Use of Single Dilution Liquid Phase ELISA (slpELISA) for the evaluation of herd immunity and vaccination efficacy against FMD virus in Argentina.

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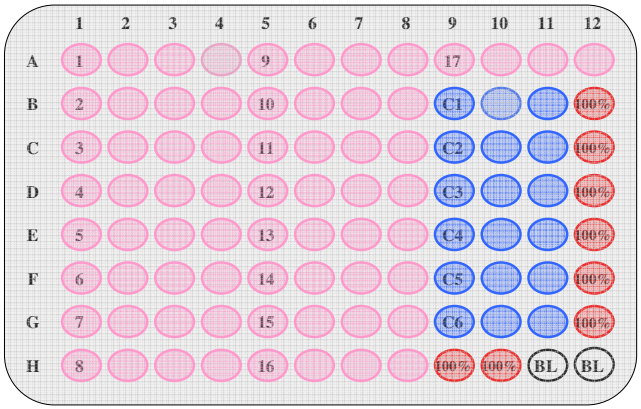
The effectiveness of the vaccination campaign depends on many factors:

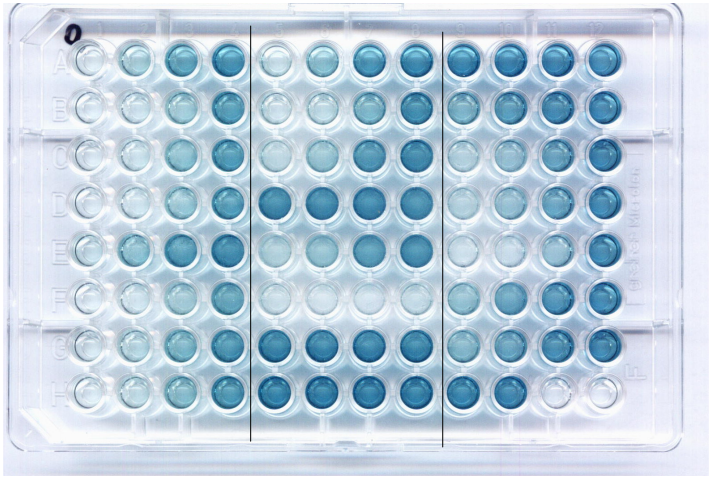
- Vaccine quality,
- Vaccination strategy
- Vaccination coverage.

- Evaluation of effectiveness in Argentina : sLP-ELISA:

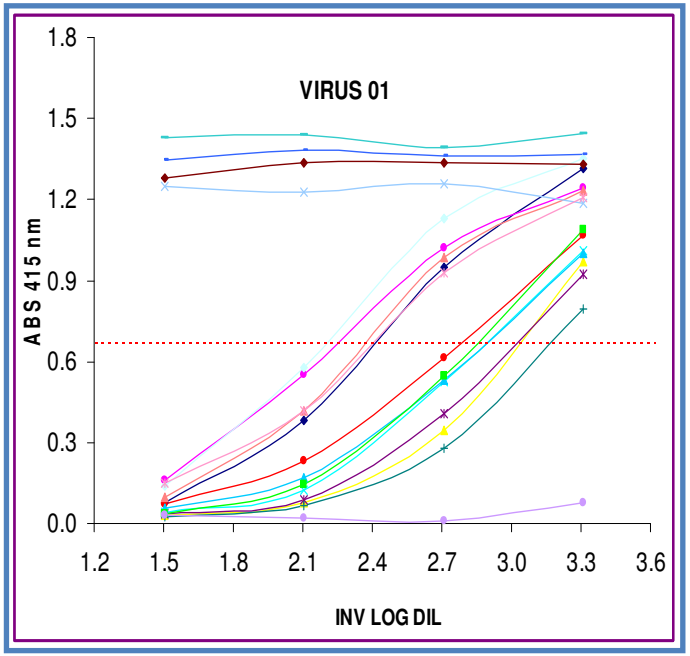
Liquid Phase ELISA (lpELISA) previously validated to measure the potency of FMDV commercial vaccines in Argentina (Periolo et al 1993, Robiolo et al.1995, Maradei et al. 2008) was adapted and standardized to be used for serological evaluation of herd immunity (Robiolo et al 2010).

