RE-ESTABLISHMENT OF FMD VACCINE PRODUCTION CAPACITY IN SOUTH AFRICA

IMMUNOGENICITY STUDIES OF THE PENTAVALENT VACCINE'S STRAINS

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BACKGROUND

- South Africa vaccinates approximately 100 000 cattle in the control zone (areas surrounding the Kruger National Park) on a biannual basis, using imported vaccines.

Because:

- In the late 2000s, a downward trend in production yields was experienced, culminating in the cessation of production activities.

- Investigations suggested possible causes as a result of:
  1. Old production infrastructure (non compliant to the then cGMP requirements)
  2. Contamination with biofilms.

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BACKGROUND

- **Embarked on a bench top scale research using commercial reagents, including water and thereby:**

1. Identified 5 virus strains representing the 3 SAT types.
   (based on their individual r 1 values)

2. Adapted the viruses for suspension based cultivation in BHK cells.

3. Optimised growth kinetics per virus strain selected.

4. Through immunogenicity studies, established (per strain):
   1. Optimal antigen payloads for vaccine formulation
   2. Vaccine stability/efficacy following storage at 4°C
   3. Potency.

5. Implemented cGMP standards for cells, virus and antigen stocks management.

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OBJECTIVE

To report on the immunogenicity of the five SAT type isolates selected for the preparation of a DOE- FMD vaccine in South Africa.

- **We have established:**
  1. Suitable antigen payloads for each of the antigens forming part of our pentavalent vaccine, that will induce protective neutralizing antibodies in vaccinated guinea pigs.
  2. Stability of vaccine when used to vaccinate guinea pigs following storage periods of 3; 6; 9 and 12 months at 4°C.
  3. Potency of the vaccine in cattle, using varying amounts of vaccine and challenging them with virulent field strains homologous to the vaccine strains.
  4. Currently establishing the duration of humoral immune response elicited by the pentavalent vaccine in cattle.

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1. SUITABLE ANTIGEN PAYLOADS

- Two pentavalent vaccines with varying amounts of antigens were prepared:

STRAIN	FULL DOSE		HALF DOSE	
SAT 1A	3,0µg	Total of 6,0 µg	1,5 µg	Total of 3,0µg
SAT1B	3,0µg		1,5 µg	
SAT 2A	3,0µg	Total of 6,0 µg	1,5 µg	Total of 3,0µg
SAT 2B	3,0µg		1,5 µg	
SAT 3	3,0µg	Total of 3,0 µg	1,5 µg	Total of 1,5µg
ADJUVANT	ISA 206 VG™ (SEPPIC, FRANCE)			
OTHERS	BUFFERS AND PRESERVATIVES			

- Following confirmation of vaccine's sterility, intact 146S particle and guinea pigs' (Gps) habituation period of 10 days, 4 groups of FMD negative Gps were immunised as follows:

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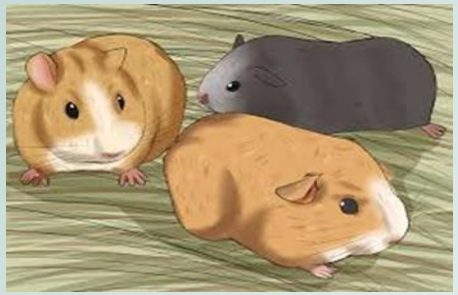
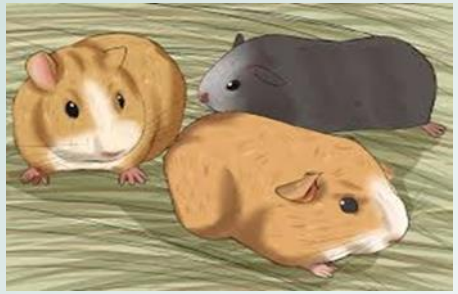
IMMUNISATION OF GPs

Full dose (3,0 µg/dose/strain)

Group 1, n =10: 0,5ml immunisation

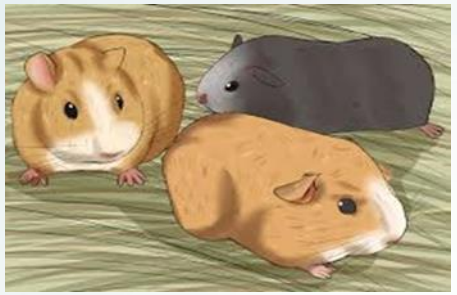


Group 2, n = 10: 0,2 ml immunisation

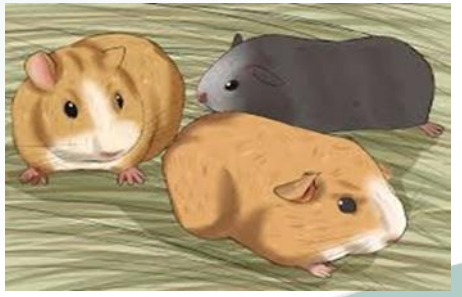


Half dose(1,5 µg/dose/strain)

Group 3, n =10: 0,5ml immunisation



Group 4, n = 10: 0,2 ml immunisation



- All animals were terminally bled 28dpv and serum samples from each blood sample subjected to virus neutralisation test against individual vaccine's strain

