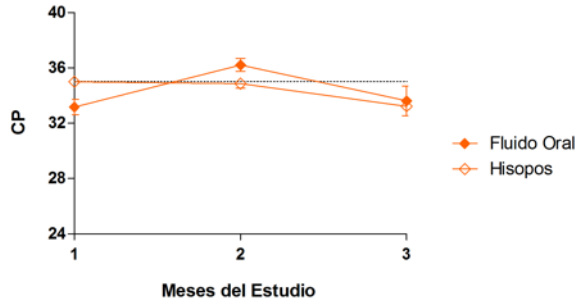
Fattening

Farm A

Variación del CP entre pruebas en la granja A en el grupo Preceba

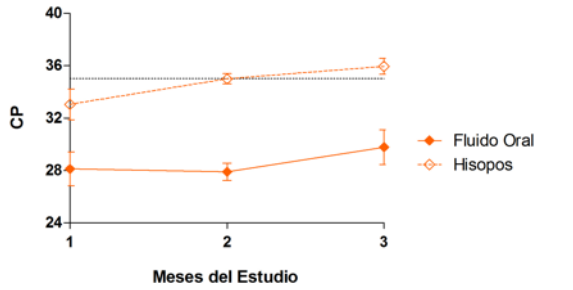


Variación del CP entre pruebas en la granja A en el grupo Ceba

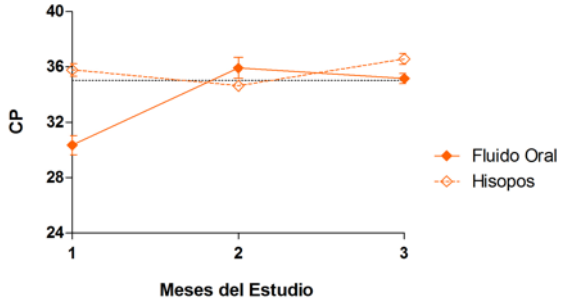


Farm B

Variación del CP entre pruebas en la granja B en el grupo Preceba

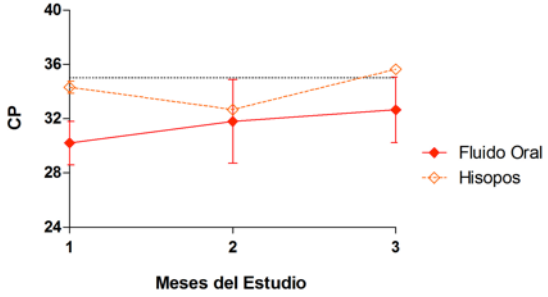


Variación del CP entre pruebas en la granja B en el grupo Ceba

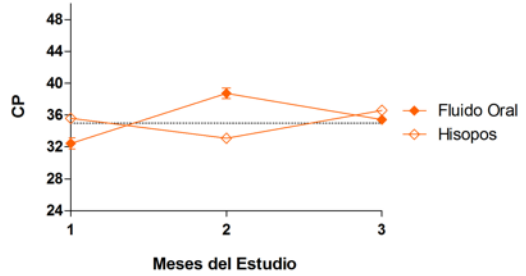


Farm C

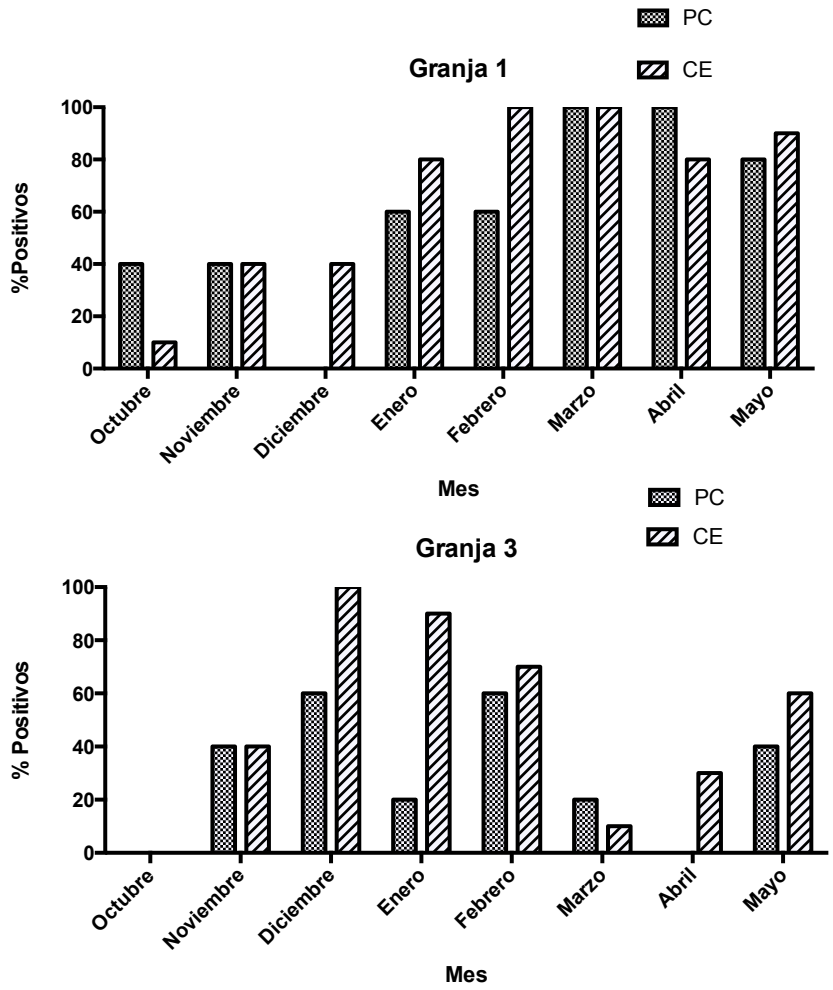
Variación del CP entre pruebas en la granja C en el grupo Preceba

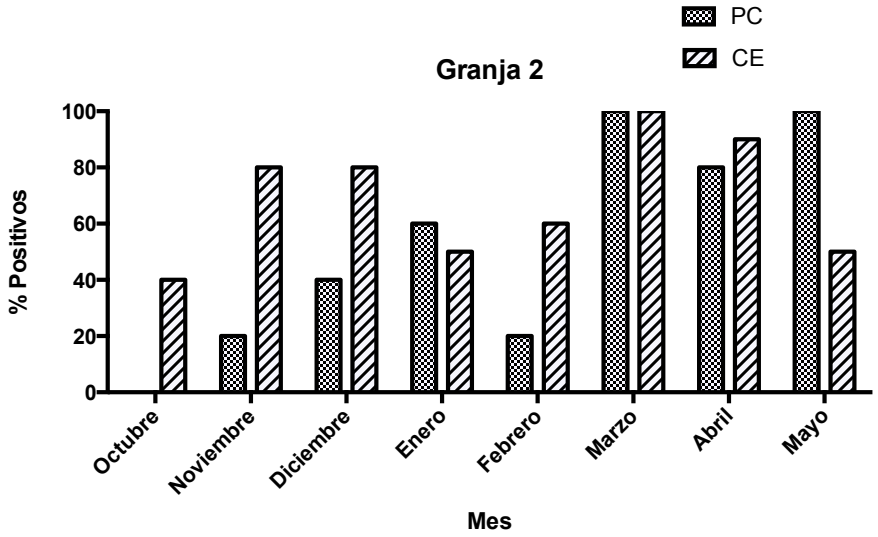


Variación del CP entre pruebas en la granja C en el grupo Ceba



Serology
Post weaning vs fattening + Seasonality





No evident seasonality

Influenza A Virus Antibody Test Kit (IDEXX®)

Chile

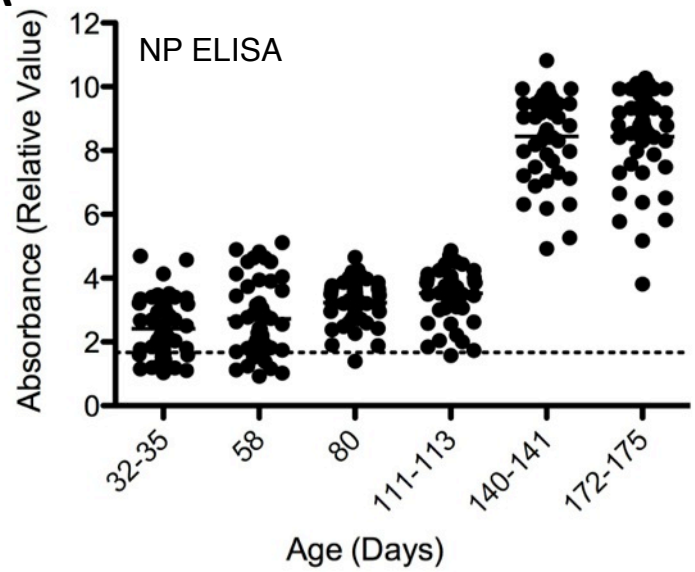
Circulation of swine influenza viruses in Chile

- Since 2010 four different strains of swine influenza viruses have been identified to be circulating in Chile: pdmH1N1-like, H1N1, H1N2 and H3N2.
- A serological study done in 2009 of 13 production sites revealed an overall seropositivity of 48% to H1N1 strains and 22% to H3N2 strains.*
- Recent studies by our group in collaboration with ASPROCER (Association of Swine Producers of Chile), have found swine farms with seropositivity to Influenza A virus ranging from 53% - 90.5%.
- High seroprevalence to influenza was detected in animals 100 days old and older, with the main susceptible/seronegative population identified in animals of 56-84 days.

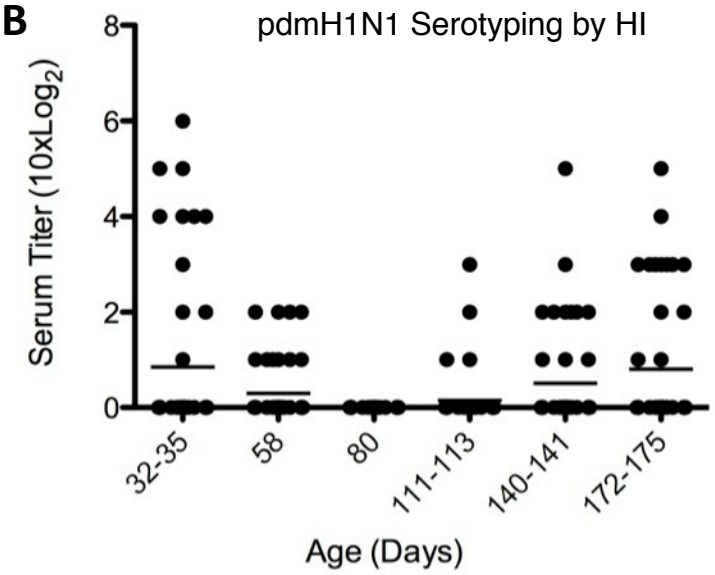
* Unpublished data, **Dr. Alvaro Ruiz**, University of Concepcion

Age distribution of seropositive animals in two farms in central Chile

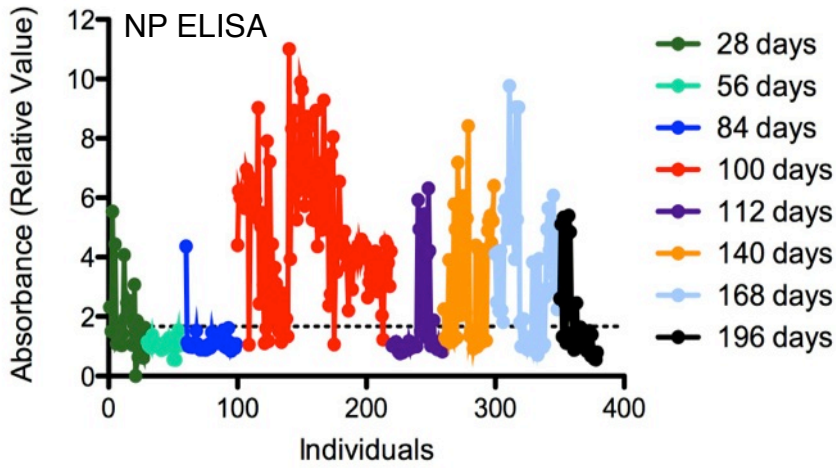
A **Site 1 (N=274)**



B



C **Site 2 (N=347)**



- Seroprevalence to pdmH1N1 ranges from 18% - 51.7%.
- Little cross-reactivity to classical swine strains.
- Serotyping to other strains currently ongoing.