Lp ELISA for Vaccine Potency





Adapted from Hambling et al. (J. Immunol.Methods 93 (1986) 115-121



•Antibody titers is expressed as the reciprocal log_{10} of serum dilutions giving 50 % of the OD recorded in the antigen control well

VALIDATION OF EACH PLATE

1. Blanks:

Average OD of Blanks <0.3

2. Antigen control:

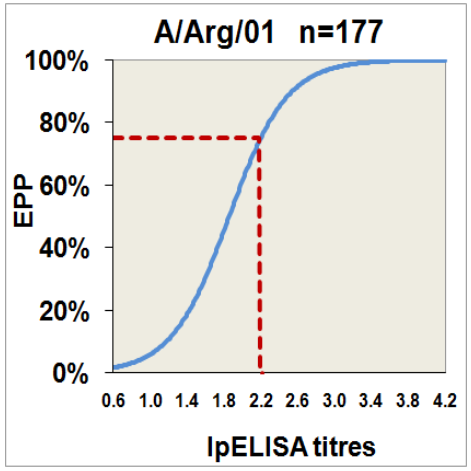
7 out of 8 antigen control should be in range accepted and do not differ by more than 0.3 OD.

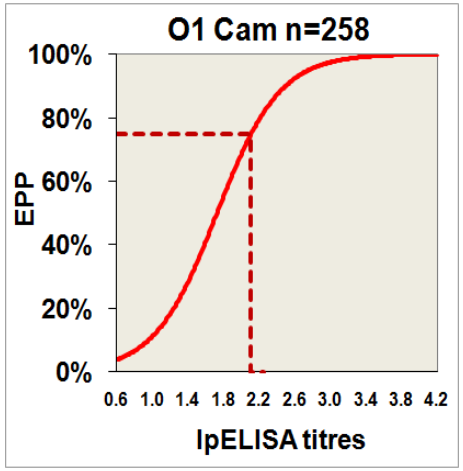
3- Control serums:

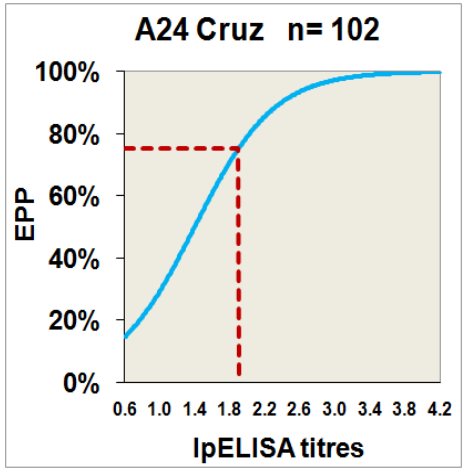
Titers of control sera should not differ in more than ±0.2 from the historical value after a factor (f) is applied . This factor is applied if at least four out of six control sera presented differences, in the same sign.

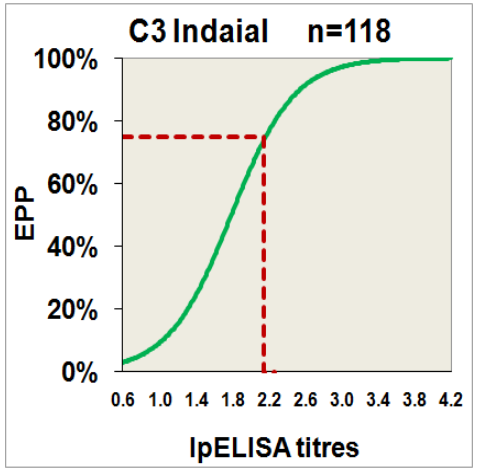
Calculations and validation of each plate were performed using proprietary software (Robiolo , unpublished)

Correlation of EPP and lp ELISA titer by logit transformation









E. Maradei et al. Vaccine 26 (2008) 6577-6586

sLP ELISA: Plate Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	66	67	68
B	2	10	18	26	34	42	50	58	69	70	71	72
C	3	11	19	27	35	43	51	59	C1			100%
D	4	12	20	28	36	44	52	60	C2			100%
E	5	13	21	29	37	45	53	61	C3			100%
F	6	14	22	30	38	46	54	62	C4			100%
G	7	15	23	31	39	47	55	63	C5			100%
H	8	16	24	32	40	48	56	64	100%	100%	BL	BL