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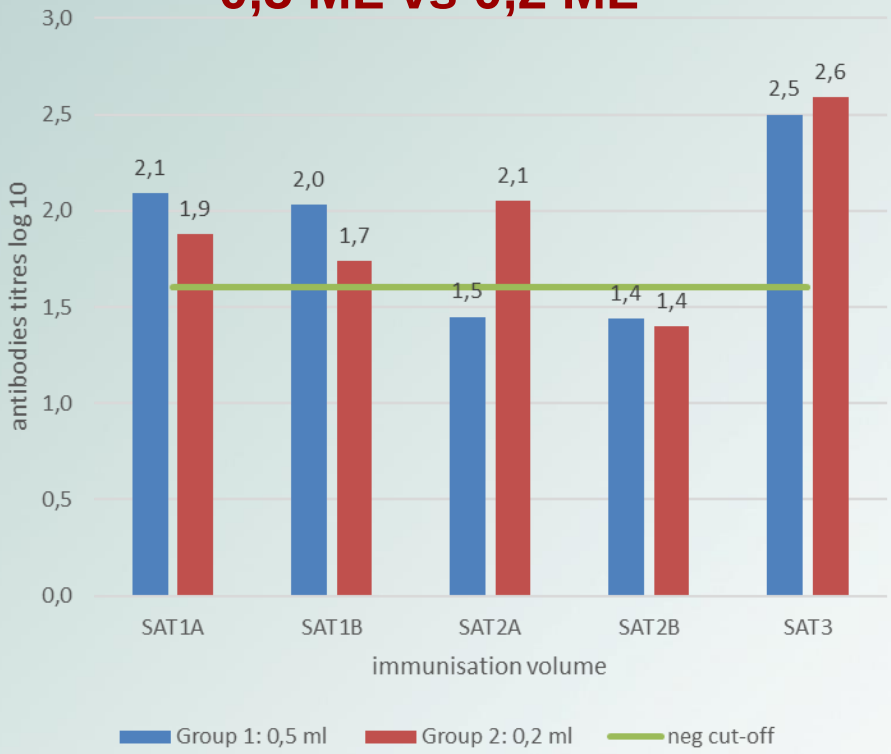
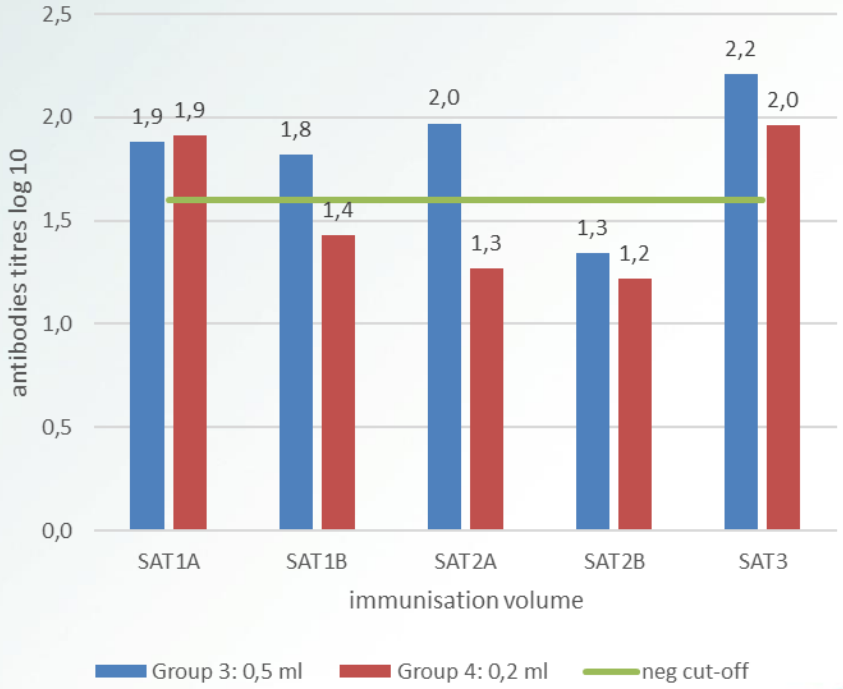
RESULTS

FULL DOSE IMMUNISATION: 0,5 ML vs 0,2 ML



HALF DOSE IMMUNISATION: 0,5 ML vs 0,2 ML



1. On average, immunisation with 0,5 ml of full dose vaccine elicited approx. 0,2 log 10 Ab titres higher than the 0,2 ml immunisation. A similar trend was observed with the half dose vaccines immunisations.
2. Questionable and unsatisfactory Ab responses against SAT 2 antigens were observed for both formulations, irrespective of volumes administered.