Circulation of swine influenza viruses in Chile

- During 2011 and 2012 part of the swine industry in Chile began to use a commercial adjuvanted vaccine made in the United States, containing five strains.

- Current serological data indicate the circulation of at least 2 influenza virus strains in most production sites, with pdmH1N1 being highly prevalent.

- HI data suggest potential drifted pdmH1N1 strains (currently under study).

- The antigenic diversity and lack of cross-reactivity of the commercial vaccine strains and the current strains circulating in the country, motivated an effort to design and make homologous vaccines.

- Current mitigation strategies in most industrialized production farms grouped by ASPROCER include the use of a homologous US made vaccine, which contains 4 components.

Guatemala

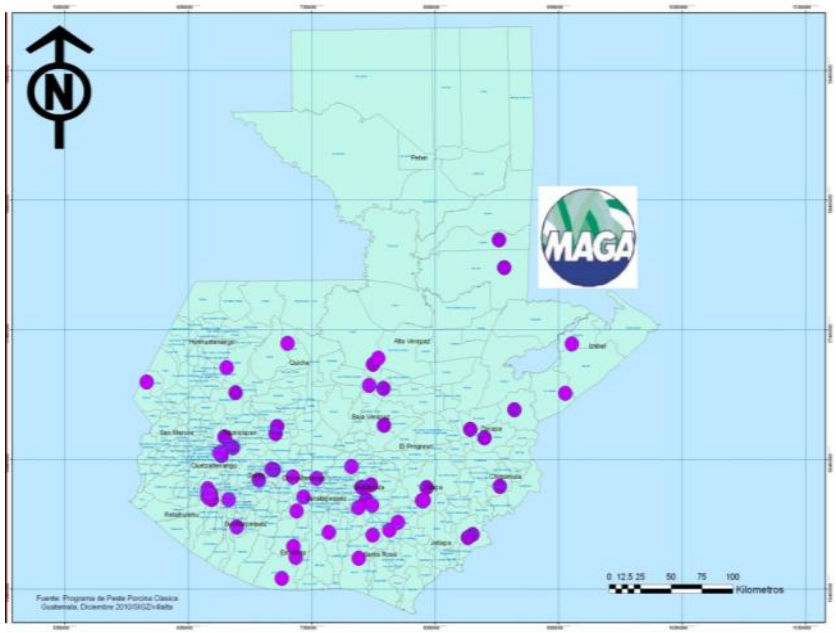
Nation-wide cross-sectional survey
multi-stage random sampling:
2010 – October (n=500) (MAGA, FAO)
2011 – June to August (n=499) (MAGA)

•Case definition: Farms or backyard pigs (pig productions units) where ≥ 10% of the exposed population have respiratory clinical signs

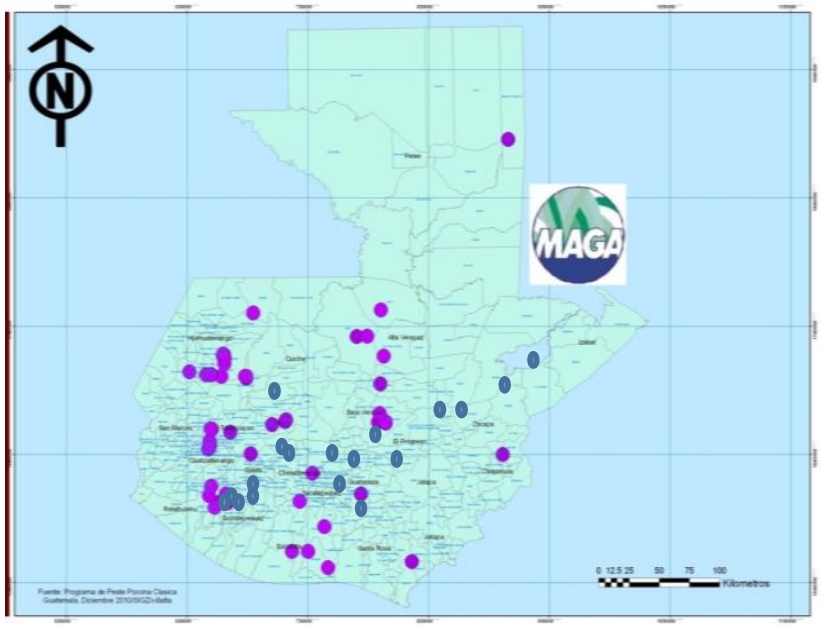
–Nasal swabs (Virus detection by rRT-PCR)

–Serum samples (Antibody detection by ELISA and HI)

Commercial farms



Backyard pigs



Spatial analysis. Global methods were used to detect the presence of purely spatial clusters of positive farms (Ripleys K function)

2) Vigilancia de influenza porcina: en 2013 no se contó con fondos asignados para continuar el muestreo en cerdos. Sin embargo, en el laboratorio en Guatemala se implementó el cultivo de células MDCK y los protocolos para aislamiento viral a partir de muestras de hisopados nasales. Así mismo se implementó el ensayo de inhibición de hemaglutinación para la identificación de anticuerpos hace distintos grupos antigénicos de influenza H1 y H3.

Las muestras colectadas en 2011 y 2012, positivas para Influenza tipo A por la prueba de rRT-PCR en tiempo real, fueron probadas para aislamiento viral. No se obtuvieron aislados virales. Los resultados fueron presentados a las autoridades de Salud Animal del Ministerio de Agricultura, Ganadería y Alimentación (MAGA) y discutidos para la mejora de futuros estudios en colaboración.

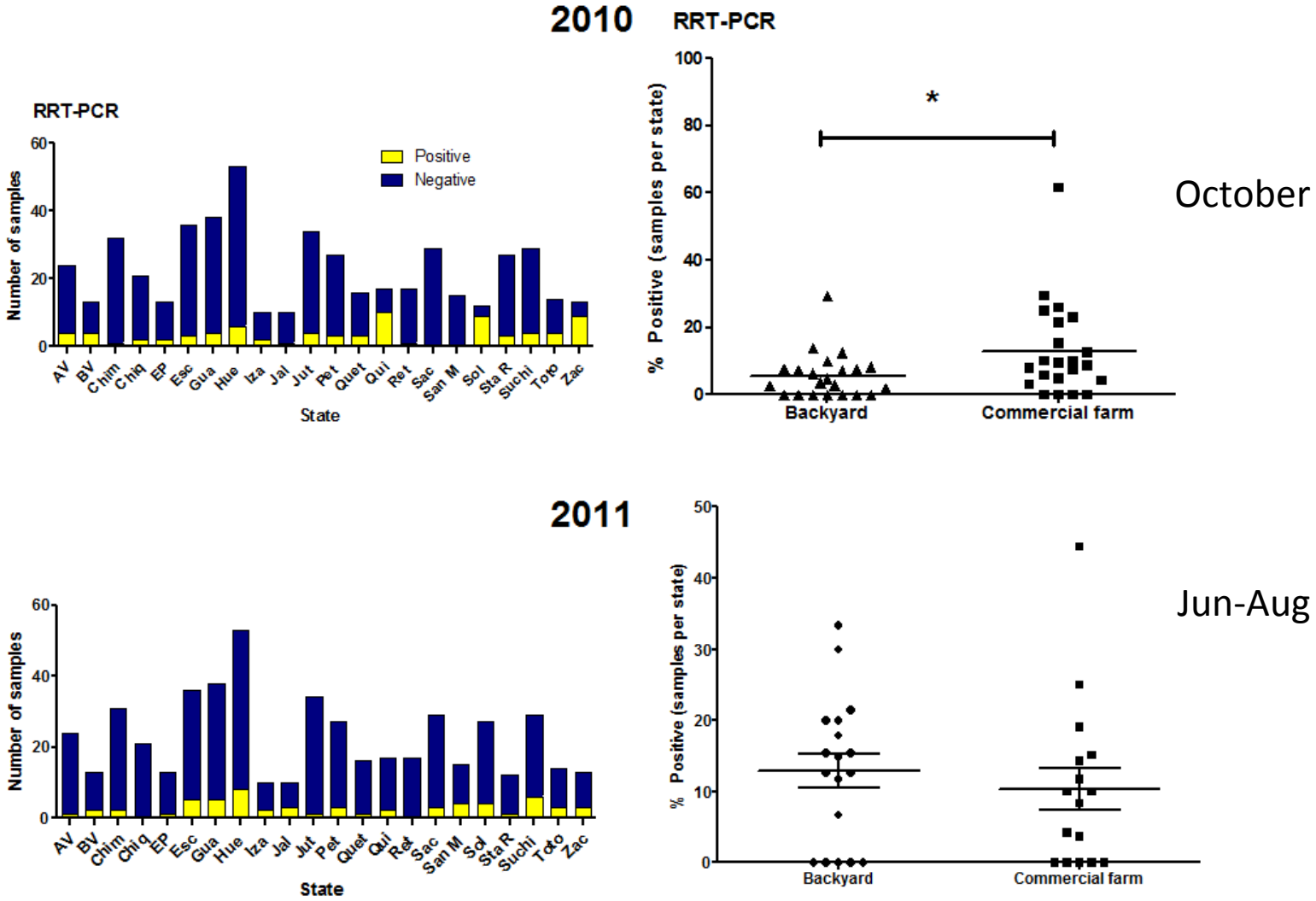
Estimated virus prevalence was comparable between years

RRT-PCR estimated Influenza A prevalence in sampled farms from Guatemala

	Prevalence		Total Average
RRT-PCR	**2010 (n=500)**	**2011 (n=499)**	
Influenza A positive	16%	12%	14%
Sick positive animals	17%	9%	13%
Healthy positive animals	10%	12%	11%
IDEXX ELISA	**2010 (n=460)**	**2011 (n=499)**	
Positive for influenza A antibodies	10%	1%	6%
Sick positive animals	9%	0%	4%
Healthy positive animals	36%	2%	19%

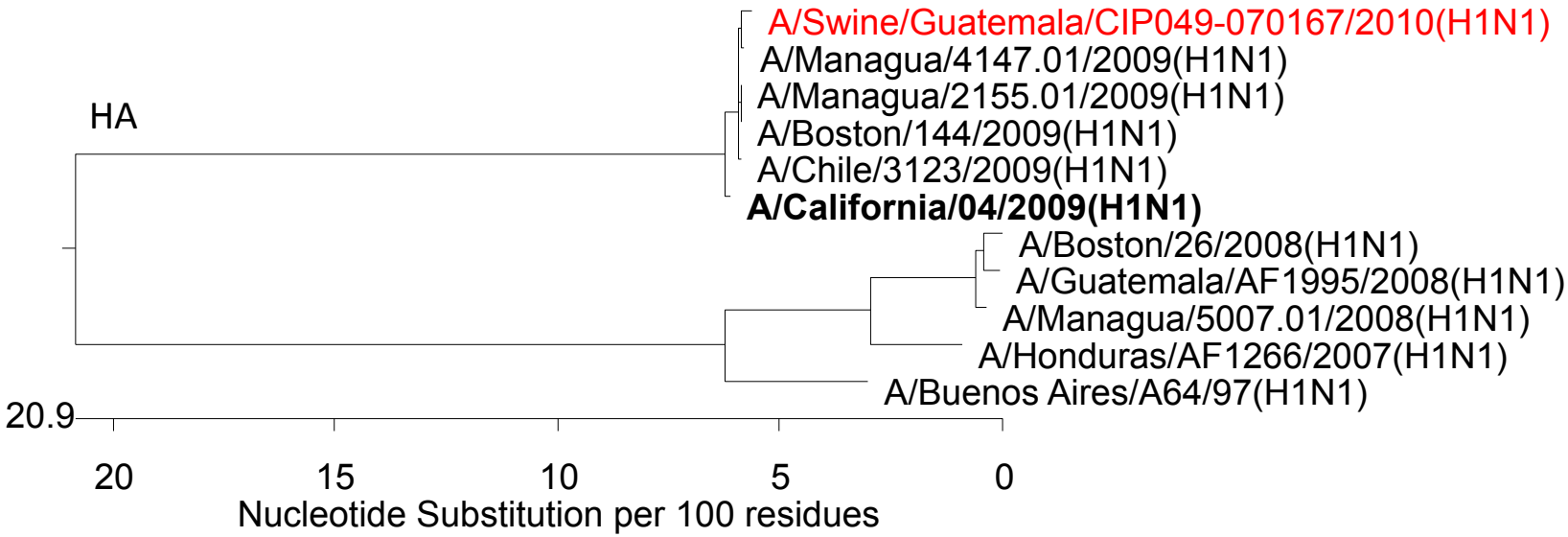
(A. González-Reiche, Unpublished)