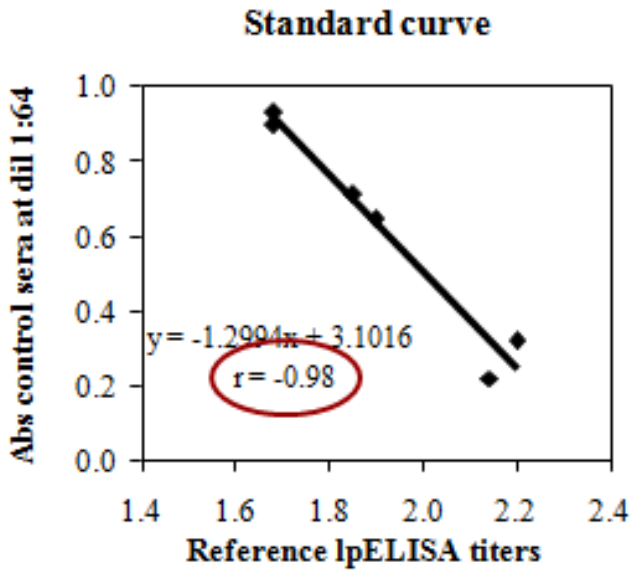
- 72 serums at a single final dilution of 1:64 can be processed
- The conditions for validation of the plate are the same as for the potency FL ELISA
- Calculation and validation of the plates is done by a Software developed by Robiolo B

STANDARD CURVE

Dilution of the control serums (1:64)were chosen so that there is a lineal relationship between its OD readings and titers calculated by final dilutions

A24/Cruzeiro

	Absorbance				Calculated	Reference
Inv. dilution	32	64	128	r	lpELISA titer	lpELISA titer
Log_{10}inv.dil.	1.50	1.80	2.10			
C1	0.028	0.321	0.702	1.00	2.14	2.20
C2	0.251	0.714	1.104	1.00	1.83	1.85
C3	0.447	0.899	1.140	0.98	1.72	1.68
C4	0.020	0.220	0.769	0.97	2.12	2.14
C5	0.188	0.646	0.986	1.00	1.90	1.90
C6	0.461	0.931	1.269	1.00	1.69	1.68



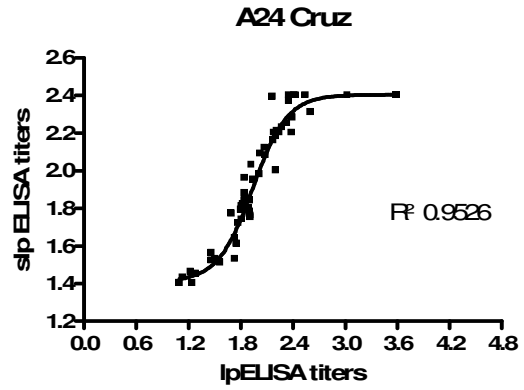
USEFUL TITER RANGE: 1.4 TO 2.4

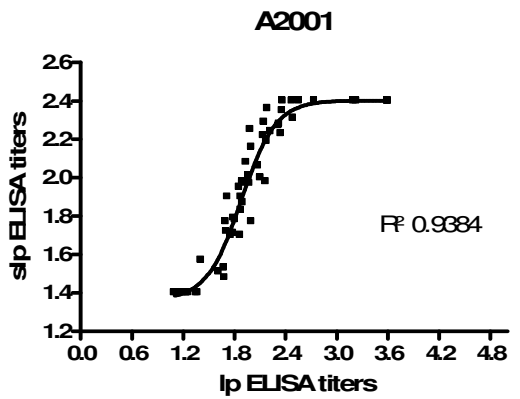
r >0.9

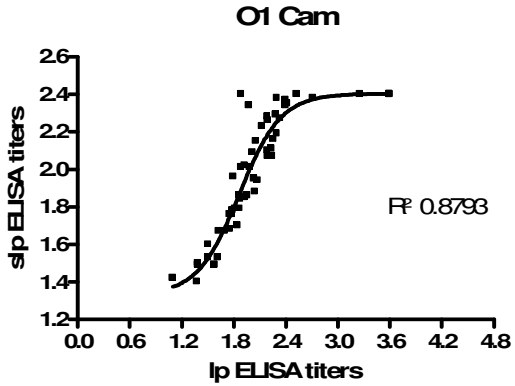
ODs of samples diluted at 1:64 are interpolated to obtain their corresponding titers