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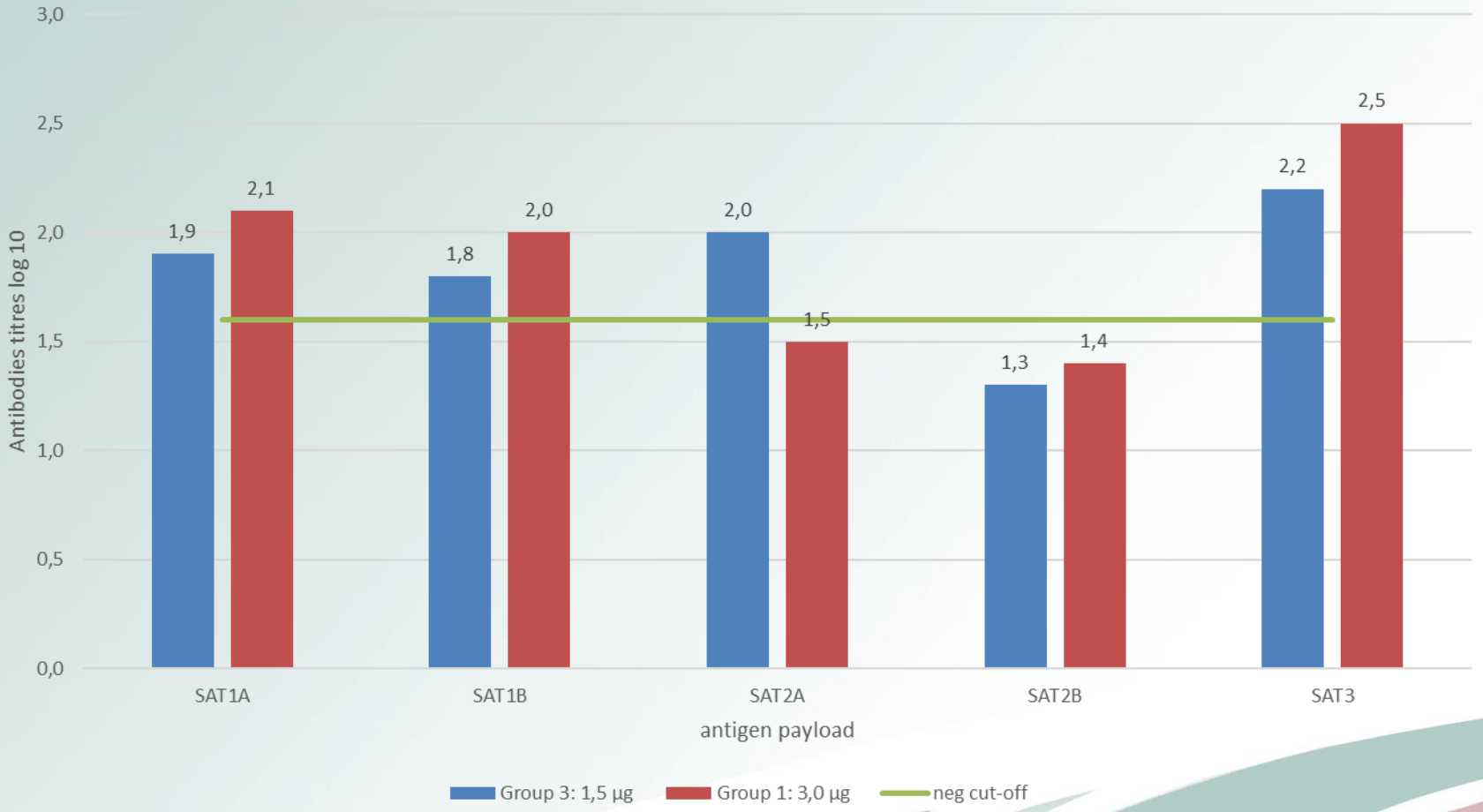
0,5 ML IMMUNISATION: FULL vs HALF DOSE



- Generally, immunisation with 0,5 ml of the full dose vaccine elicited higher neutralising Ab than the immunisation with the same volume of the half dose vaccine.

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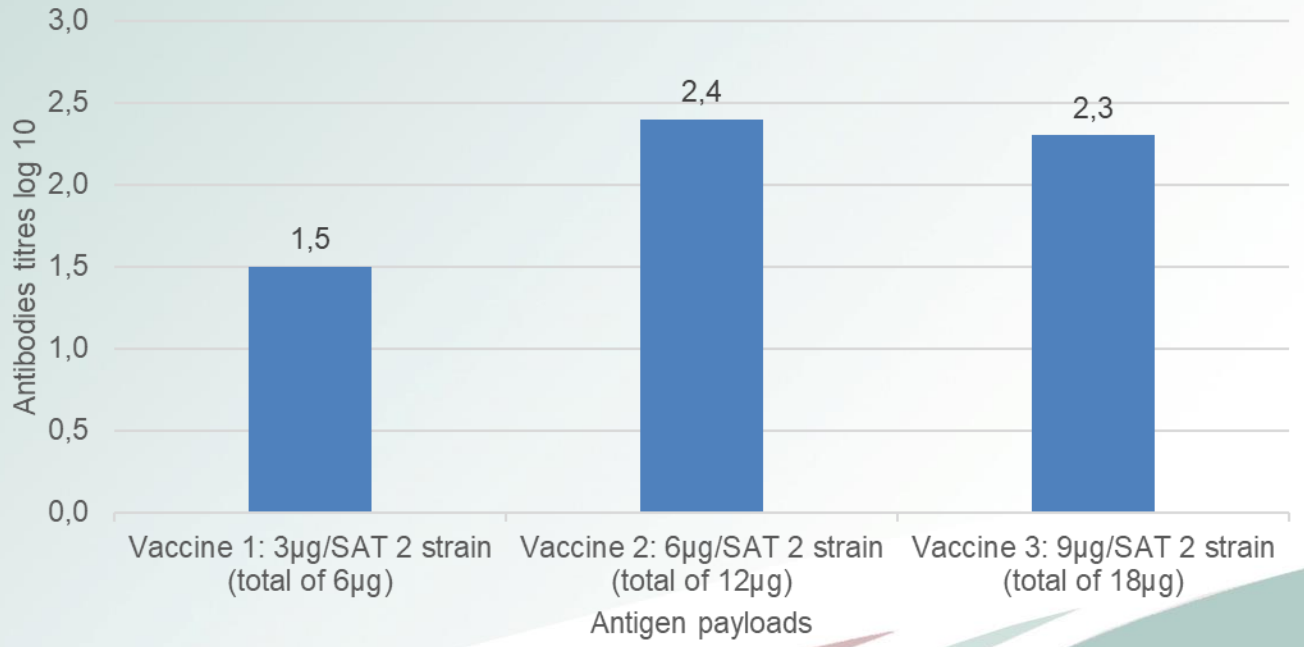
OPTIMAL SAT 2 ANTIGEN PAYLOAD

