Assessing protective antibody levels in buffaloes using novel and traditional tests in the presence of maternal antibodies

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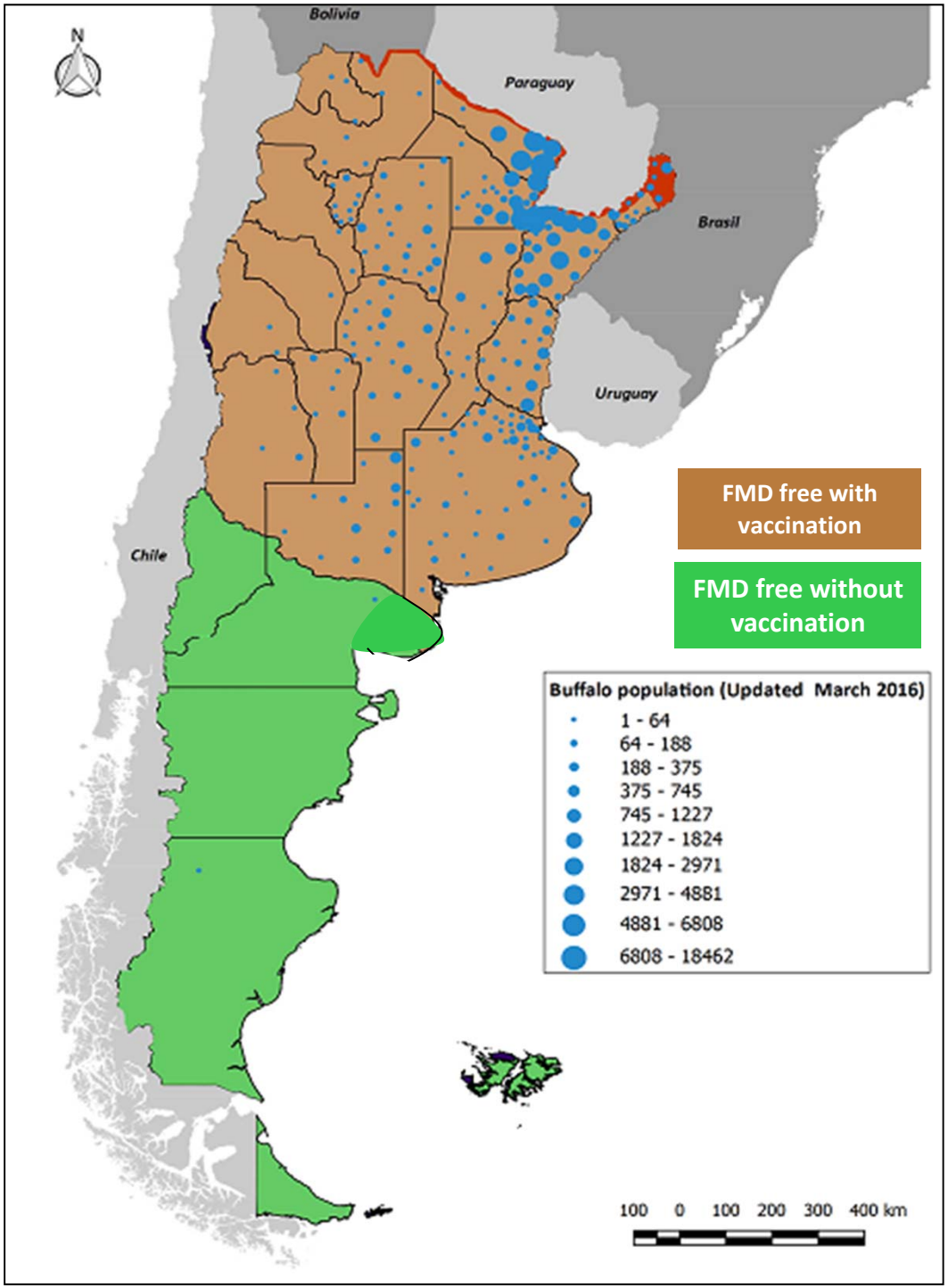


Why buffaloes?

- Water buffalo (*Bubalus bubalis*) population in Argentina is exceeding 100,000 heads (2016)
- It is the third population of South America; after Brazil and Venezuela.
- Buffaloes are located in the north-east of the country within the FMD vaccination territories
- The farms are neighboring areas close to border with Paraguay, where the latest regional FMDV- type O outbreak occurred in 2011

Evaluation of effective vaccination is assessed indirectly using serology

Goal: correctly assessing protective antibody levels

Protection induced by vaccination is indirectly assessed using serology

Antibody titers by Virus Neutralization Test or Liquid Phase Blocking ELISA are correlated with protection in cattle

75% Expected Protection

- Not much work performed with buffalo samples… do they behave as cattle samples?

What does serology tell us?

VNT

Virus neutralization test

Antibodies that prevent virus entry to cultured cells.

Readout: Ab titer preventing 50% of infection

LPBE

Liquid-phase blocking ELISA

Total antibodies, any Ab able to bind the virus. Regardless their isotype, or affinity.