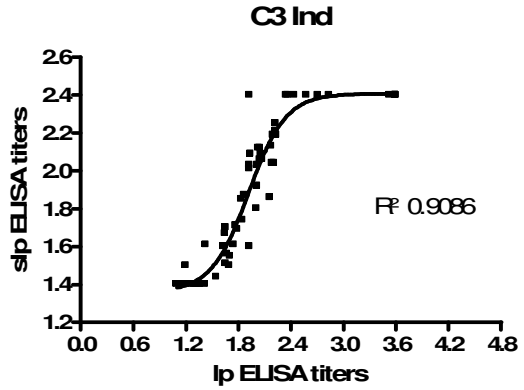
VALIDATION OF slpELISA : CORRELATION WITH lp ELISA

N:60







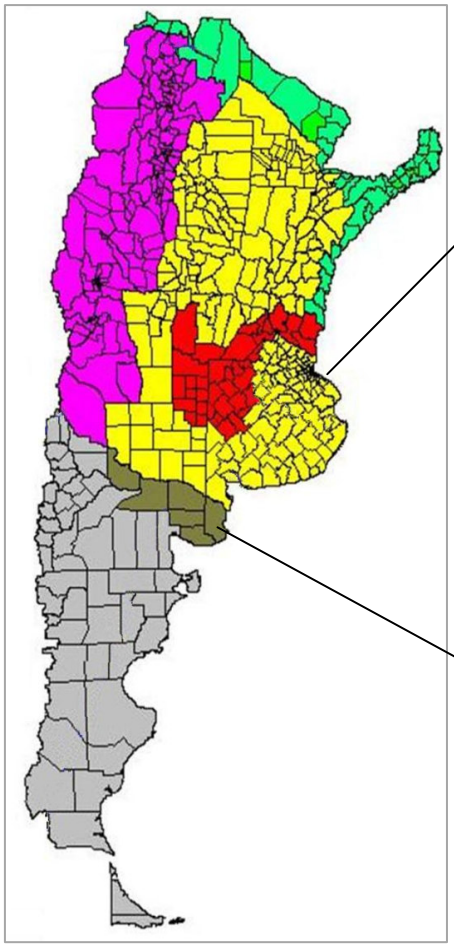


HERD IMMUNITY

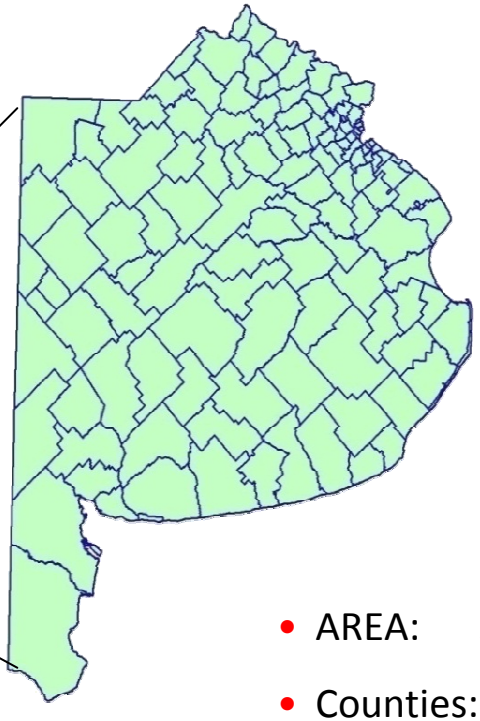
374 VACCINATION UNITS (FARMERS ASSOCIATION) TRAINED AND CONTROLLED BY SENASA

Circa 55.000.000 BOVINES

Circa 1/3 CALVES <1 YEAR



BUENOS AIRES PROV.