- Following indications that insufficient neutralising Ab were elicited against SAT 2 antigens for both formulations,
- 3 fresh pentavalent vaccines comprising of a total of 6, 12 and 18 µg/dose for SAT 2 antigens (i.e. 3,6,9 µg/SAT2 strain/dose) were tested in Gps.



- We opted for a pentavalent vaccine comprising of 6,0 µg/SAT2 strain/dose as well as 3,0 µg/SAT 1 & 3 strains/dose for future vaccine formulations.

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2.VACCINE EFFICACY FOLLOWING STORAGE AT 4°C & POSSIBLE VACCINE STABILISERS

- We formulated 3 pentavalent vaccines,

Vaccine constituent	Vaccine 1	Vaccine 4	Vaccine 5
Total SAT 1	6 µg/dose		
Total SAT 2	12 µg/dose		
Total SAT 3	3 µg/dose		
Standard components	x	x	x
HEPES Buffer		x	x
$MgCl_2$		x	

- and used them to immunise 3 groups of Gps following vaccines' storage periods of 1, 3, 6, 9 and 12 months at 4°C.
- At each testing period, we collected blood samples and terminated the Gps 28 dpv.
- VN tests were conducted on serum samples after each test period, to measure the level of Ab against individual vaccine strains.

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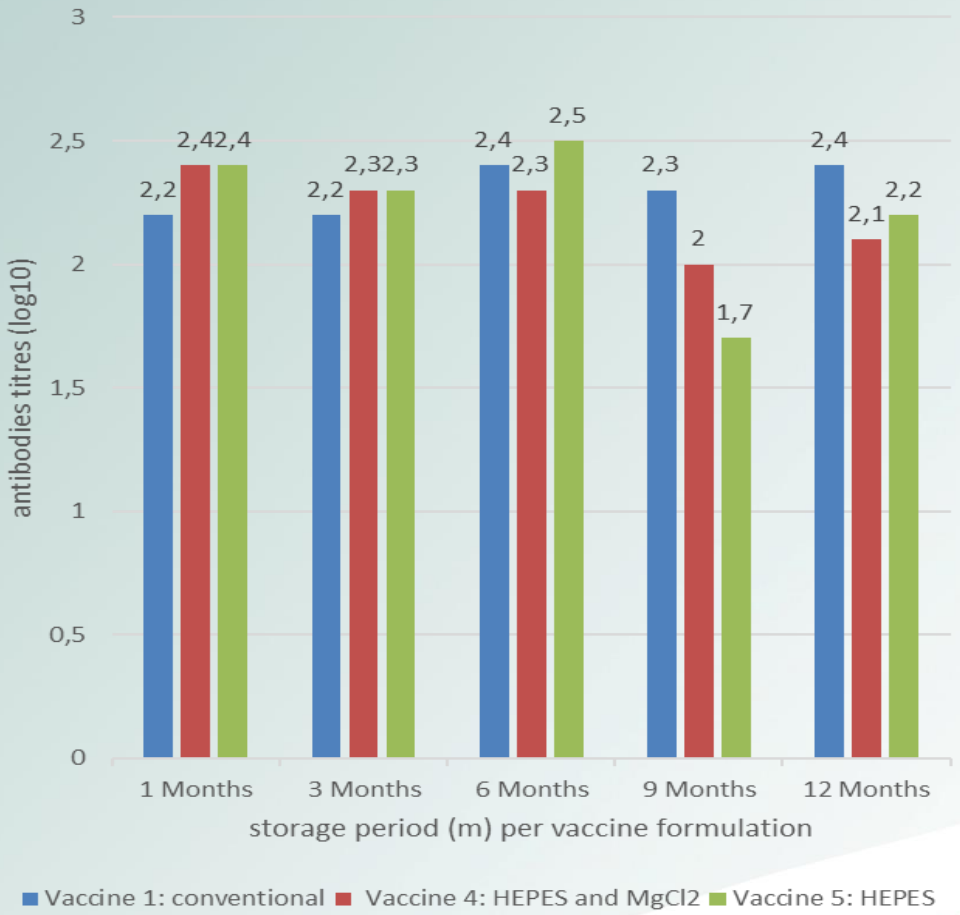
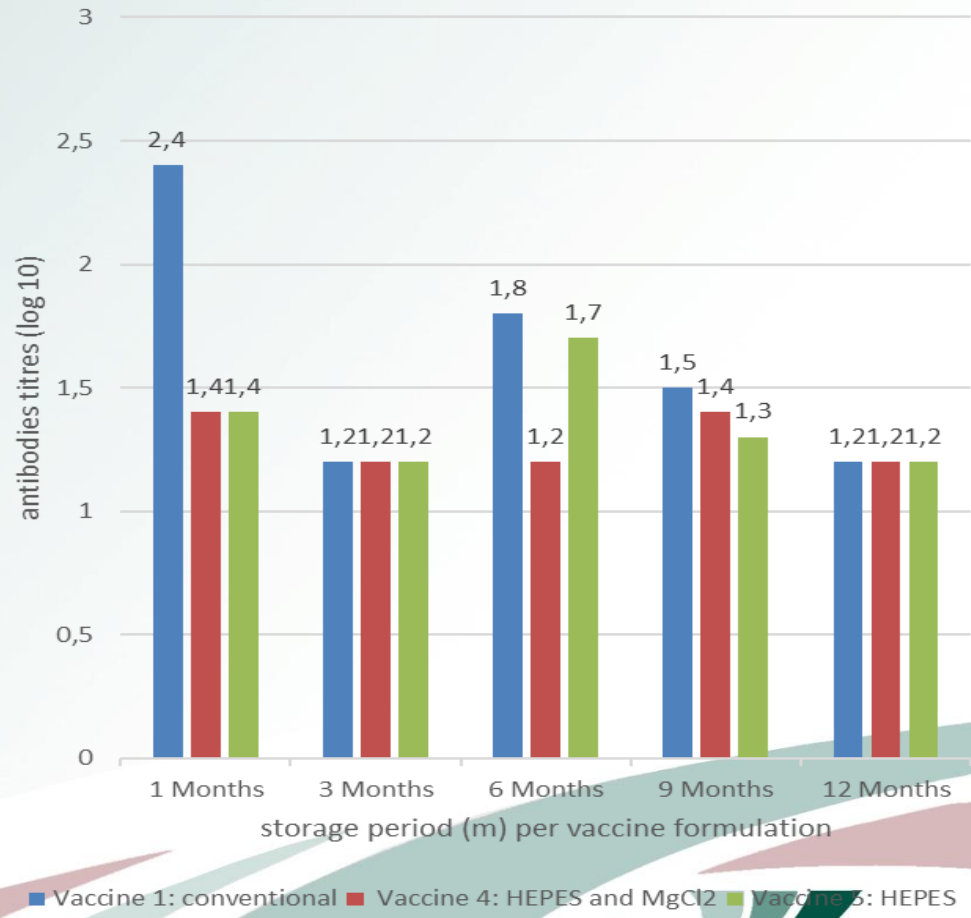
RESULTS: IMMUNE RESPONSE AGAINST SAT 1 & 2 POST VACCINATION