Readout: Ab titer blocking 50% of detector Ab binding

Those “sticky” buffalo antibodies

Gold standard: VNT

LPBE: over-estimates level of protective antibodies

Assay	Sensitivity	Specificity	κ value
LPBE	84.6 (75.5 – 91.3)	**48.6** (97.8 – 59.2)	0.35

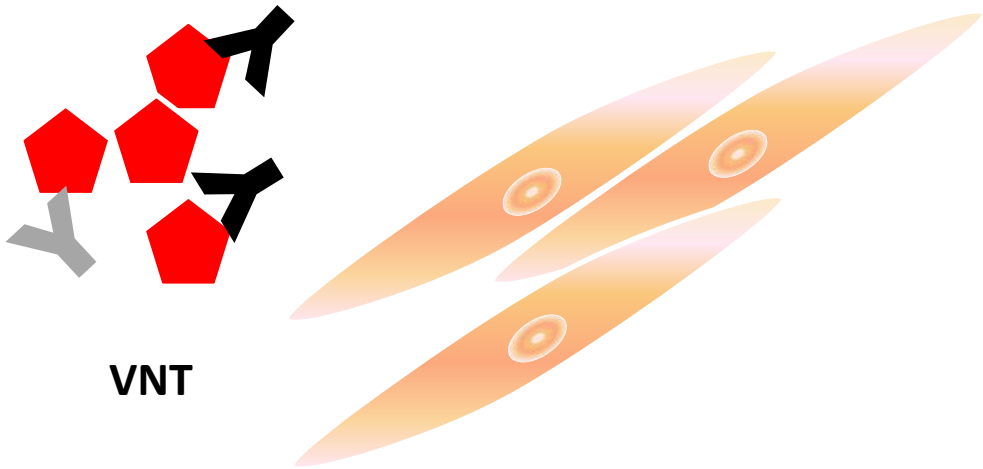
Washing with urea gives an OD value that matches better VNT results.

Assay	Sensitivity	Specificity	κ value
AVIDITY ELISA	74.6 (64.5 – 83.3)	**93.83** (85.0 – 98.8)	0.62

- ***GFRA 2015***
- ***J. Appl. An. Res.* 2017.** Sala, JM et al. Alternatives for the serological assessment of foot-and-mouth disease vaccine immunity in buffaloes (*Bubalus bubalis*). DOI: 10.1080/09712119.2017.1335641

“Sticky” non-specific binders: the buffaloes’ nightmare

High avidity binders Low avidity binders



VNT

LPBE

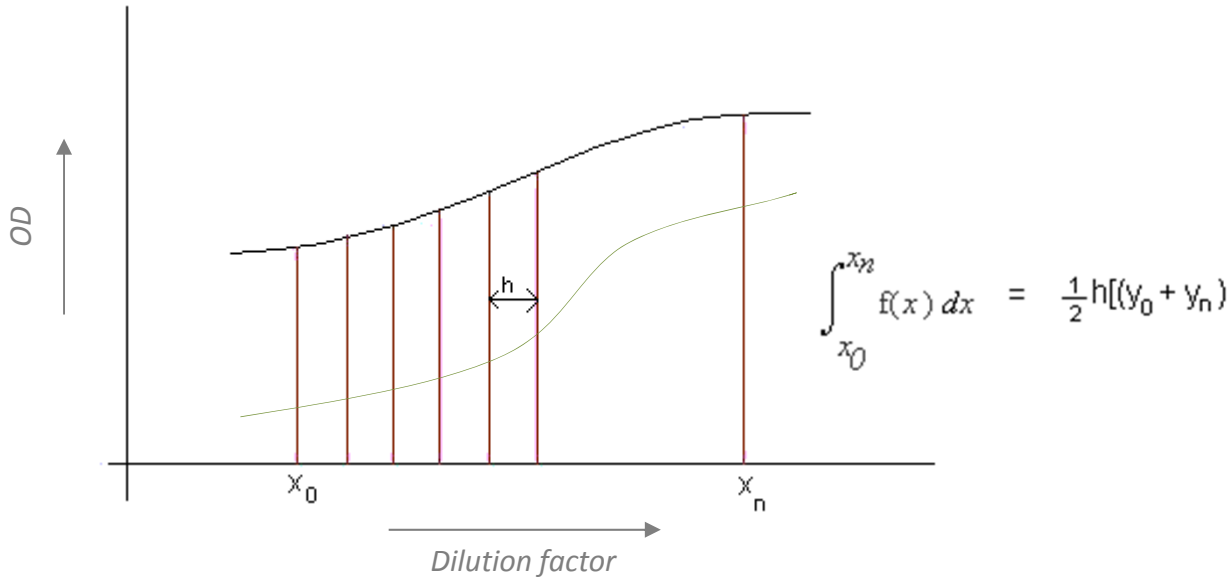
AVIDITY ELISA

The urea washing step detaches low avidity binders

Washing binders with urea gives an OD value that matches better VNT results

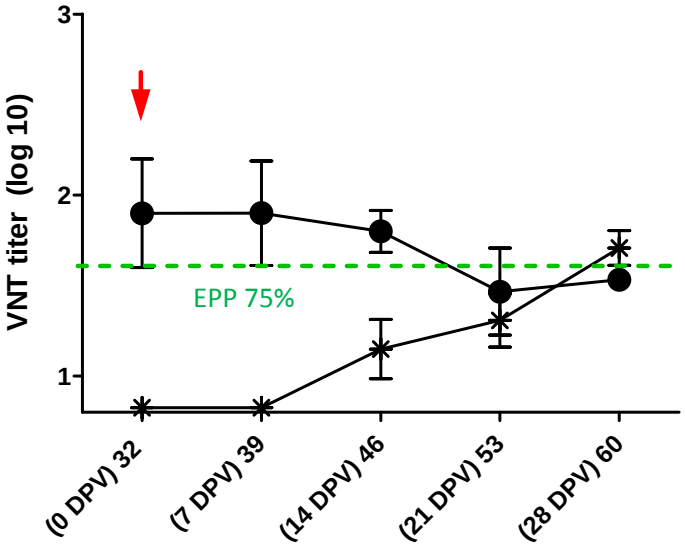
- Single dilution
- Works well comparing all data at a certain time point