Influenza A RRT-PCR prevalence in pigs varies between states and type of production unit



(A. González-Reiche, Unpublished)

The viruses…
fully pandemic H1N1 viruses were isolated from pigs

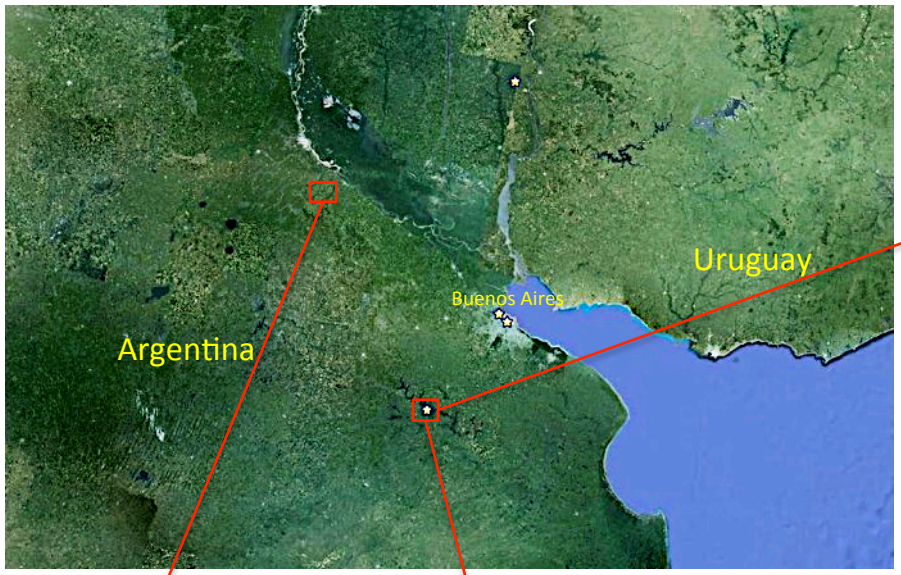


H3N2 isolates are related to seasonal human viruses

A/Swine/Guatemala/CIP049-040078/2010(H3N2)

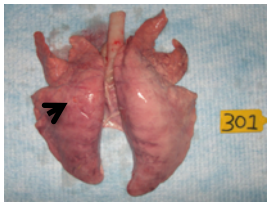
Segment	Most Similar Sequence in BLAST	%ID
PB2	A/Mexico City/WRAIR3580T/2010(H3N2)	99%
PB1	A/Mexico City/WRAIR3577T/2010(H3N2)	98%
PA	A/Thailand/CU-B657/2009(H3N2)	99%
HA	A/California/NHRC0004/2011(H3N2)	99%
NP	A/Mexico City/WRAIR4139N/2010(H3N2)	97%
NA	A/Mexico City/WRAIR4139N/2010(H3N2)	97%
M	A/Uganda/MUWRP-070/2009(H3N2)	100%
NS	A/Mexico/UASLP-013/2008(H3N2)	99%

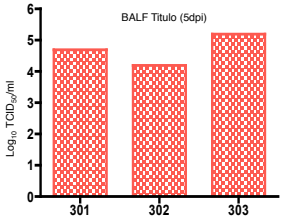
ARGENTINA

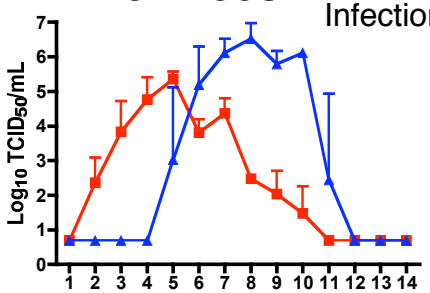


Evidence of a non-contemporary human H3N2 adapted to be transmitted among pigs

Farm A
Buenos Aires
6000 sows
Nov-2008







Infection and Transmission of A2/08 (H3N2)
Infected
Direct Contact

Journal of General Virology (2011), 92, 2871–2878 DOI 10.1099/vir.0.036590-0

Outbreak of swine influenza in Argentina reveals a non-contemporary human H3N2 virus highly transmissible among pigs

Javier A. Cappuccio,[1]† Lindomar Pena,[2]† Marina Dibárbora,[3] Agustina Rimondi,[3] Pablo Piñeyro,[1] Lucas Insarralde,[1] María A. Quiroga,[1] Mariana Machuca,[1] María I. Craig,[3] Valeria Olivera,[3] Ashok Chockalingam,[2] Carlos J. Perfumo,[1] Daniel R. Perez[2] and Ariel Pereda[3]

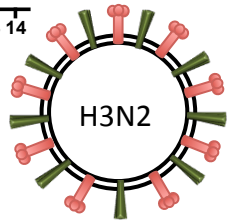
Farm B
Santa Fe
4000 sows
May-2010

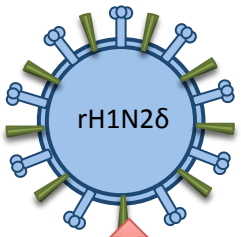
Farm C
Buenos Aires
9000 sows
Oct-2009

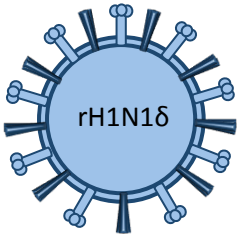
DISPATCHES

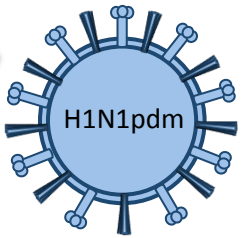
Pandemic (H1N1) 2009 Outbreak on Pig Farm, Argentina

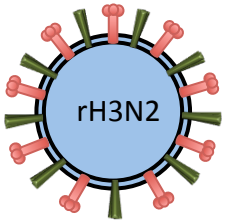
Ariel Pereda,[1] Javier Cappuccio,[1] María A. Quiroga, Elsa Baumeister, Lucas Insarralde, Mariela Ibar, Ramón Sanguinetti, María L. Cannilla, Débora Franzese, Oscar E. Escobar Cabrera, María I. Craig, Agustina Rimondi, Mariana Machuca, Rosa T. Debenedetti, Carlos Zenobi, Leonardo Barral, Rodrigo Balzano, Santiago Capalbo, Adriana Risso, and Carlos J. Perfumo











Influenza Other Respi Viruses. 2011 Nov;5(6):409-12. doi: 10.1111/j.1750-2659.2011.00246.x. Epub 2011 Apr 7.

Evidence of reassortment of pandemic H1N1 influenza virus in swine in Argentina: are we facing the expansion of potential epicenters of influenza emergence?

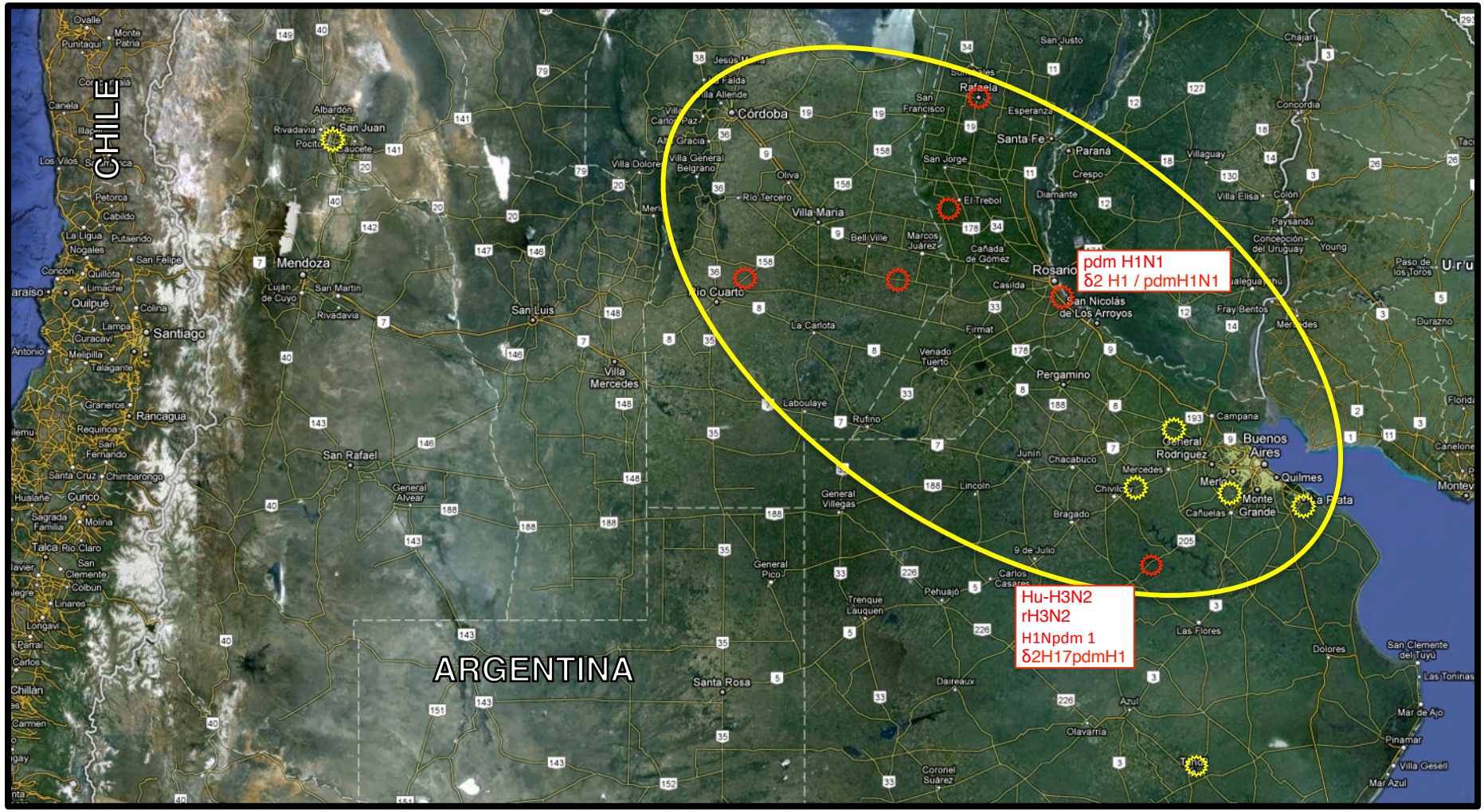
Pereda A, Rimondi A, Cappuccio J, Sanguinetti R, Angel M, Ye J, Sutton T, Dibárbora M, Olivera V, Craig MI, Quiroga M, Machuca M, Ferrero A, Perfumo C, Perez DR.