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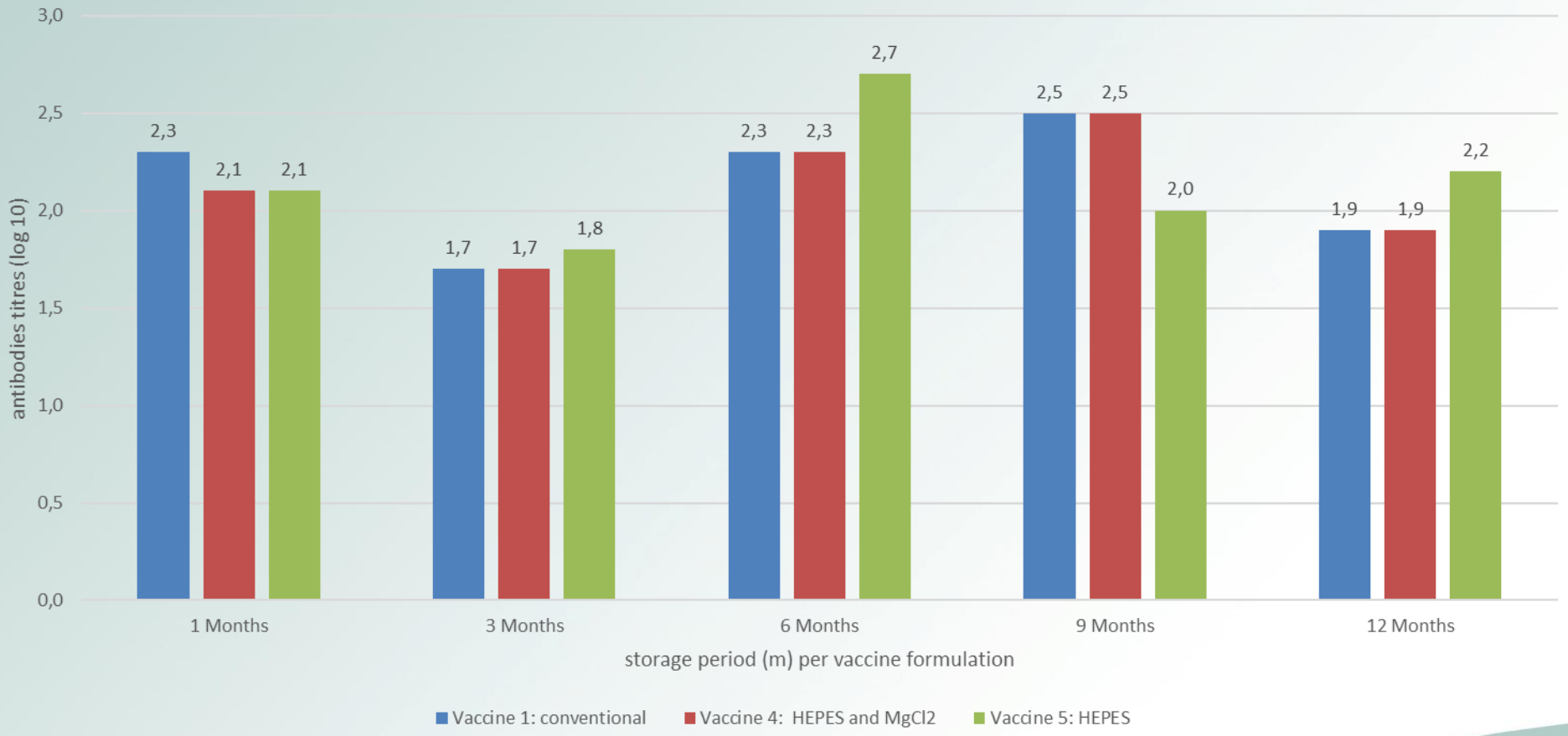
IMMUNE RESPONSE AGAINST SAT 3 POST VACCINATION