To measure AVIDITY INDEX in a kinetics response we diluted serum samples and the trapezium-rule was used to compute the residual area after urea treatment

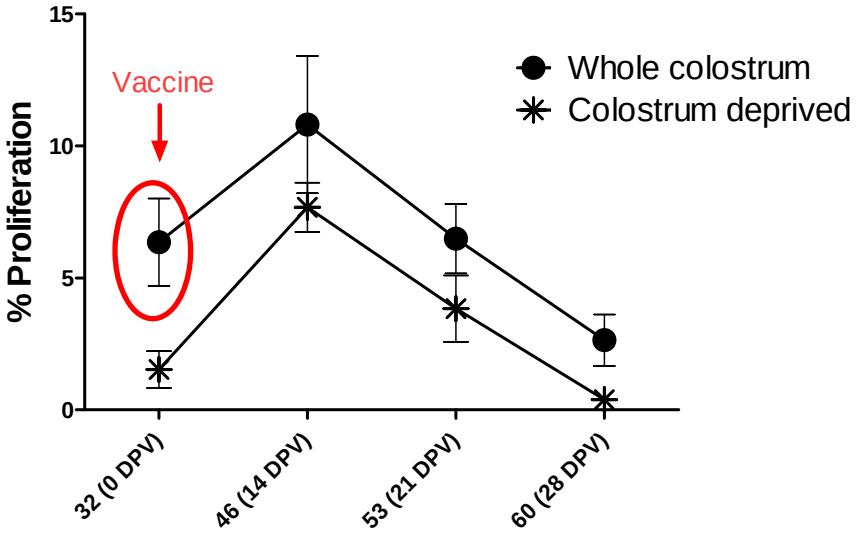


Problems with buffalo serum samples: not *just* the sample

Maternal neutralizing Abs interfere with Ab responses



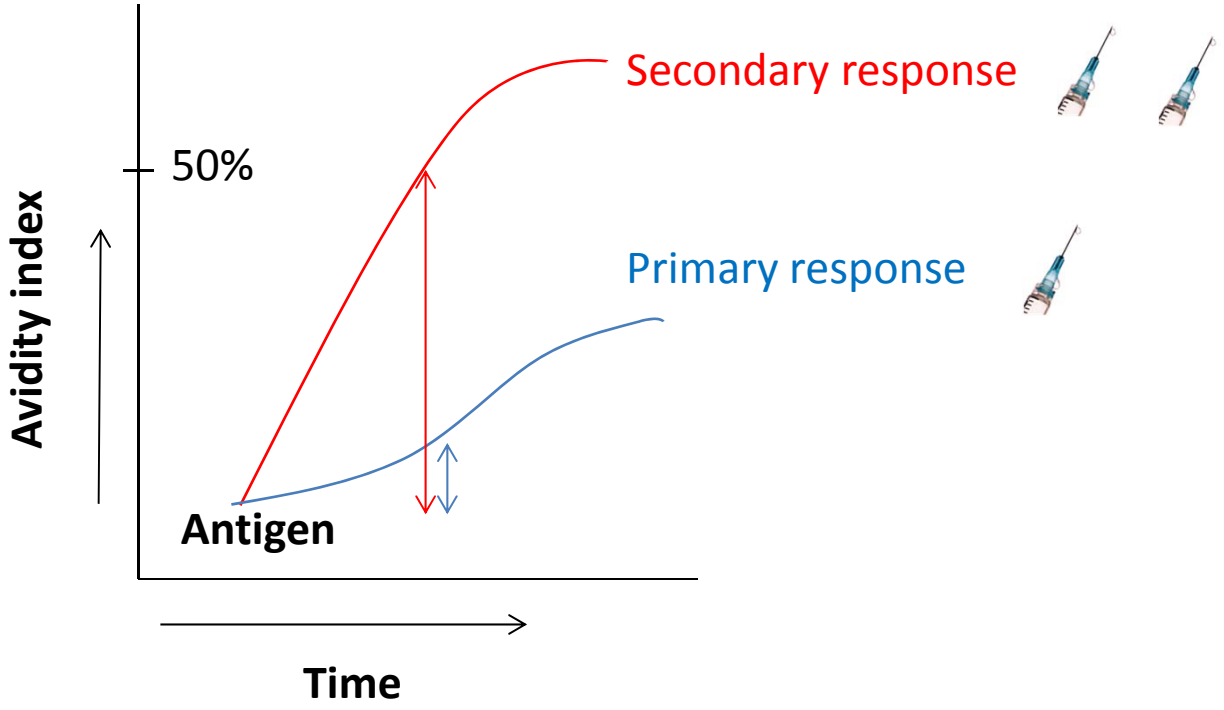
Maternal immunity does not affect T-cell responses