Laboratorio Aves y Porcinos, Instituto de Virología CICVyA-Instituto Nacional de Tecnología Agropecuaria, Buenos Aires, Argentina. apereda@cnia.inta.gov.ar

Farm D
Buenos Aires
9000 sows
Jan-2012

Recombinant H3N2 + pdm

GITEP study 2012
Sampling sites



rRT-PCR/isolate = Positive - Serology = Positive

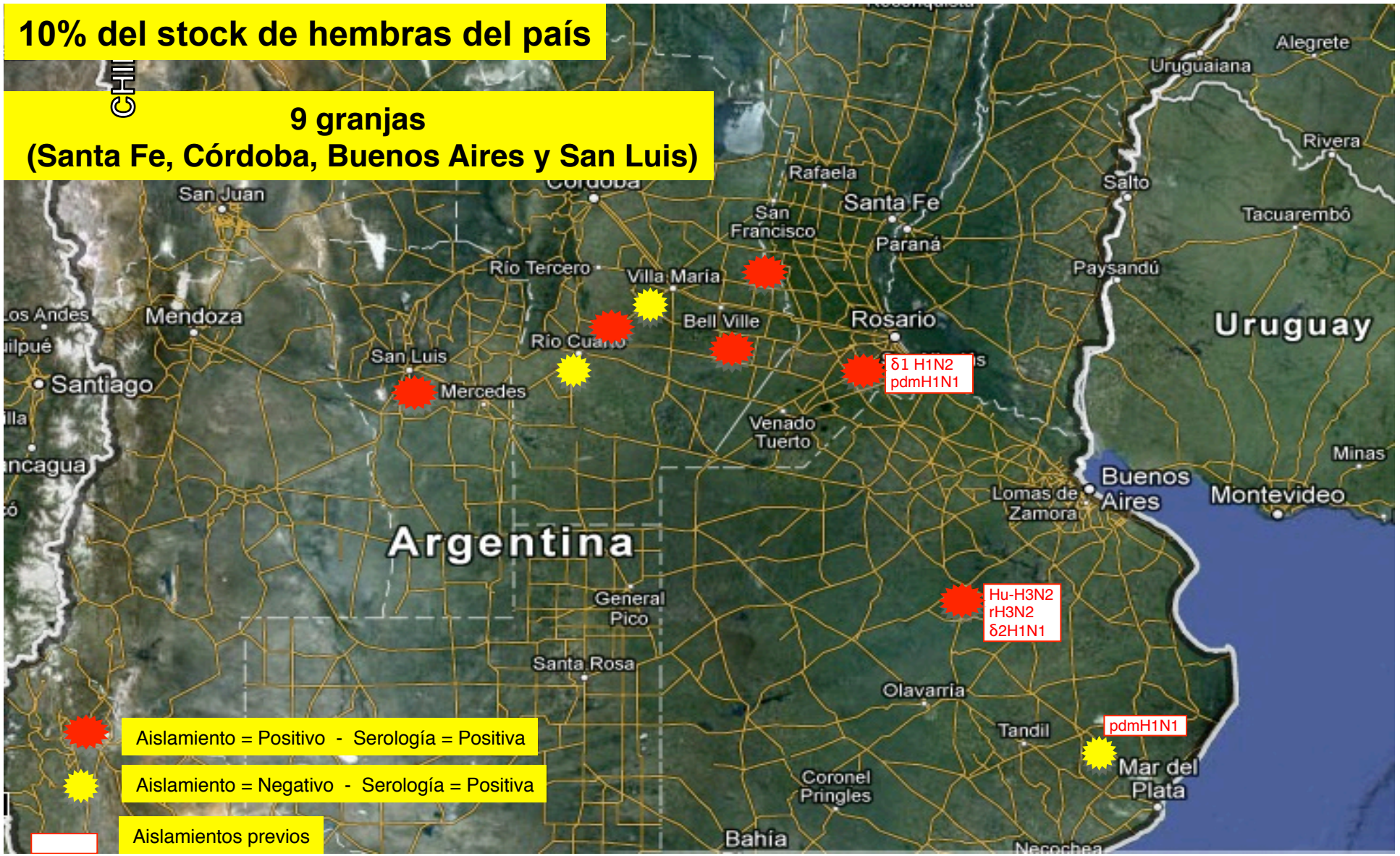
rRT-PCR/isolate = Negative - Serology = Positive

Analyzed samples

- 1199 nasal and/or bronchial swabs
- 59 lungs

10% of sows stock in Argentina

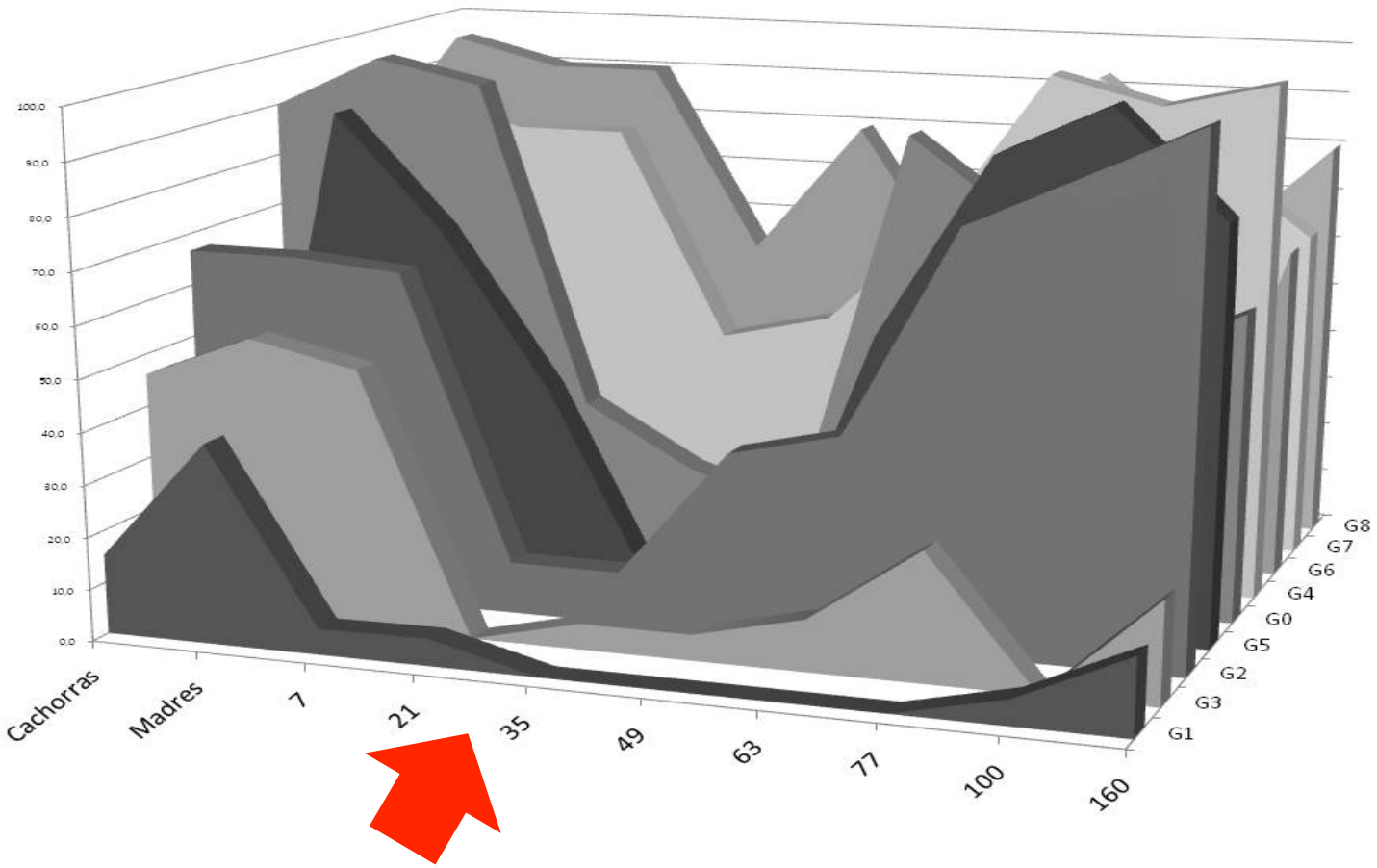
2012 : Estudio del comportamiento de la infección por el virus de influenza en cerdos previa implementación de vacunas en Argentina.



Granja	Signos clínicos	Edad (días)	Aislamiento
G0	SI	63	SI **(rH3N2)**
G1	NO		NO
G2	SI	49 y 63	SI **(pH1N1)**
G3	SI	35 y 49	SI **(pH1N1)**
G4	SI	21 y 140	SI **(pH1N1)**
G5	SI	50 y 120	NO
G6	SI	25	NO
G7	SI	35	SI **(pH1N1)**
G8	SI	35	NO

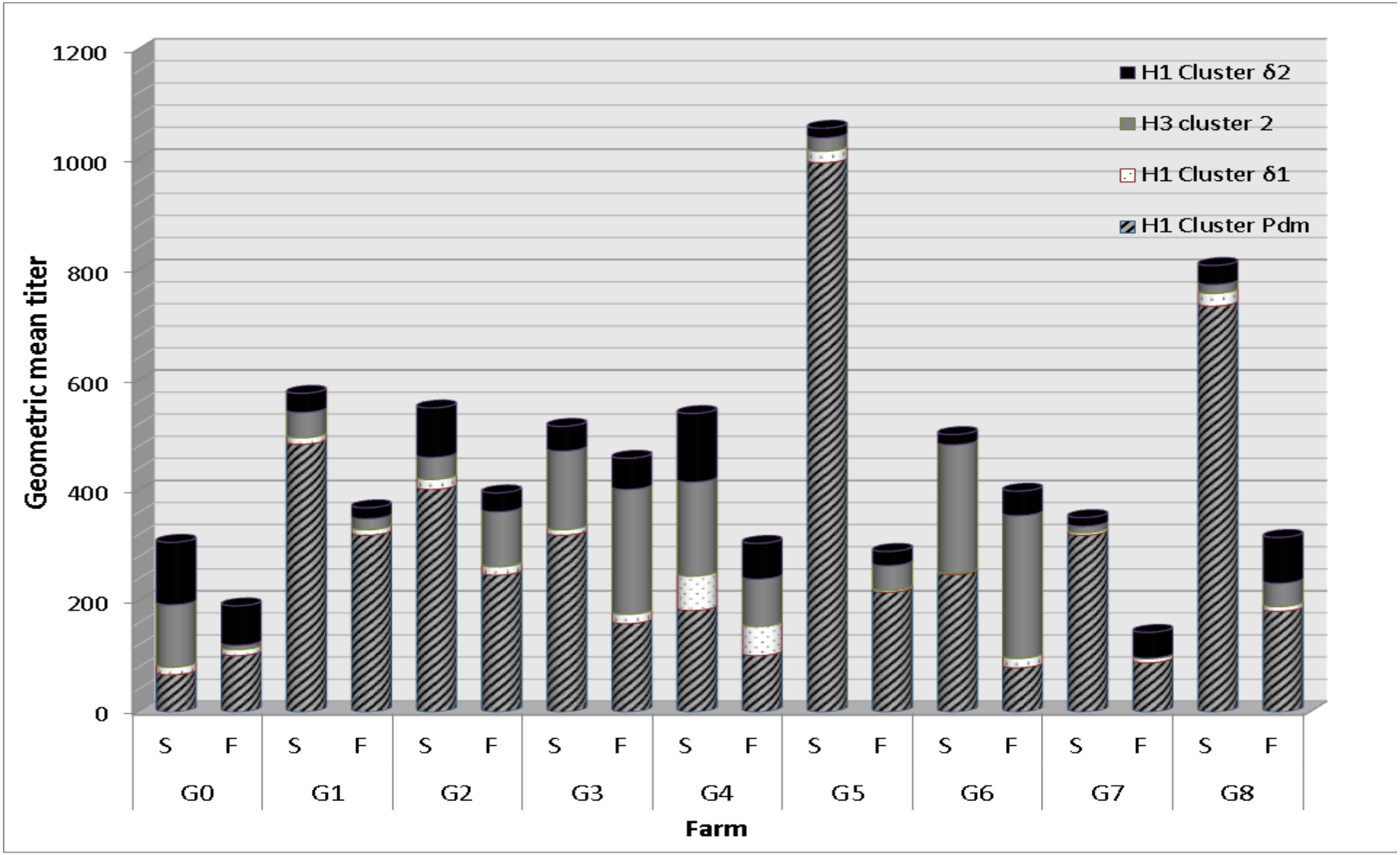
8/9 granjas signos clínicos en destete
2/9 granjas signos clínicos en engorde

Granja	Lesiones Macroscópicas	Lesiones Microscópicas	rt PCR +	Aislamiento
G2	2/9	3/9	1/2	1
G4	4/7	3/7	1/3	1
G7	1/1	1/1	1/1	1
G8	5/17	1/5	1/3	0



- 48% del total de los sueros positivos
- Se observó una caída de anticuerpos calostrales hacia los 21 a 35 días y un aumento entre los 49 y los 100 días.
- El porcentaje de cerdas positivas varió entre 25-100%, mientras que en el engorde se observaron porcentajes de entre 15 a 100% de animales positivos.