- AREA: 300,000 km^2
- Counties: 105
- BOVINES :20 millions
- Farms: 60 000

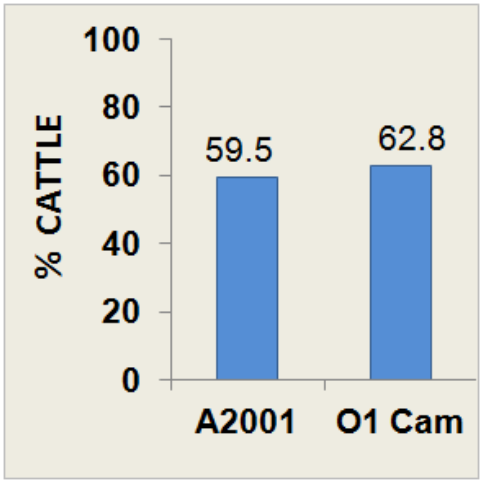
HERD IMMUNITY

SINGLE SERUM DILUTION slpELISA IN NSP NEGATIVE SERA

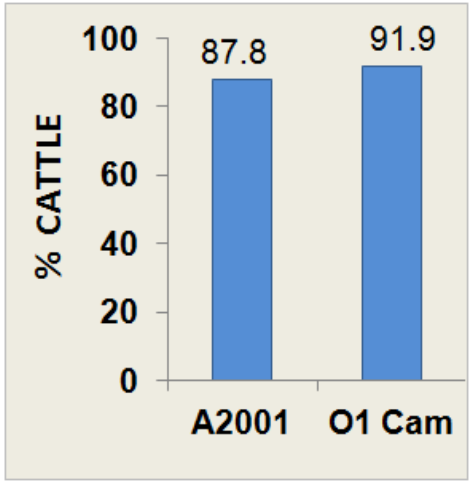
2004 N:20742 39 Counties

% OF ANIMALS WITH slpELISA TITERS COMPATIBLE WITH EPP ≥75%

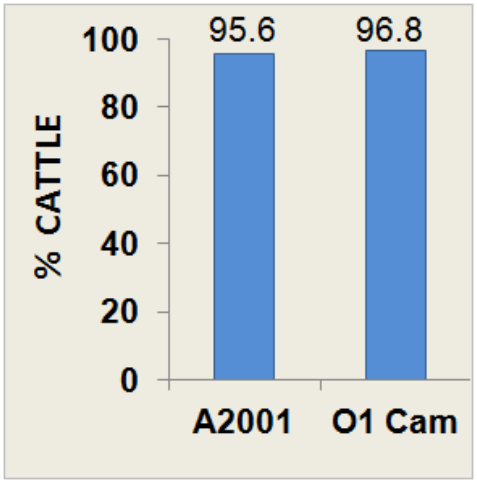
6-12 months
n= 13832



12-24 months
n= 4118



>24 months
n= 2792



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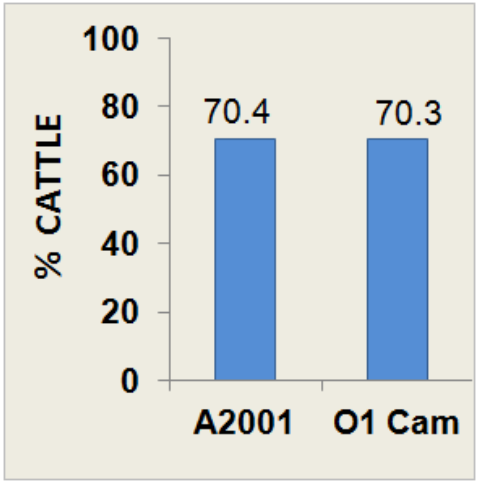
HERD IMMUNITY