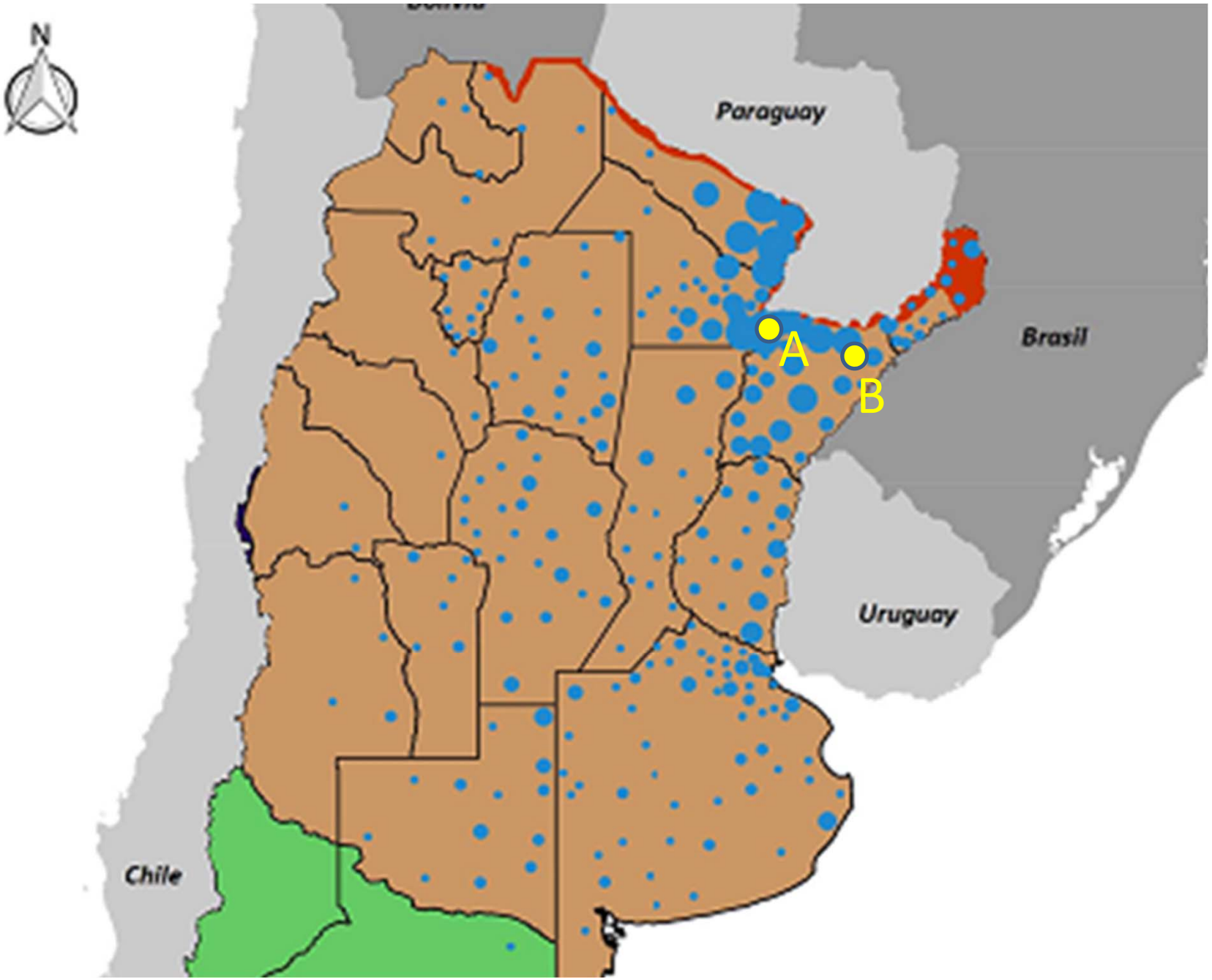
✓ **Maternal immunity (cells and Abs) does not affect the ability of the calf to elicit a primary immune response to vaccination**

- ***GFRA 2013.*** *Influence of antibodies transferred by colostrum in the immune responses of calves to current foot-and-mouth disease vaccines. Capozzo, Bucafusco et al*
- ***Vaccine . 2014.*** *Influence of antibodies transferred by colostrum in the immune responses of calves to current foot-and-mouth disease vaccines. Bucafusco el al.*