Subtipos circulantes (HI)



Conclusiones trabajo 2012

•Existe una infección endémica de virus de influenza que afecta particularmente en la etapa de posdestete a lo largo del año

•Los estudios virológicos demuestran, que si bien existe una predominancia de H1N1 pdm (4 aislamientos), existe circulación de otros subtipos y recombinaciones entre ellos (rH3N2)

•En relación a la serología se observó una caída de anticuerpos hacia los 21 a 35 días y un aumento entre los 49 y los 100 días

•Los resultados del estudio de HI indican una co-circulación de más de un subtipo viral.

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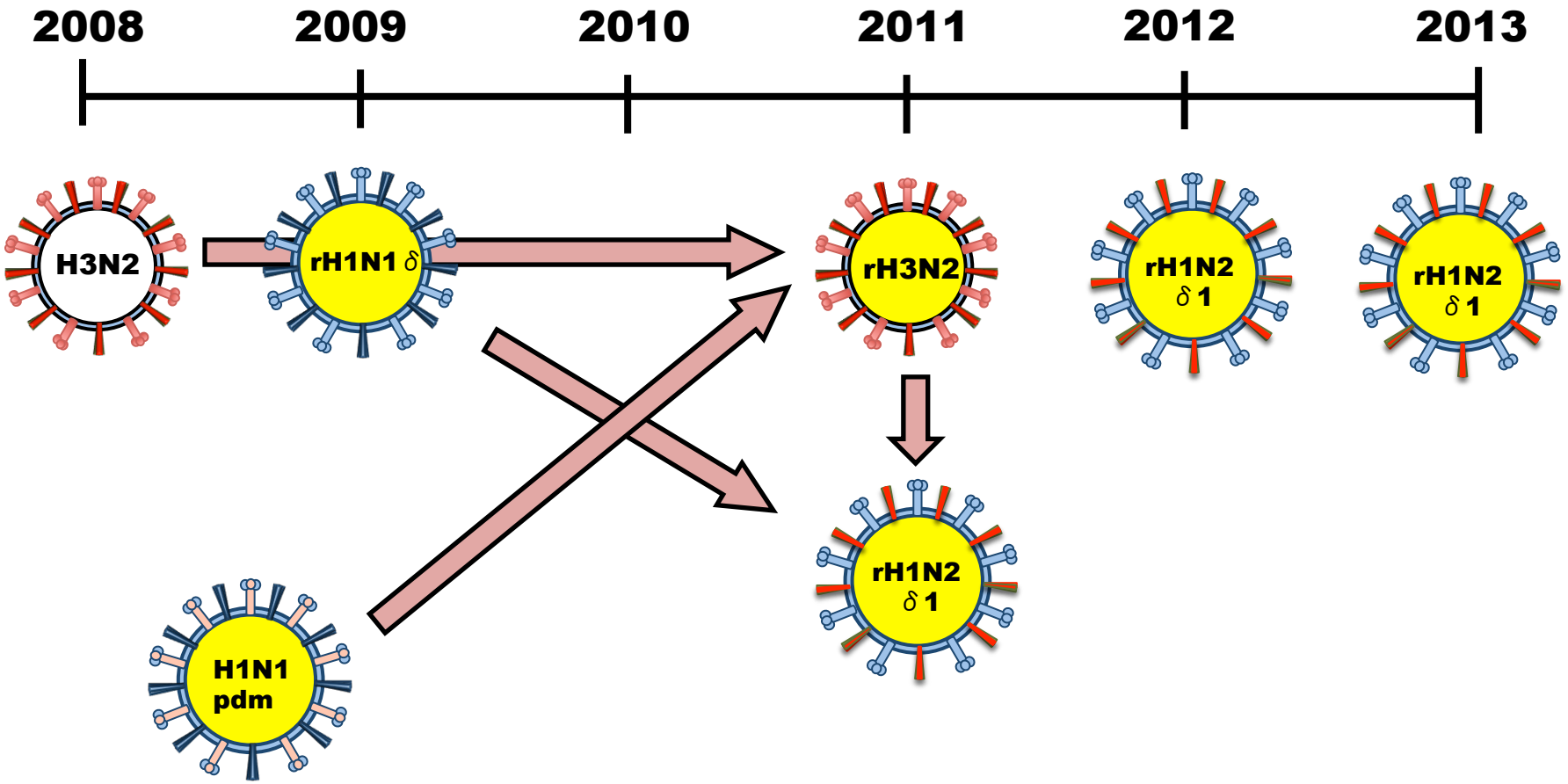
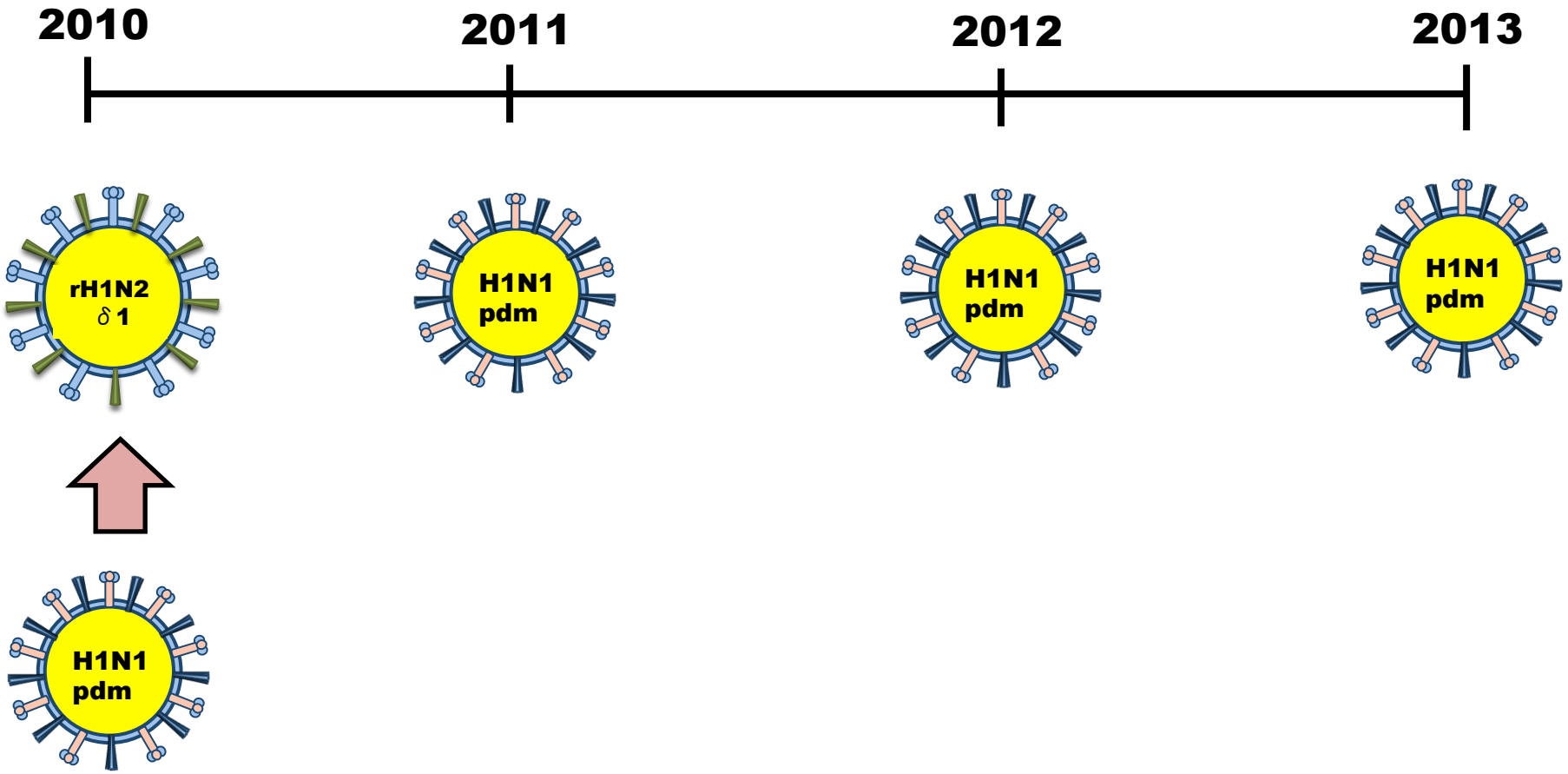
Centro de Investigación en Ciencias Veterinarias y Agronómicas
Instituto de Virología "Dr. Scholein Rivenson"

Relevamiento continuo de virus de Influenza en granjas porcinas

Granja 1



Granja 2


2010
2011
2012
2013
rH1N2
δ 1
H1N1
pdm
H1N1
pdm
H1N1
pdm
H1N1
pdm

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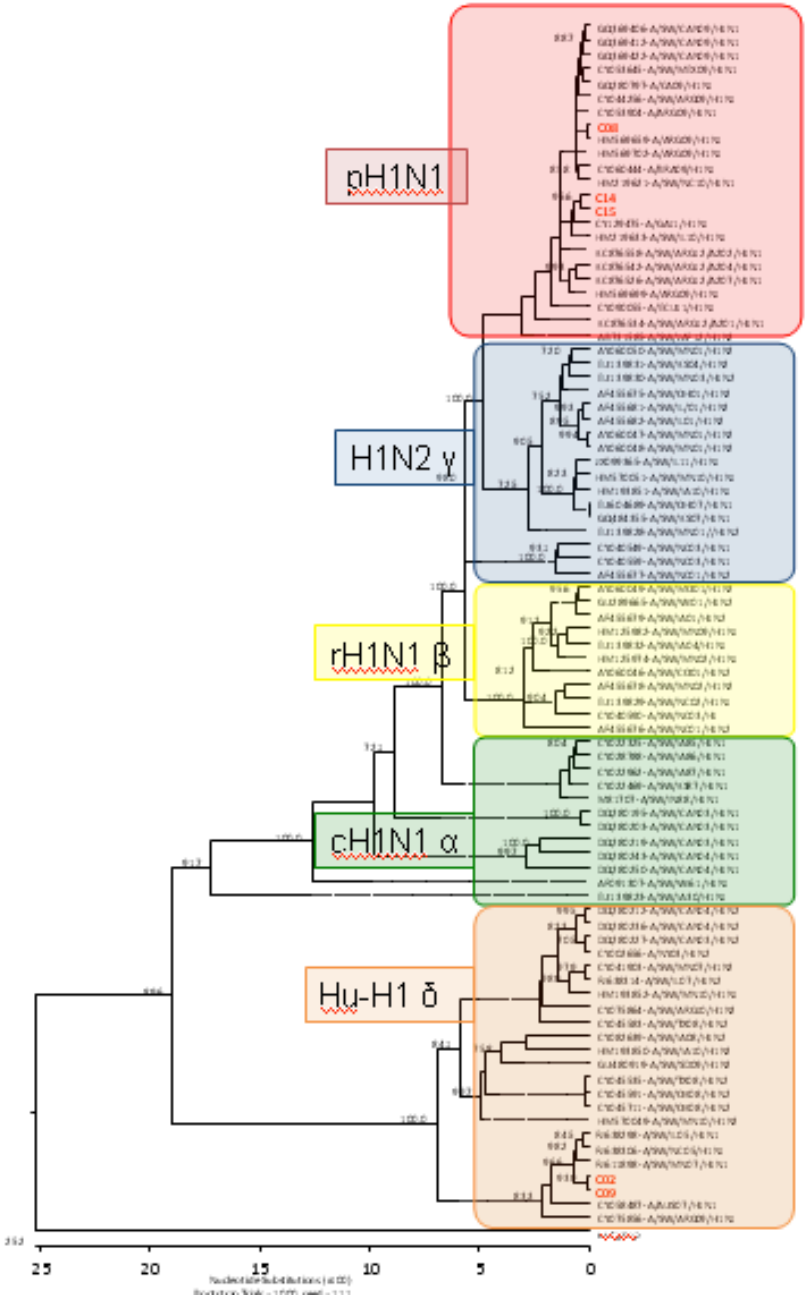
Vigilancia Influenza porcina 2013