SINGLE SERUM DILUTION slpELISA IN NSP NEGATIVE SERA

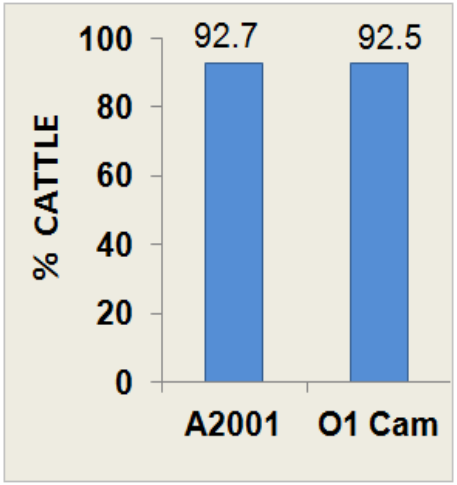
2011 N: 11480 22 Counties

% OF ANIMALS WITH slpELISA TITERS COMPATIBLE WITH EPP ≥75%

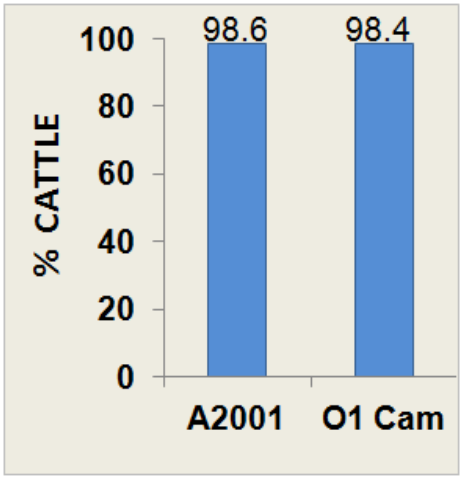
6-12 months
n= 8540



12-24 months
n= 2513

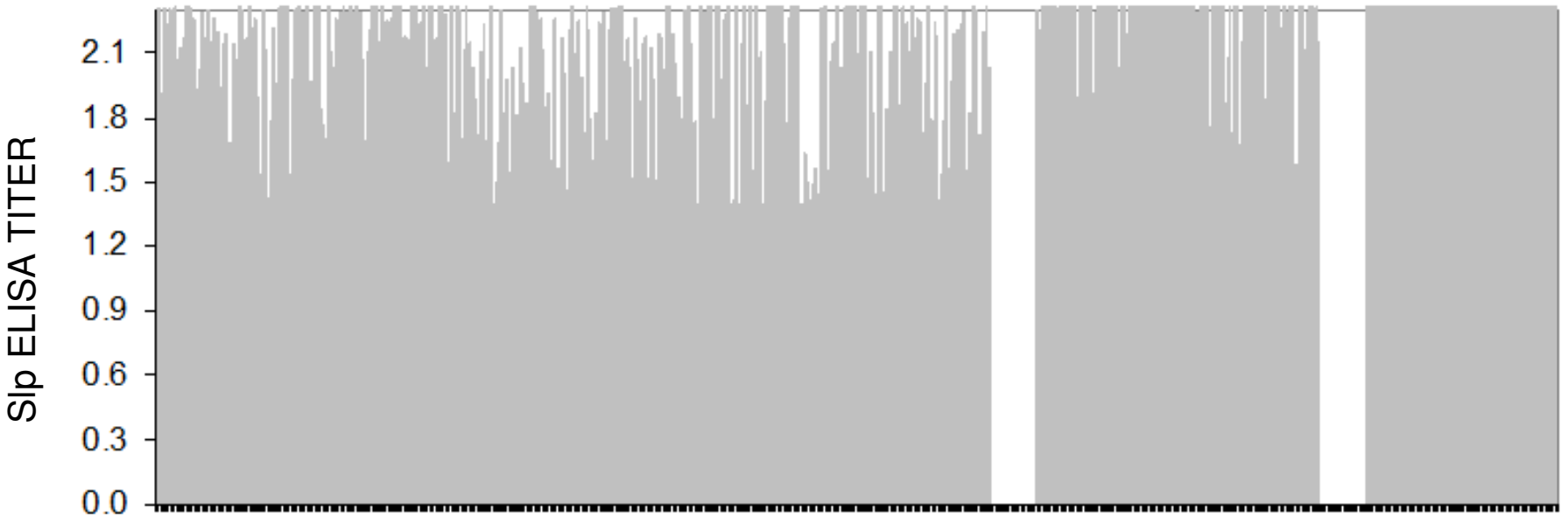


>24 months
n= 427



HEARD IMMUNITY IN BUENOS AIRES COUNTY

SEROTYPE A2001



6 to 12 MONTHS
N: 366

12 to 24 MONTHS
N: 125

> 24 MONTHS
N: 84

Final Remarks

•The slp ELISA presented in this work provides a simple method for evaluation of many samples and is suitable to measure herd immunity .

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•The validation of each plate through blank , antigen and 5 to 6 control serums allows a high **repeatibility.**

•The **reliability** of the method is supported by:

1.The previous correlation between PPG and Ab levels for Argentine Vaccines determinated by lp ELISA (Periolo et al 1993, Robiolo et al.1995, Maradei et al. 2008).

2. The correlation between titres obtained by lp ELISA and slp ELISA

• It provides an adequate method for establish reference levels of protection, for each animals age category in the field and monitor the effectiveness of vaccination campaigns.