Avidity is an useful parameter for detecting secondary responses

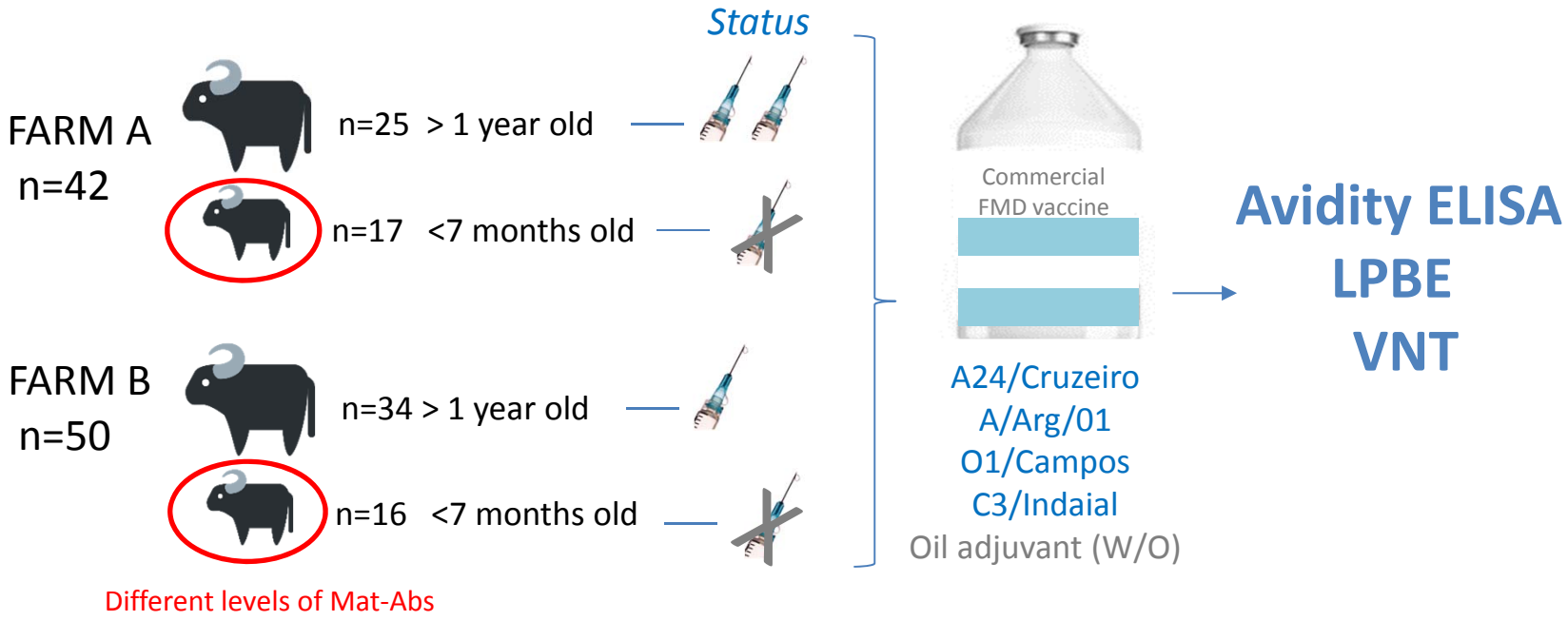


Can FMD-Avidity ELISA be used to detect secondary responses in the presence of maternal antibodies?


N
Paraguay
Brasil
Uruguay
Chile
A
B

Experimental design

Water buffaloes (*Bubalus bubalis*)