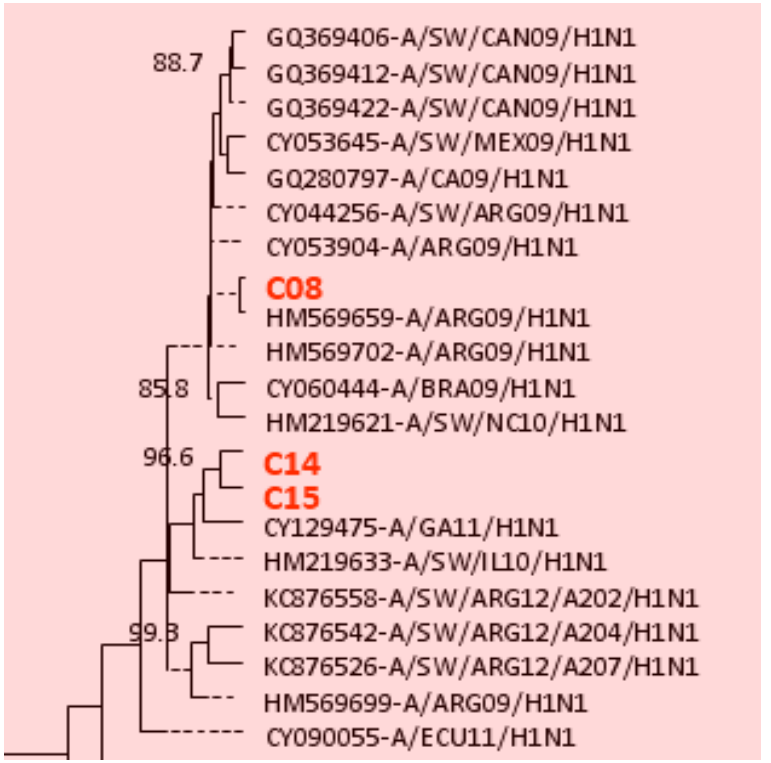
Nº de muestras procesadas: 320 **Nº de muestras positivas: 120/320**

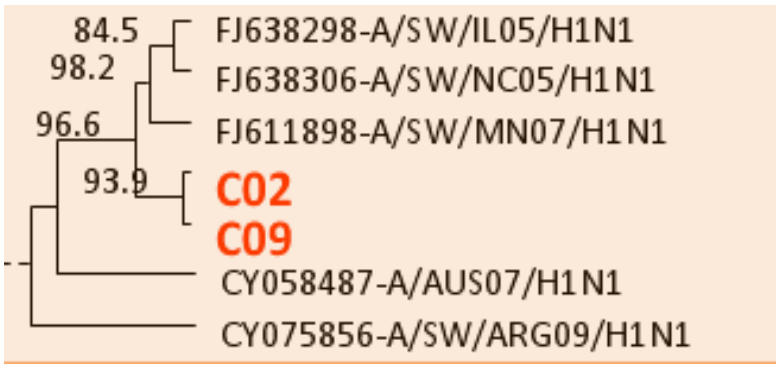
Nº Total de aislamientos: 33

Protocolo	Fecha	Procedencia	Subtipo
C02	Feb-13	BsAs	**δH1N2**
C07	Abr-13	BsAs	**rH3N2**
C08	Jun-13	Sta.Fe	**pH1N1**
C09	Jun-13	BsAs	**δH1N2**
C14	Jul-13	Sta.Fe	**pH1N1**
C15	Ago-13	Sta.Fe	**pH1N1**