1. Ab responses against SAT 2 antigens inconsistent .
2. HEPES buffer and $MgCl_2$ do not significantly improve vaccine stability during storage (based on SAT 1 and 3 Ab responses over 12 months).
3. Test to be repeated due to Ab responses against SAT 2 antigens.
4. Meanwhile, future vaccines to be formulated conventionally.

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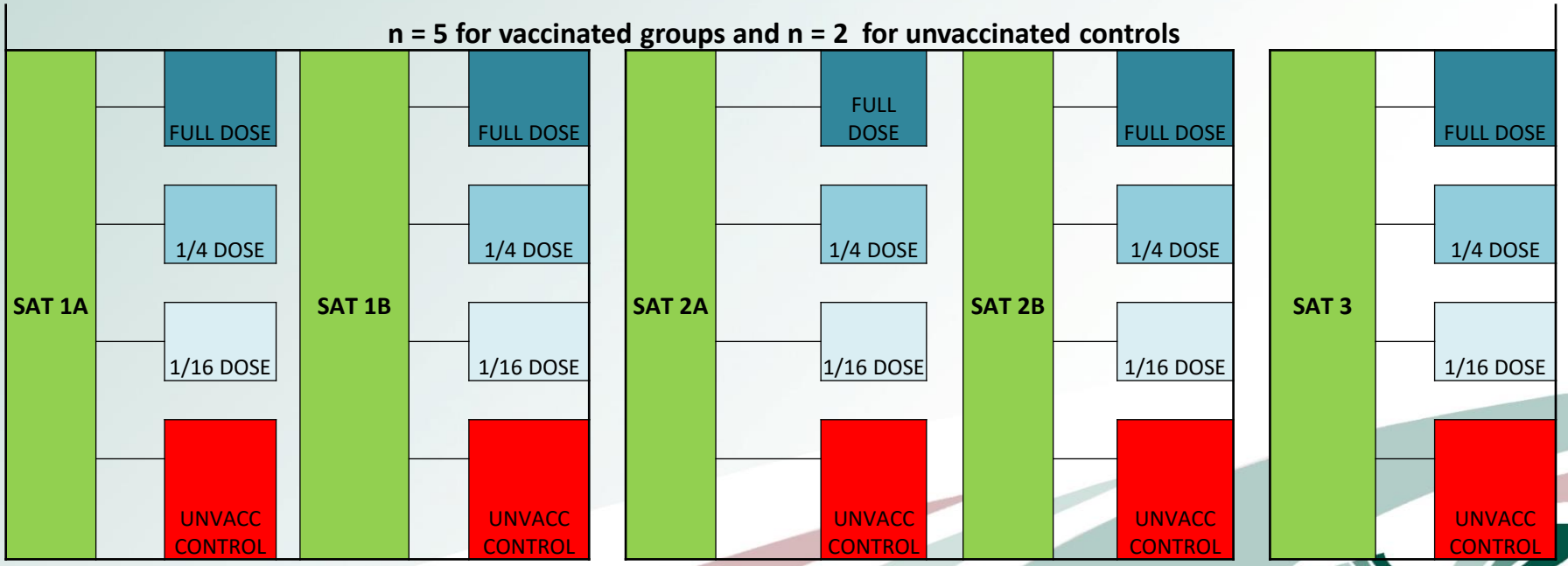
3. VACCINES' PD50 TEST IN CATTLE

- The study was conducted according to the method described in the OIE manual of diagnostic tests and vaccines for terrestrial animals.

- Five monovalent vaccines representing the pentavalent vaccine's antigens were formulated and used to immunise groups of 6 months old cattle per strain with reducing volumes of respective vaccines.



- 10^4 BID was used to challenge 17 cattle/strain, 28 dpv and monitored for 10 dpc.