Kinetics of antibodies

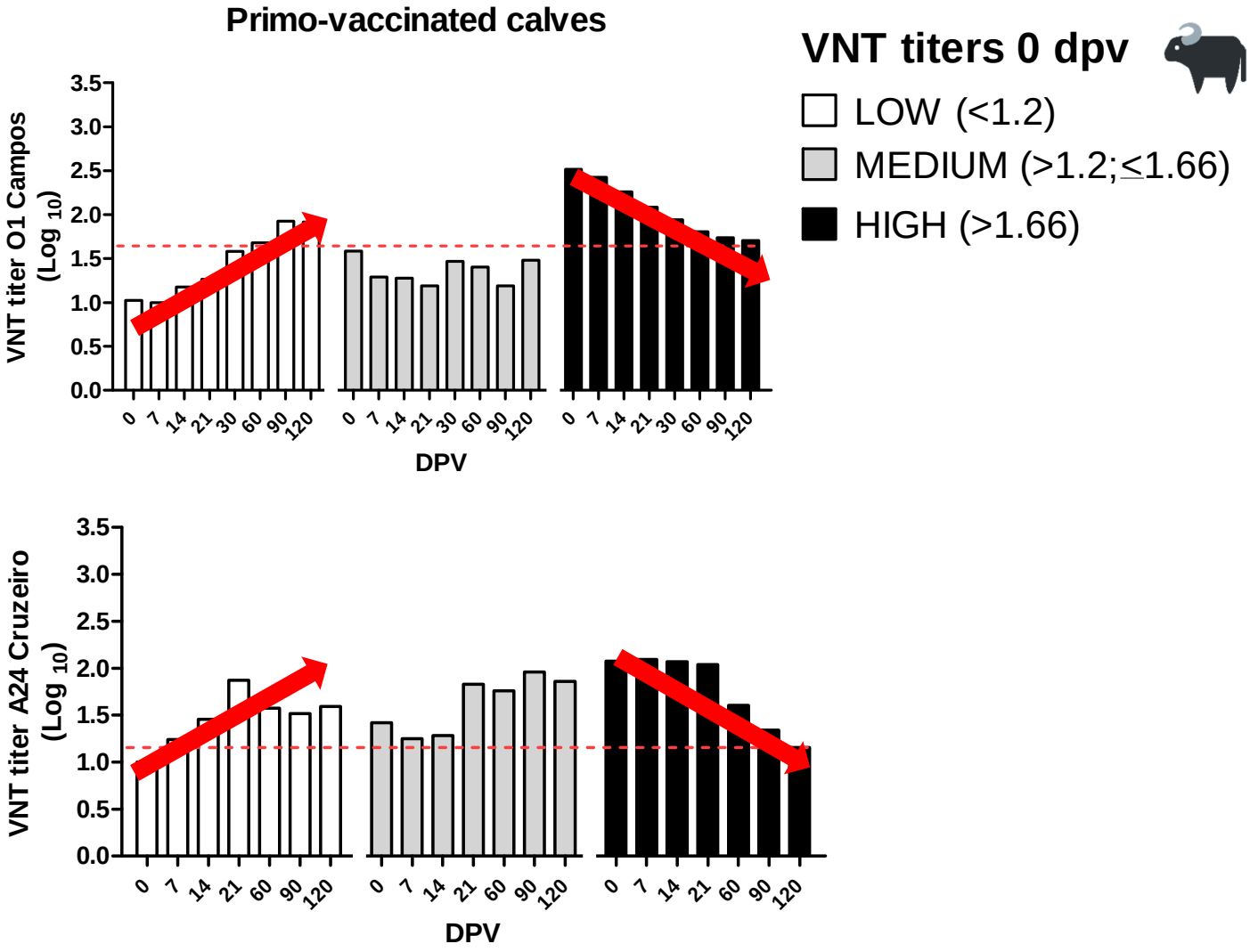
against O1/Campos and A24/Cruzeiro

Naive n=33

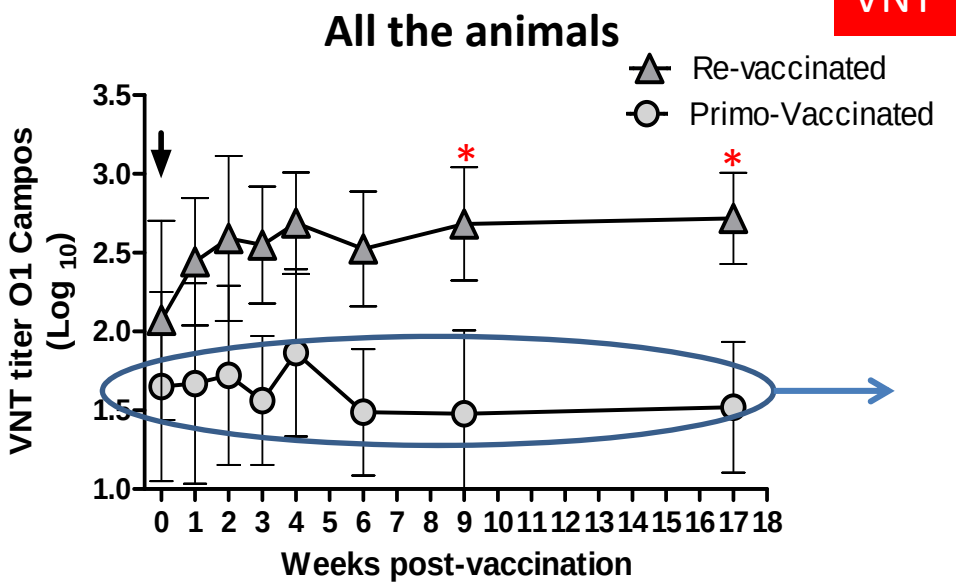
Immune n=59

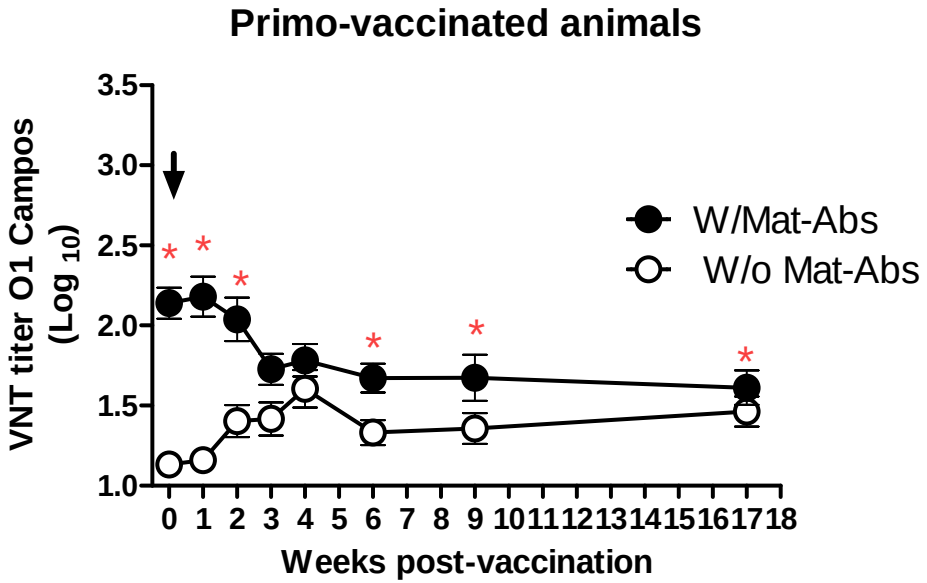
0, 1, 2, 3, 4, 6, 9 and 17 weeks post-vaccination