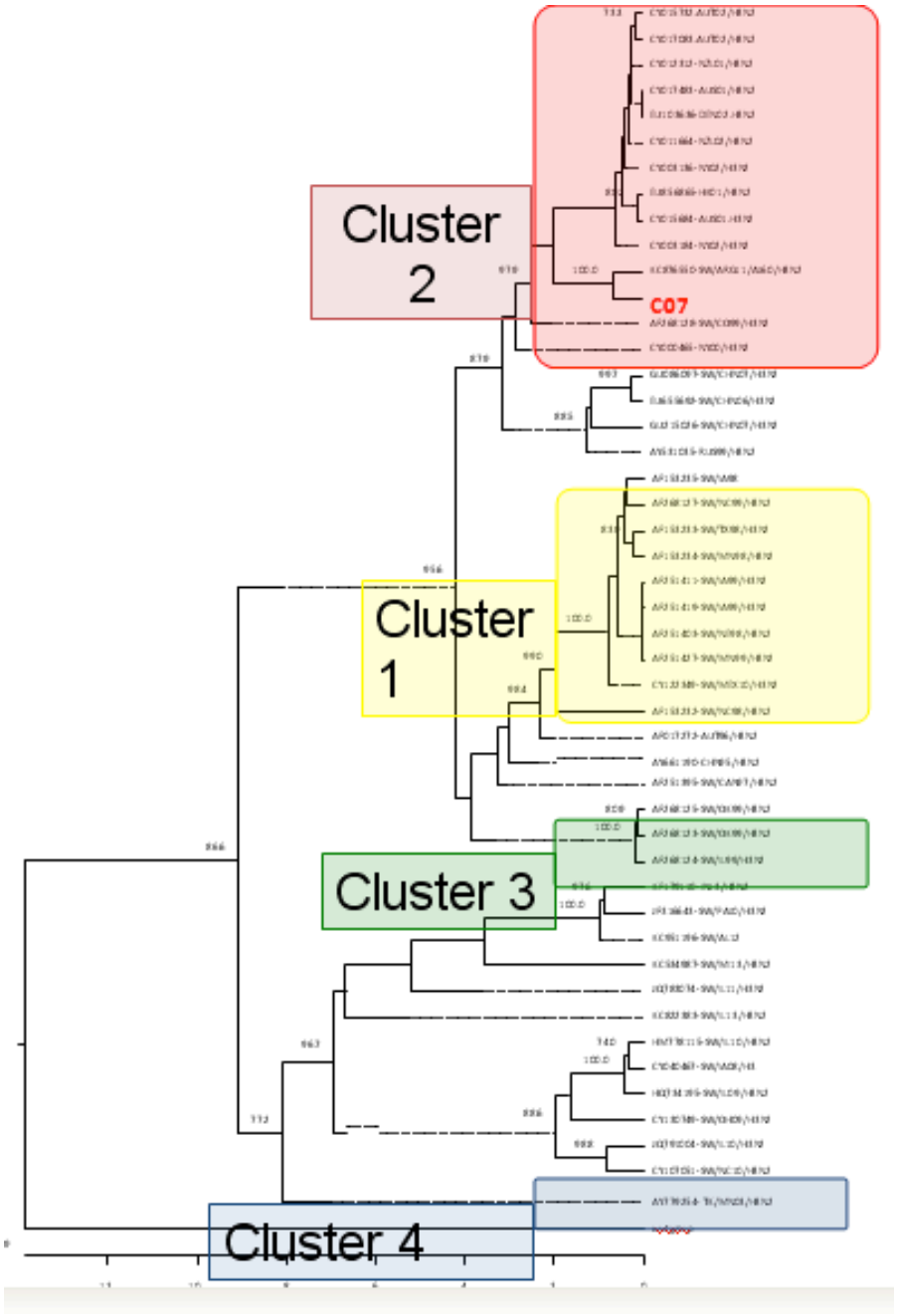
Árbol filogenético para H1

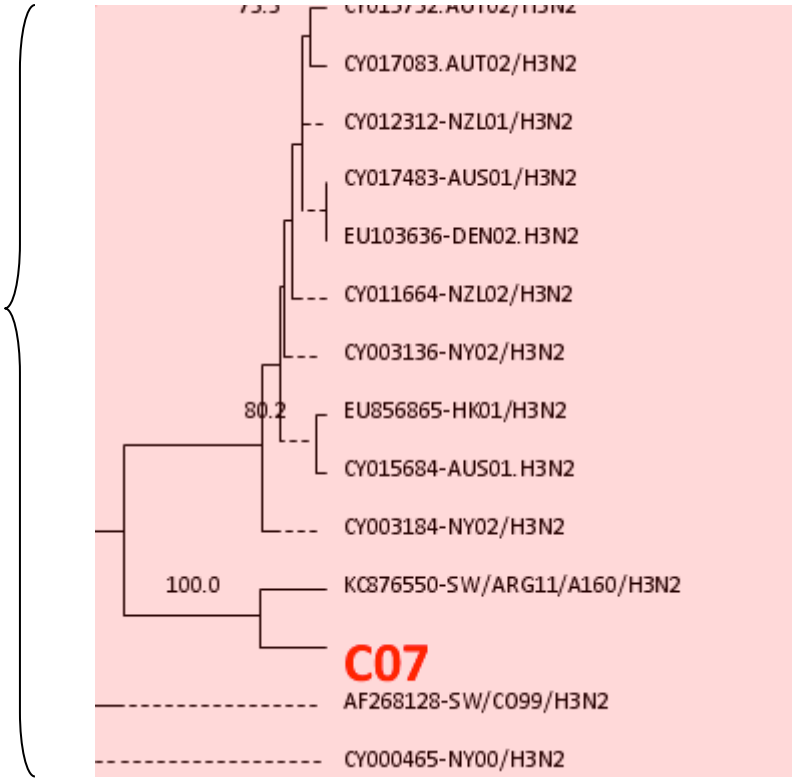




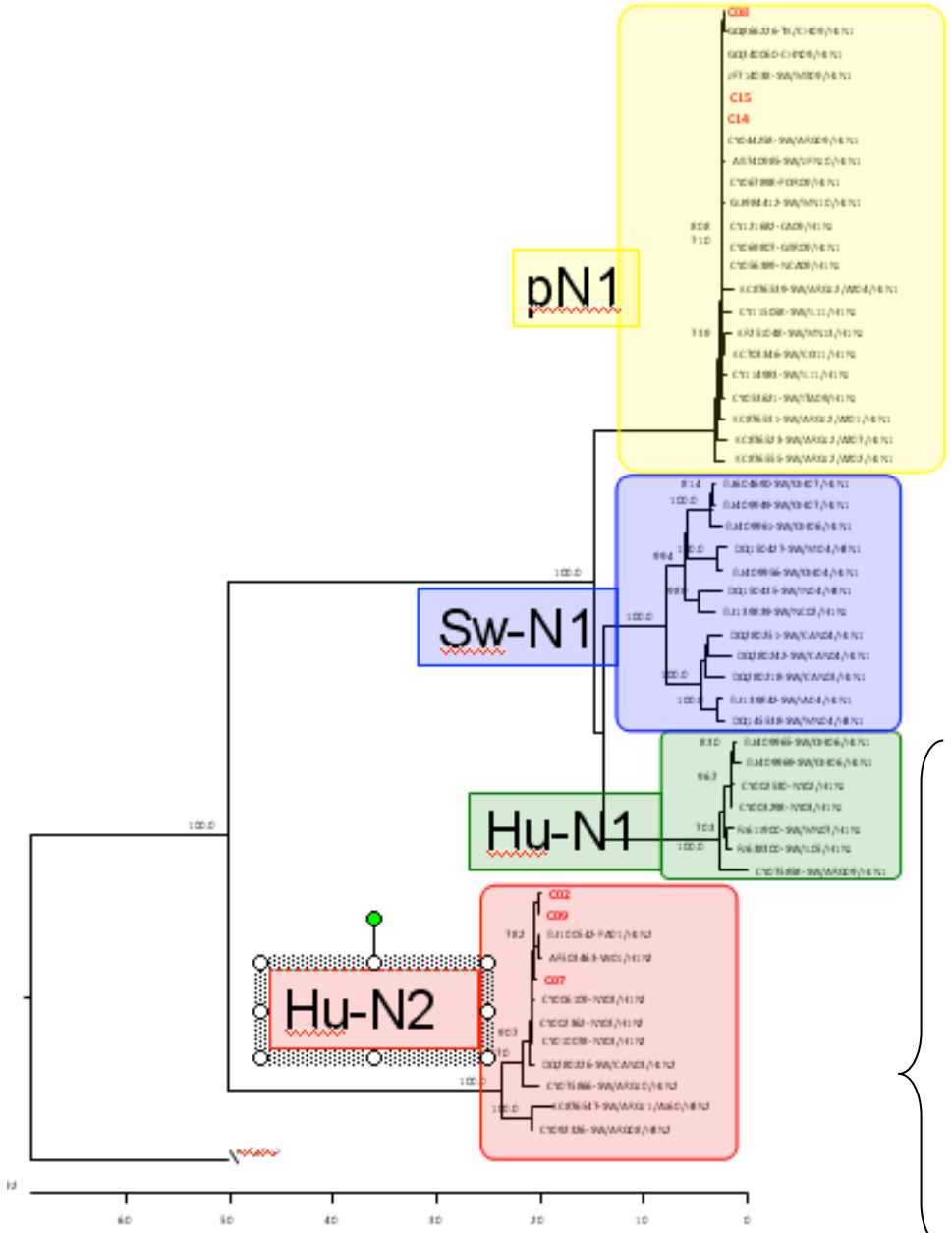


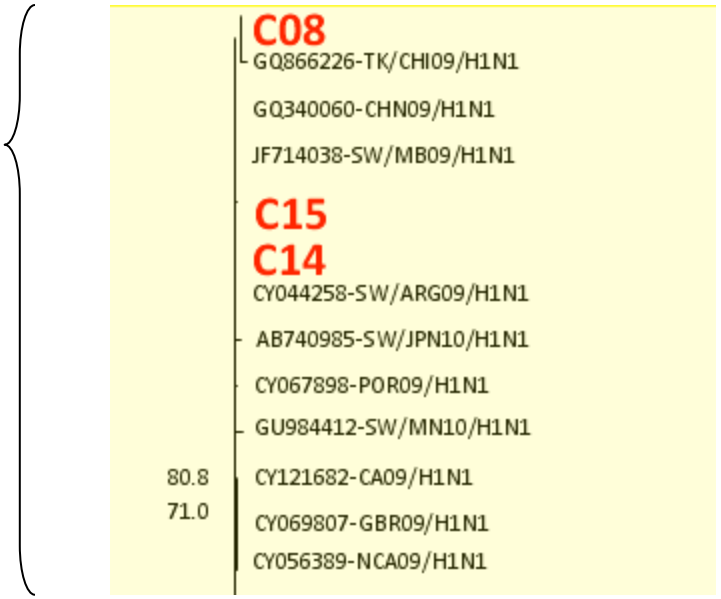
Árbol filogenético para H3

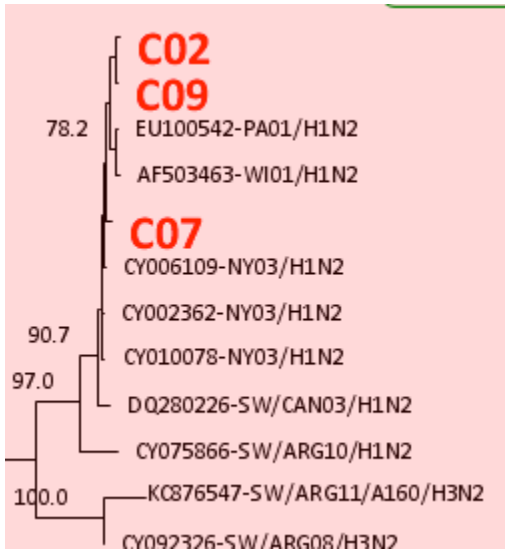




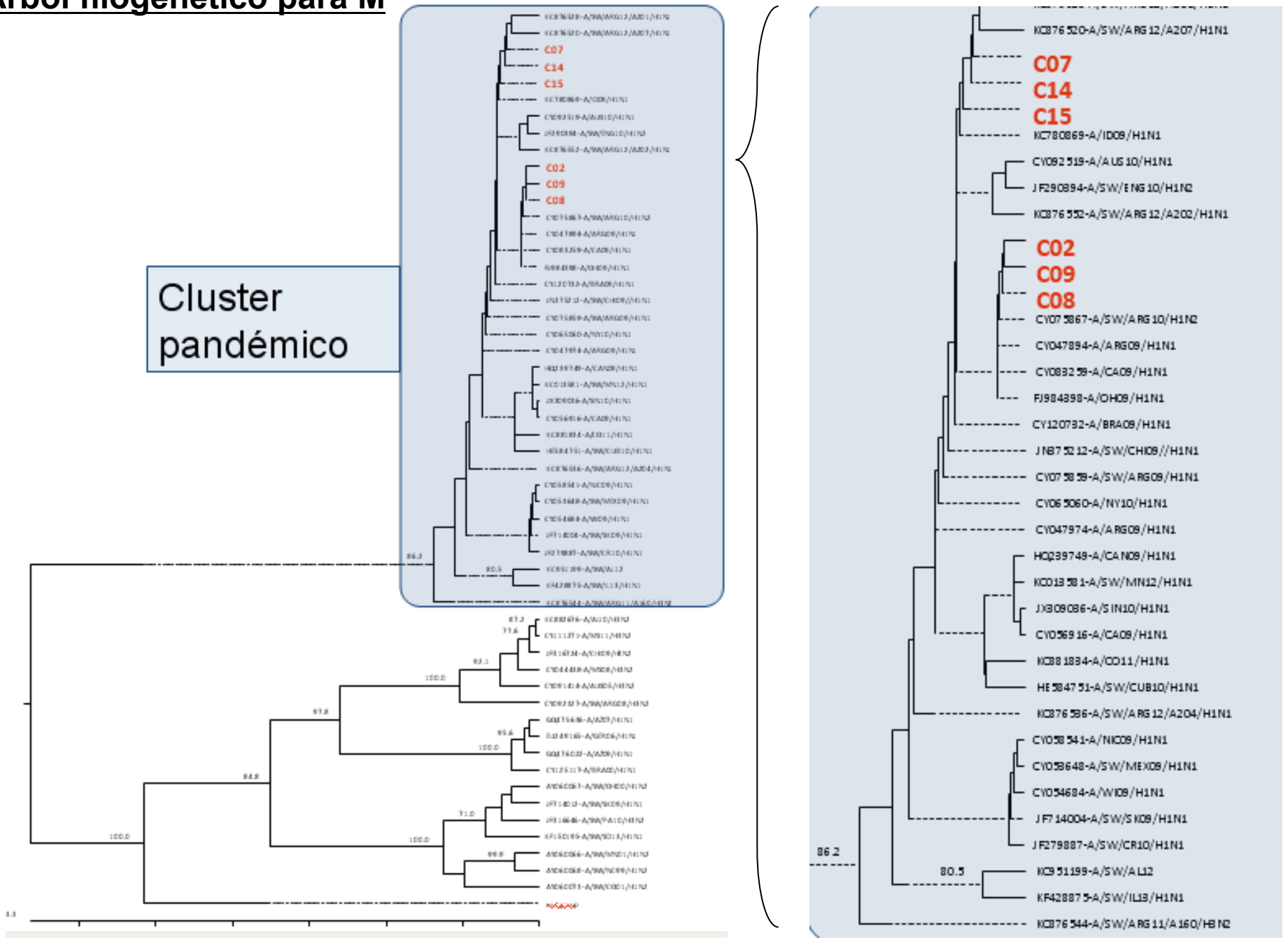
Árbol filogenético para NA



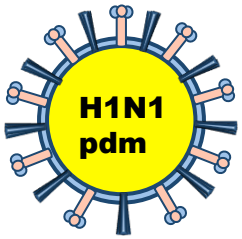


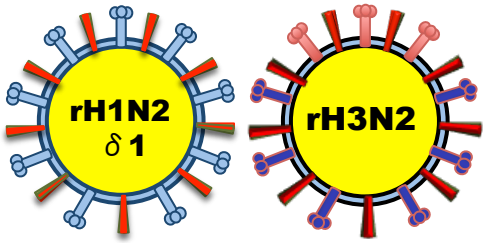


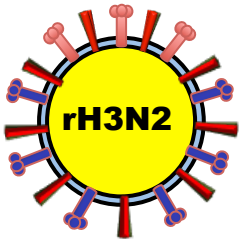
Árbol filogenético para M



Conclusiones 2013







Subtipo predominante en Argentina

Nuevas evidencias de reasociación

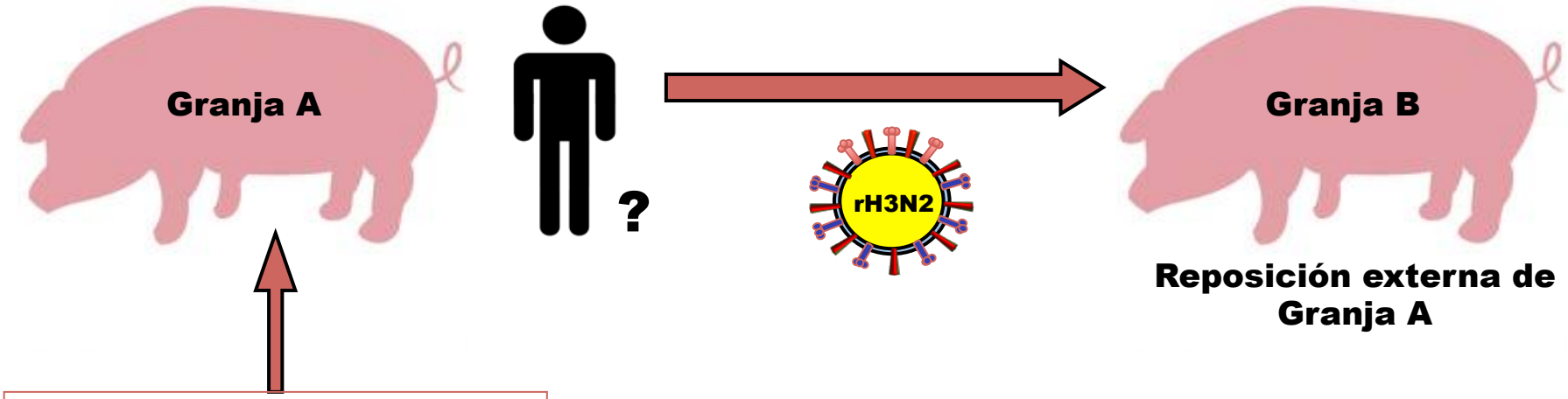
Alta capacidad de transmisión

¿Eficacia de las vacunas?