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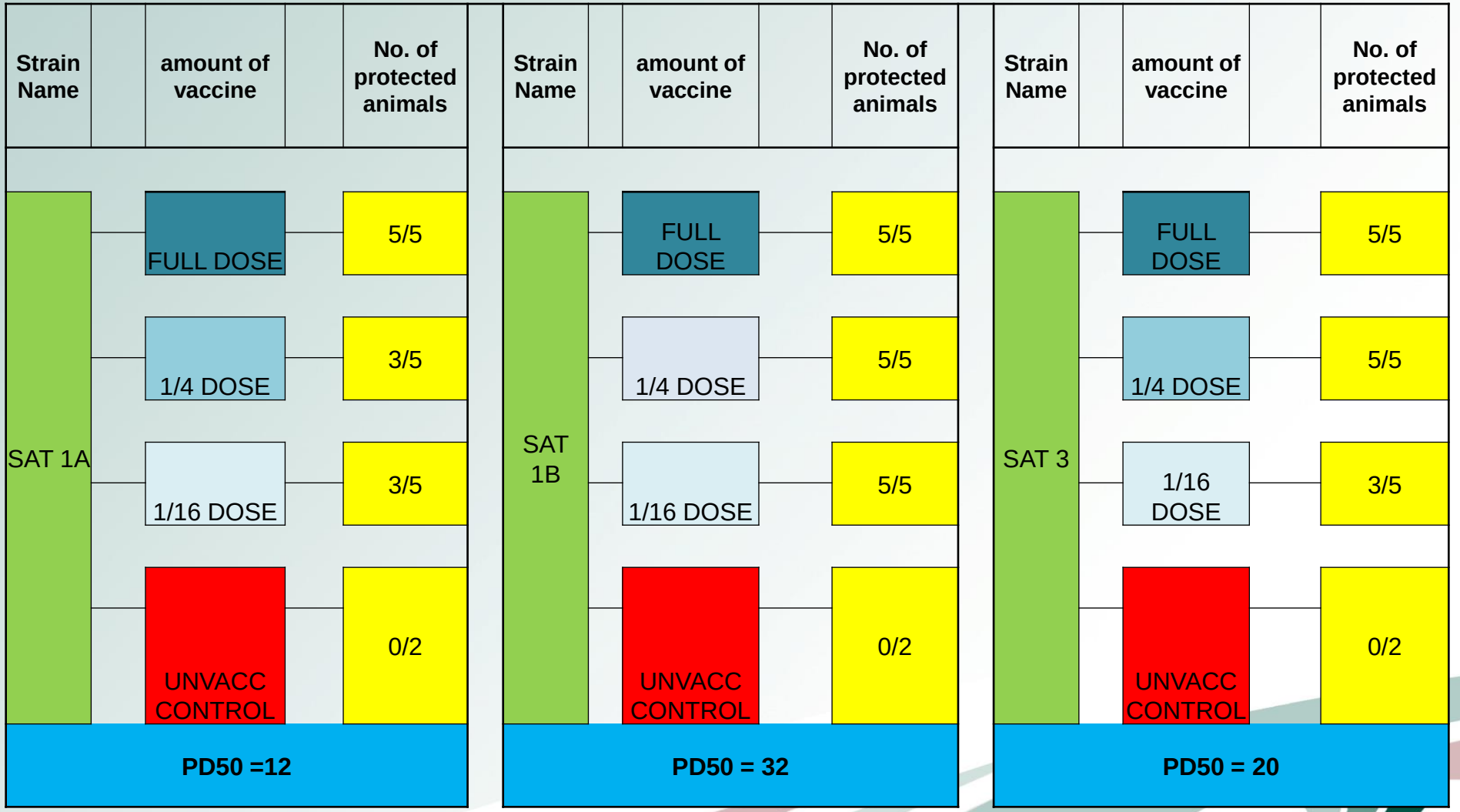
PD50 RESULTS PER STRAIN