Maternal neutralizing Abs interfere with vaccination



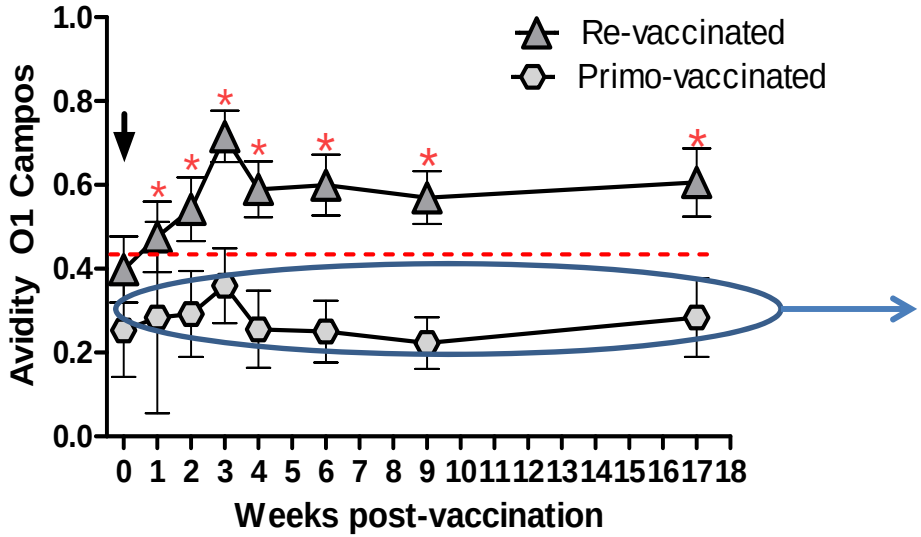
VNT

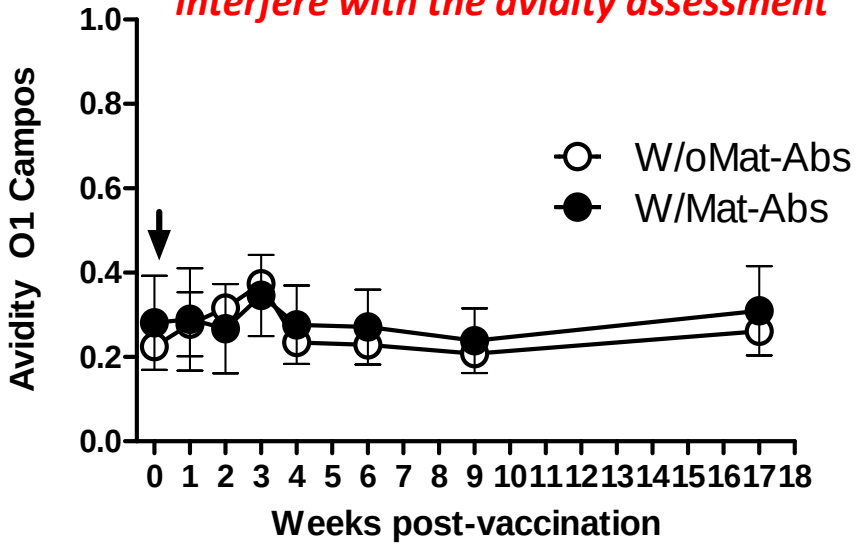




AVIDITY INDEX

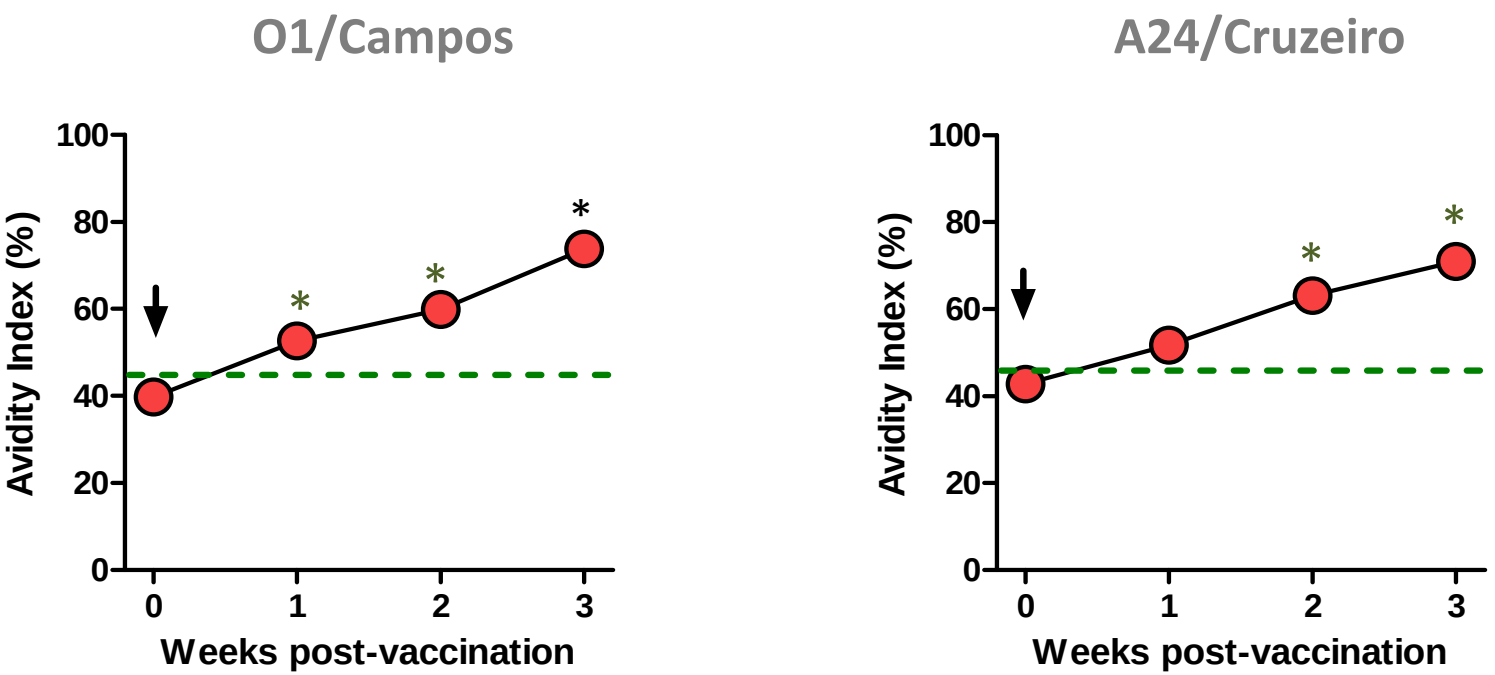
The presence of Mat-Abs does not interfere with the avidity assessment





* Differences were significant between groups ($p<0.05$)