Instituto Nacional de Tecnología Agropecuaria
Centro de Investigación en Ciencias Veterinarias y Agronómicas
Instituto de Virología "Dr. Scholein Rivenson"

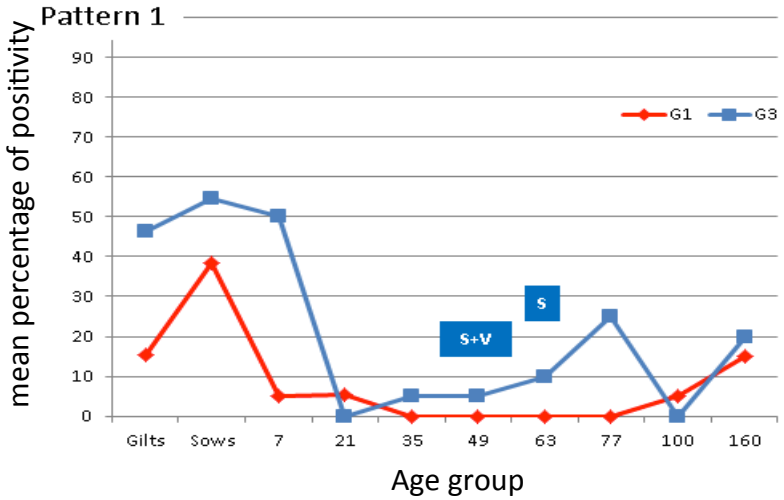
Evidencia de infección con el mismo virus en dos granjas NO relacionadas

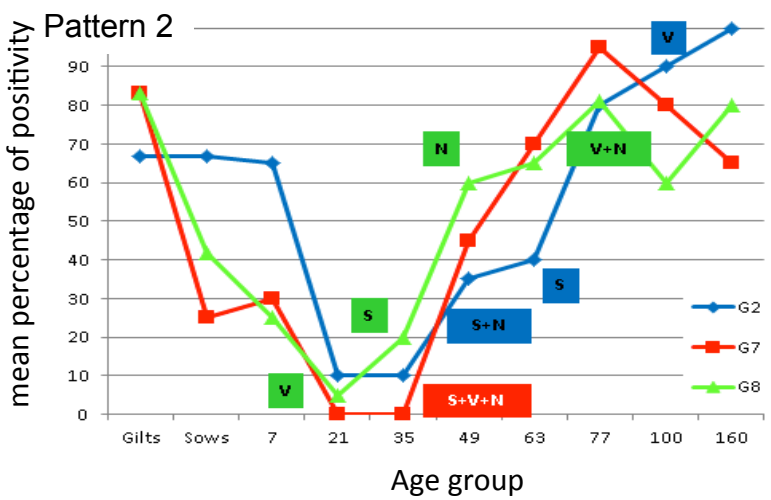
- Abril 2013
- **Granja B** Pcia. BsAs 300 madres, reposición externa
- Brote agudo sintomatología respiratoria (tos -Tº 40-42)
- Todas las categorías afectadas
- Rápida transmisión entre galpones y rápida recuperación de la granja (15-20ds)
- Aislamiento virus de Influenza en todas las categorías: **rH3N2**



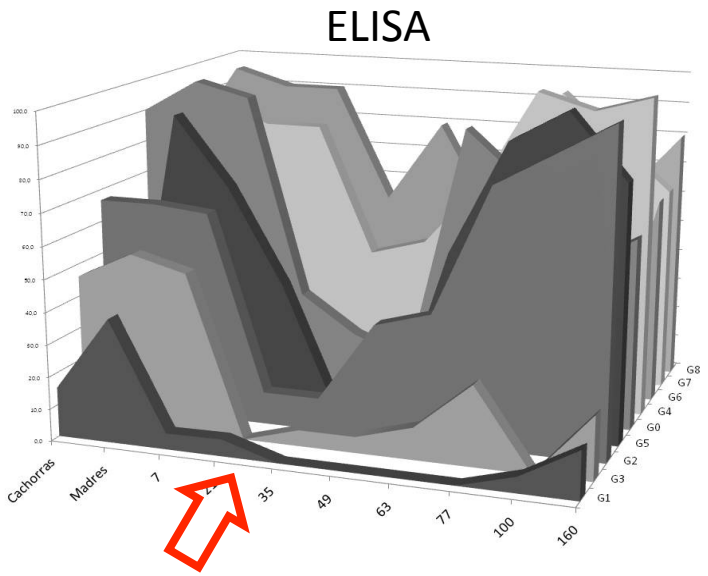
Subtipos aislados en Granja A :

Description	Max score	Total score	Query cover	E value	Ident	Accession
Influenza A virus (A/swine/Argentina/CIP051-A160/2011(H3N2)) segment 4 hemagglutinin (HA) ge	3024	3024	99%	0.0	99%	KC876550.1
Influenza A virus (A/swine/Argentina/CIP051-A2/2008(H3N2)) hemagglutinin (HA) gene, complete	2952	2952	99%	0.0	98%	CY092324.1

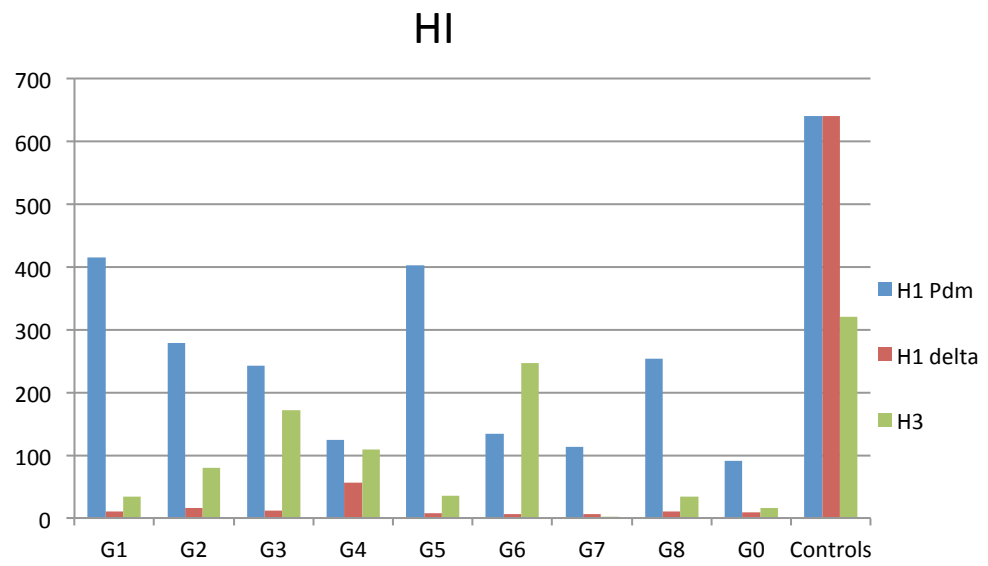




S: clinical signs. V: virus isolation. N: virus isolation from pneumonic lung lesions at necropsy



10% rH3N2
90% H1N1pdm



Isolated Viruses in Argentina

- **Non contemporaneous human origin H3N2**
  A/swine/Argentina/CIP051-A2/08 (H3N2)

- **pdm H1N1**
  A/swine/Argentina/SAGiles-31215/2009 (H1N1)

- Reassortant Viruses :

  • **Human Like δ2 H1 SIV (HA+NA) – pdm H1N1 (internal genes)**
  A/Swine/Argentina/CIP051-BsAs76/2009 (H1N1)
  A/Swine/Argentina/CIP051-SantaFe/2010 (H1N2)

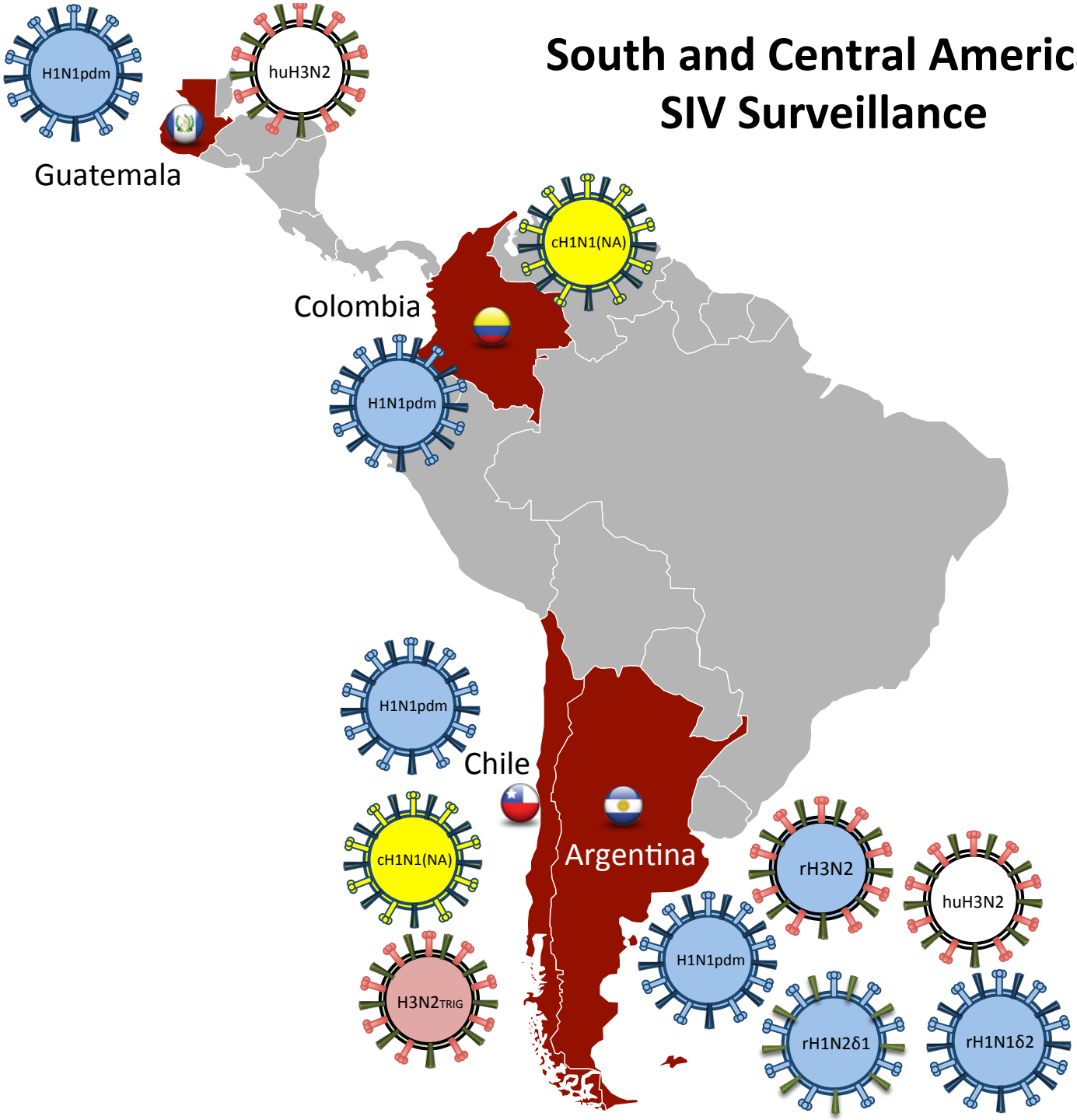
  • **Pig adapted Hu H3N2 (HA+NA) – pdm H1N1 (internal genes)**

Antropozoonoses

Summary and perspectives:

- **From collected data we know that:**
  - Influenza circulates in pigs in Central and South America: year to year variation suggests that levels of influenza transmission may vary along the year.
  - Mostly of the viruses isolated have a human origin and the principal subtype is the pandemic H1N1 virus.
  - These viruses started to reassort between them, at least in Argentina (and Brazil).
  - In some countries, the sera collected before the 2009 pandemic outbreak have very low HI reactivity against the pandemic virus, which explains the spread of this virus, but also demonstrate the lack of activity of this virus in the region before this pandemic virus appears in 2009.
- **But we still don't know:**
  - The seasonality of influenza transmission in pigs.
  - The ecological factors important for transmission.
  - Relatedness of human cases of influenza to circulation of the viruses in pigs
  - Antigenic relatedness.
- **What we need for the region:**
  - More training in epidemiological/risk assessment and lab testing.
  - More awareness of the relevance of SIV by institutional presence (an agenda between FAO and/or OIE local vet officers with OFFLU).
  - More financial support to ensure the continuity of these efforts.
  - Local reference laboratory for South and Central America.

South and Central America SIV Surveillance



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