CEVAN

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Virginia Sammartino

INTA

Sergio Duffy

Emilio León

COPROSA-Buenos Aires

Adriana Torres

Sanitary Units

SENASA

Intermediate precision of slpELISA
A24/Cruzeiro, A/Arg/01, O1/Campos and C3/Indaial

FMDV strain	Serum Nº	Mean titer[a]	SD	CV%	FMDV strain	Serum Nº	Mean titer[a]	SD	CV%
A24/Cruz	1	≤1.40	0.00	0.0	O1/C	1	≤1.40	0.00	0.0
	2	1.83	0.09	5.0		2	2.09	0.10	4.7
	3	1.54	0.06	4.2		3	1.66	0.06	3.8
	4	1.57	0.16	10.2		4	1.92	0.08	4.0
	5	1.68	0.12	7.4		5	1.68	0.06	3.3
	6	1.46	0.07	5.0		6	1.52	0.07	4.9
	7	2.12	0.21	10.0		7	2.22	0.13	5.9
	8	1.93	0.07	3.8		8	2.12	0.14	6.6
	9	≥2.40	0.00	0.0		9	≥2.40	0.00	0.0
	10	≥2.40	0.00	0.0		10	≥2.40	0.00	0.0
A/Arg/01	1	≤1.40	0.00	0.0	C3/Ind	1	≤1.40	0.00	0.0
	2	1.89	0.09	4.8		2	1.49	0.04	2.8
	3	1.53	0.06	3.8		3	1.48	0.09	5.8
	4	1.71	0.07	4.4		4	1.59	0.17	10.5
	5	2.00	0.10	5.2		5	1.56	0.10	6.2
	6	1.48	0.16	10.9		6	2.38	0.03	1.3
	7	2.20	0.11	5.2		7	1.73	0.12	6.7
	8	2.33	0.05	2.2		8	2.25	0.13	5.7
	9	≥2.40	0.00	0.0		9	≥2.40	0.00	0.0
	10	≥2.40	0.00	0.0		10	≥2.40	0.00	0.0