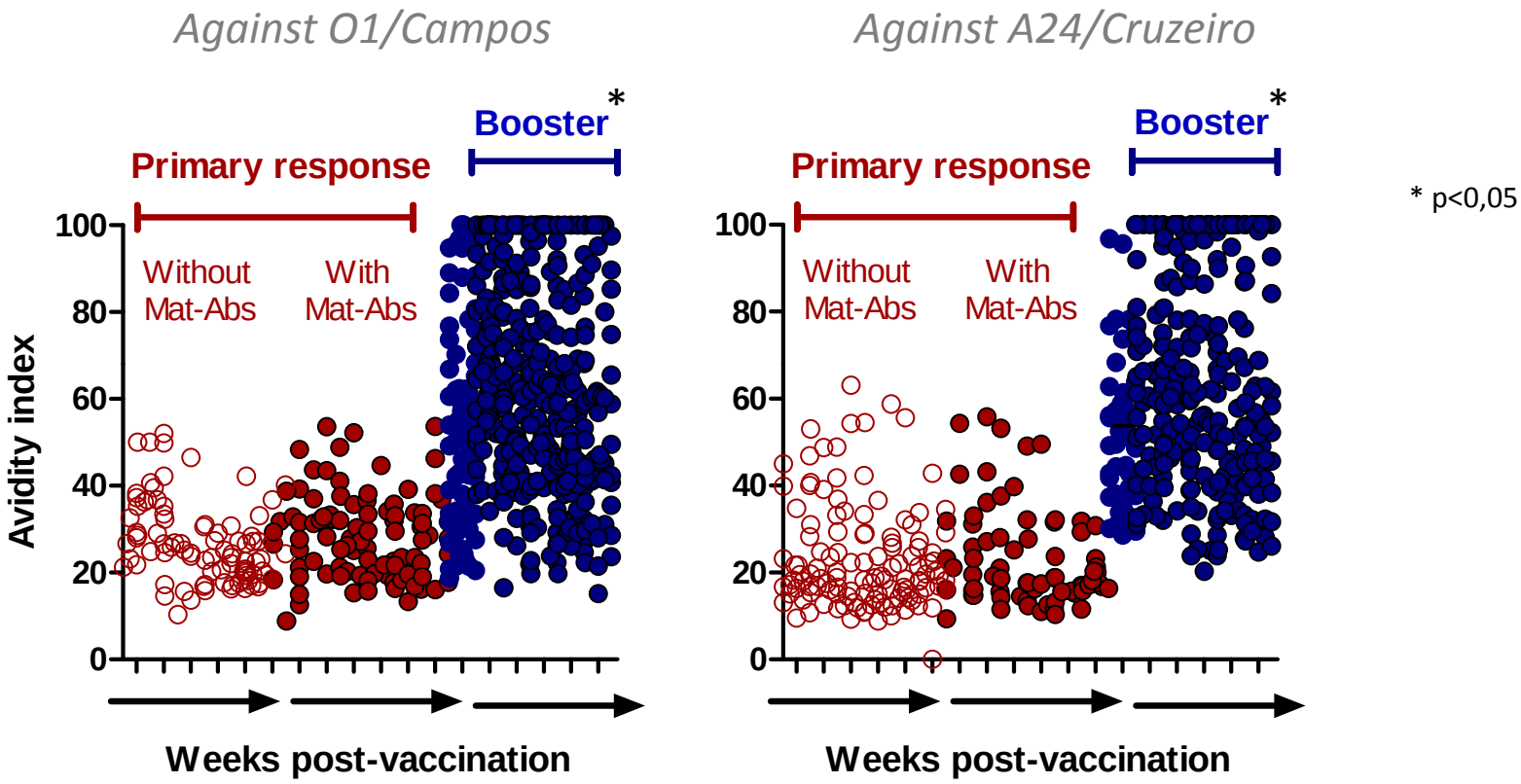
Booster effect



** p<0.05; Significantly different from 0 dpv (ANOVA, Tukey)*

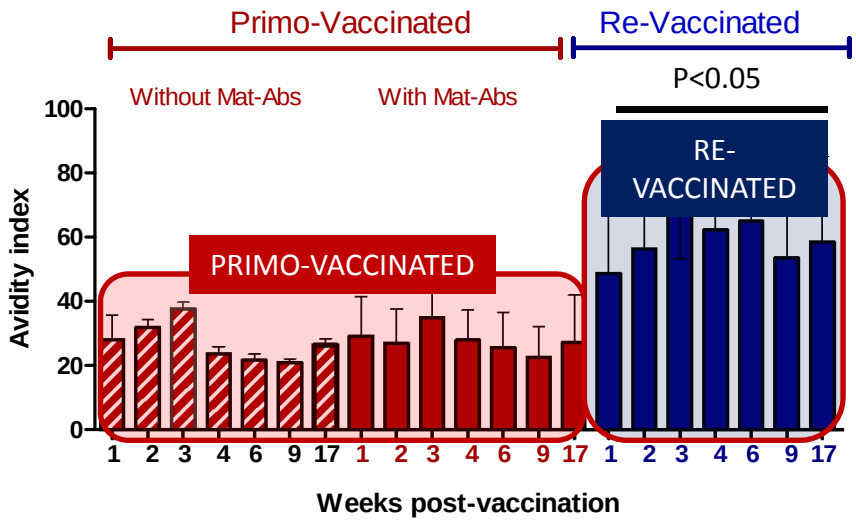
Avidity reveals a booster response 7 to 14 days after the onset

Avidity ELISA discriminated between primo from booster responses even in the presence of Mat.Abs



The presence of Mat-Abs does not interfere with the avidity assessment

Avidity ELISA: detects animals receiving a booster



Indirect correlates of homologous protection

- VNT:
  - good, but difficult for high-throughput
  - do not discriminate primo vs. re-vaccinated in the presence of Mat Abs
- LPBE
  - May not work so well for buffaloes, excellent for cattle and pig samples

AVIDITY ELISA: advantages

- Can be used in SINGLE DILUTION
- Avidity INDEX can be estimated by serial dilutions
- Uses sucrose-gradient purified virus: no need to standardize capture or detector antibodies.
- Applicable to any new strain
- Already tested with bovine, pig and buffalo samples
- Easy to set up : protocol already deployed to several Labs

AVIDITY ELISA: possible applications

- Alternative to VNT, particularly for buffalo samples
- Rapid identification of booster responses
- Discriminate primo from re-vaccinated animals
- Not interfered by maternal immunity.
- Can help identifying vaccinated-infected animals: *in the case of an outbreak vaccinated animals will show an increase in AI even before eliciting detectable anti-NSP Ab.*

Avidity ELISA arises as a useful tool for discriminating between primo from booster responses, due to vaccination or infection, regardless the presence of maternal immunity

Acknowledgements

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Capozzo´s Lab (3 missing)