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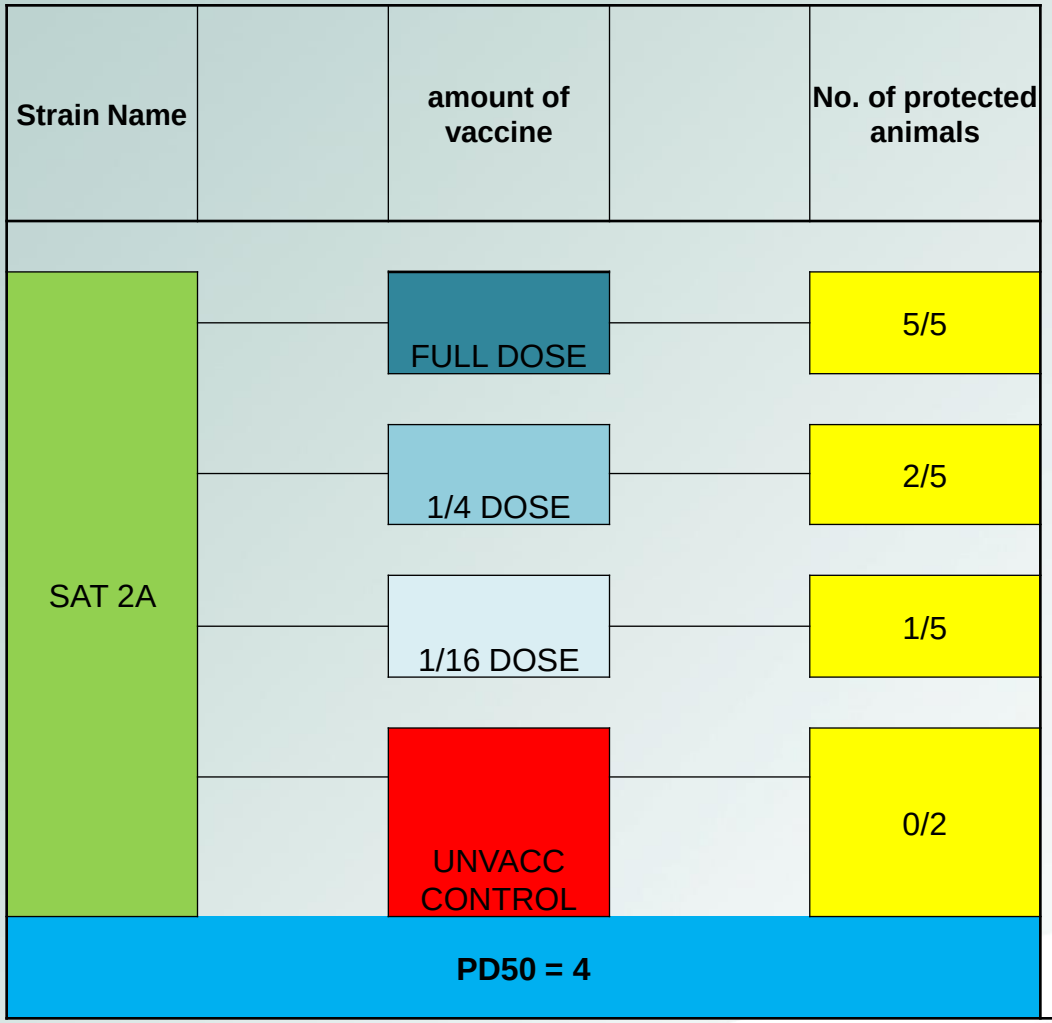
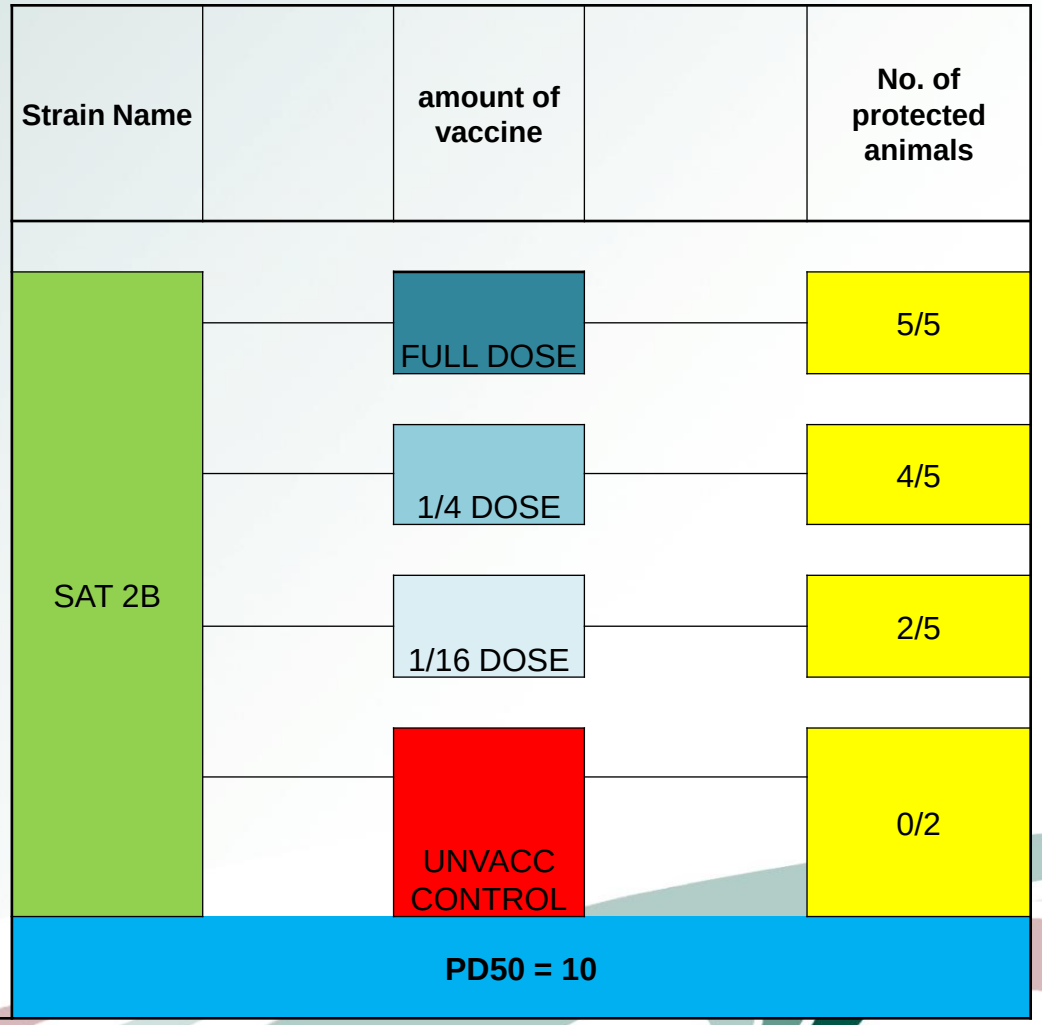
PD50 RESULTS PER STRAIN





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CONCLUSIONS

- The pentavalent could potentially offer full protection against circulating strains in the field (due to high potency) and;
- Could potentially be used successfully for emergency vaccinations.
- An independent study in goats has shown full protection against heterologous challenge.
- Preliminary indications of the study of the duration of immunity in cattle are that the vaccine offers:
  - 100% protection against SAT 2 heterologous challenge after 42 days of primary vaccination;
  - 100% protection, when challenged 3 months after primary vaccination and booster vaccination at 42 days post primary vaccination and;
  - 80% (1 out 5 cattle) protection when booster vaccinations are not administered 42 days post primary vaccination.

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