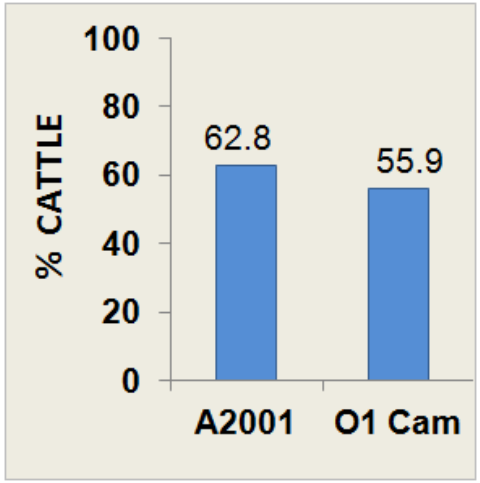
HERD IMMUNITY

SINGLE SERUM DILUTION slpELISA IN NSP NEGATIVE SERA

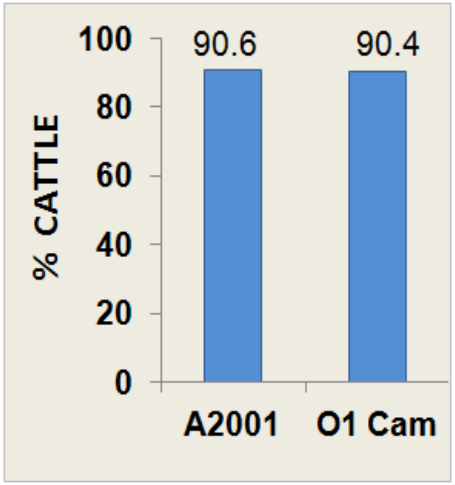
2008 N: 5480 11 Counties

% OF ANIMALS WITH slpELISA TITERS COMPATIBLE WITH EPP ≥75%

6-12 months
n=



12-24 months
n=



>24 months
n= 0

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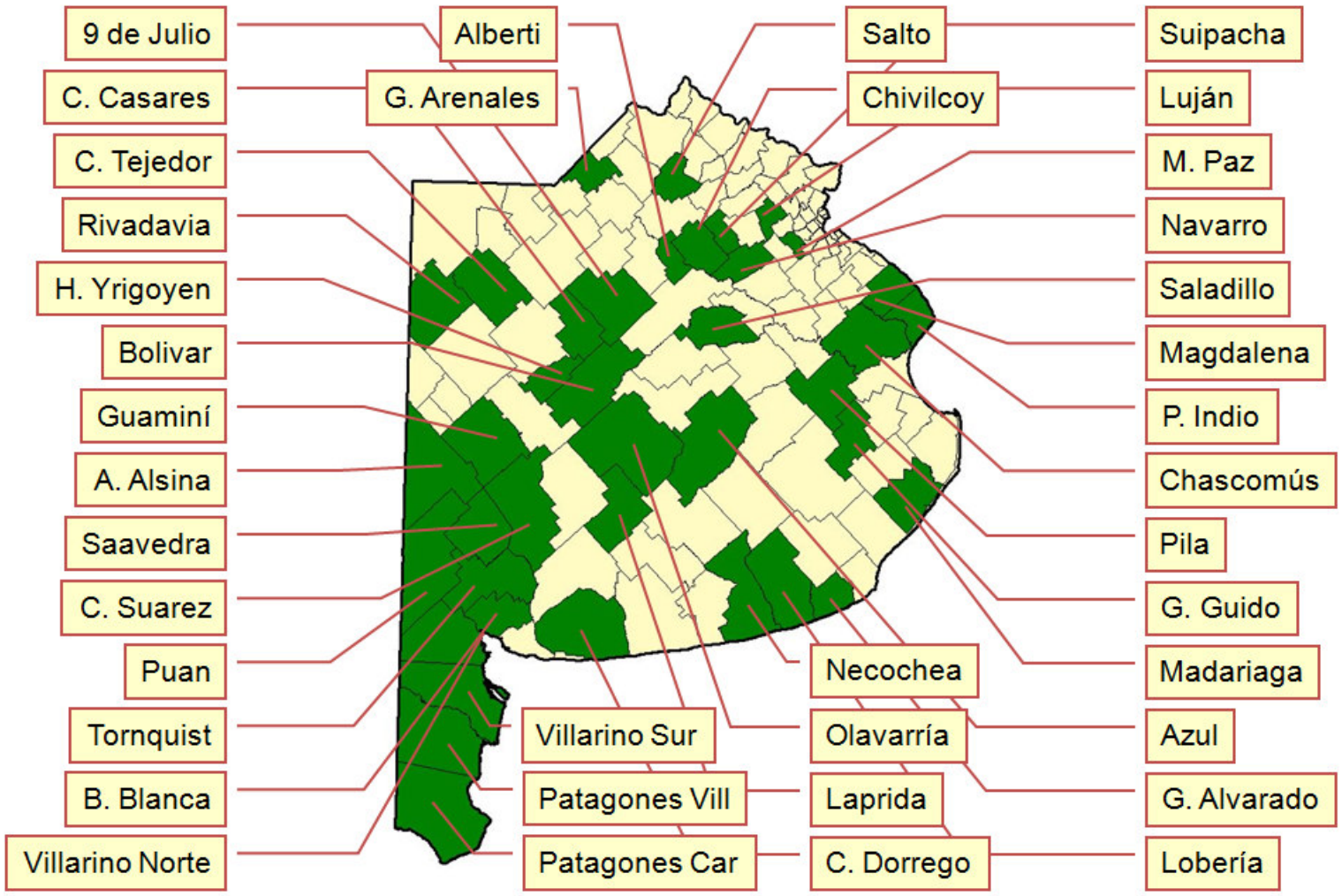
❖ Counties that participate in 2004 (n: 39)



Sampling size

Farms were selected with probability proportionate to the number of animals
Equal number of bovines were selected from each farm.
Cattle population was divided in three categories:

Category	Expected %of protected animals	Max. Acceptable error	N animals/ farm	N Farms per Sanitary units
6 - 12 months	65%	10%	10	42
12 - 24 months	86%	10%	3	42
> 24 months	90%	10%	2	43
				42

- Confidence level 95%
- Homogeneity: low

PRINCIPLE

•Each serum is incubated with a pretitrated dose of the corresponding virus strain .

• Free virus is trapped in an ELISA plates coated with specific PC rabbit serum and detected by a pool of MAbs

•The binding is revealed with Antimouse IgG conjugated and the corresponding color substrate.

Adapted from Hambling et al. (J. Immunol.Methods 93 (1986) 115-121

LIQUID PHASE ELISA