Is Aluminum replaceable in vaccines?
A sheep model for new biodegradable vaccine adjuvants

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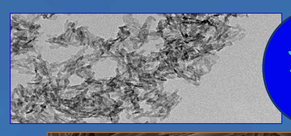
Aluminum hydroxide (Al) has been used as a non-biodegradable adjuvant in human and veterinary vaccines for more than 100 years. It is associated with local reactions at the injection site (IS) and causes neurological and autoimmune-inflammatory symptoms in humans and animals. An example of associated adverse reactions was observed after **bluetongue virus (BTV)** vaccination campaigns in sheep. **Calcium phosphate nanoparticles (CPNP)** and **microcrystallized L-tyrosine (MCT)** are biodegradable and biocompatible adjuvants that have been tested in mice with promising results. They could be future alternatives to Al. MCT, approved in human for antigen-specific immunotherapy techniques, has recently demonstrated efficacy in prophylactic vaccines and CPNPs are in the human first clinical phases. The aim of this work was to study the serologic response and IS reaction of BTV vaccines prototypes using MCT and CPNP as adjuvants and compare the results with those obtained with Al and non-adjuvanted BTV (NAV).

MATERIAL & METHODS

VACCINE FORMULATION



- Calcium phosphate nanoparticles (CPNP) + BTV4
- Microcrystallized L-tyrosine (MCT) + BTV4
- Aluminum hydroxide (Al) + BTV4
- Non-adjuvanted BTV4 **(NAV)**
- PBS — Phosphate buffer saline

5 groups of 6 lambs

ANIMALS

Rasa Aragonesa breed
13 month

INOCULATION SITES (IS):
Right & left flanks
Subcutaneous route

VACCINATION SCHEDULE, SAMPLING & IS PALPATION

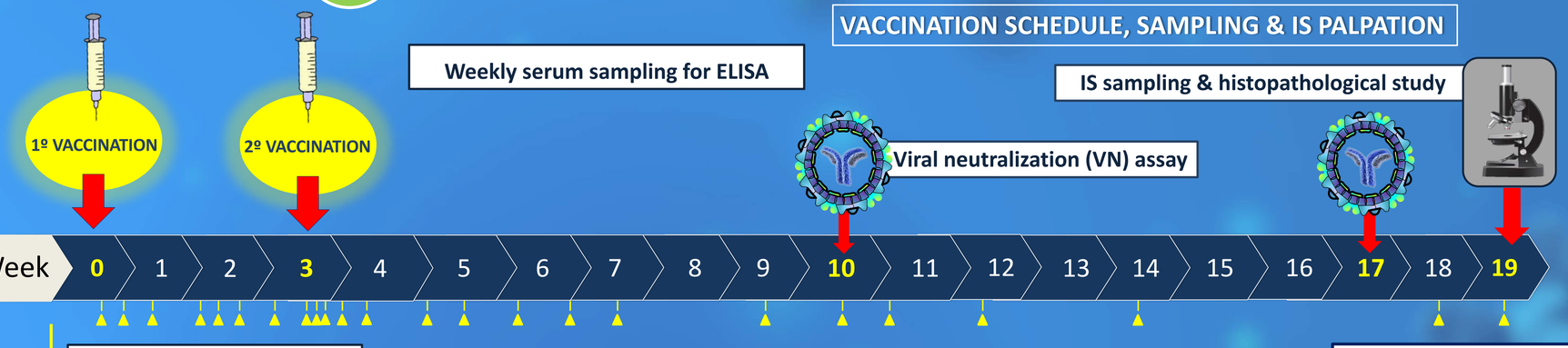


Table 1. Categories and criteria used for evaluation of local reactions

CATEGORIES	DESCRIPTIVE CRITERIA
NOT FOUND	Not detected by palpation
SMALL	Can not be retained between thumb and forefinger. Mild bump
MEDIUM	Can be retained between thumb and forefinger with difficulty. Moderate bump
LARGE	Can be easily retained between thumb-forefinger. Severe bump

RESULTS

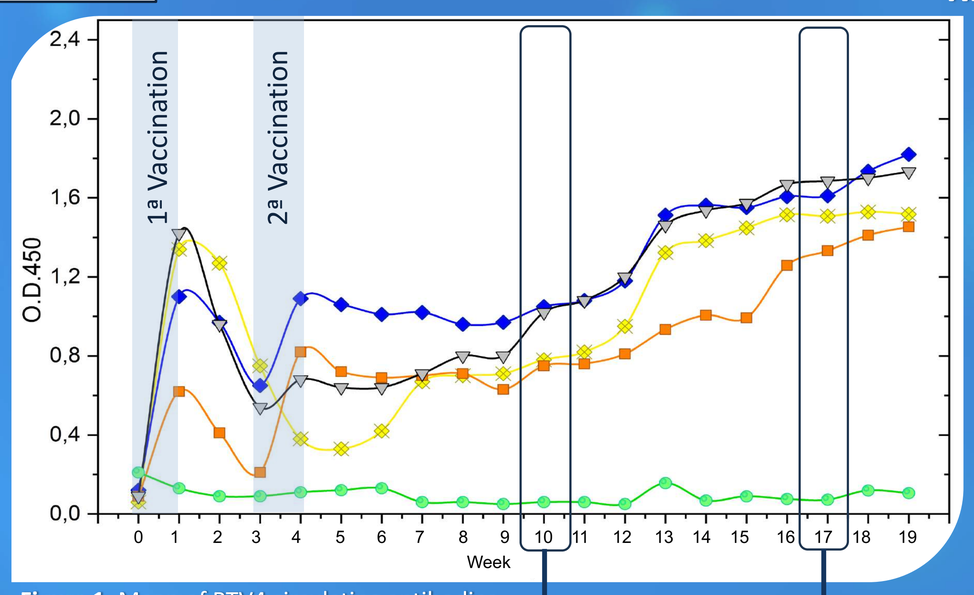


Figure 1. Mean of BTV4 circulating antibodies

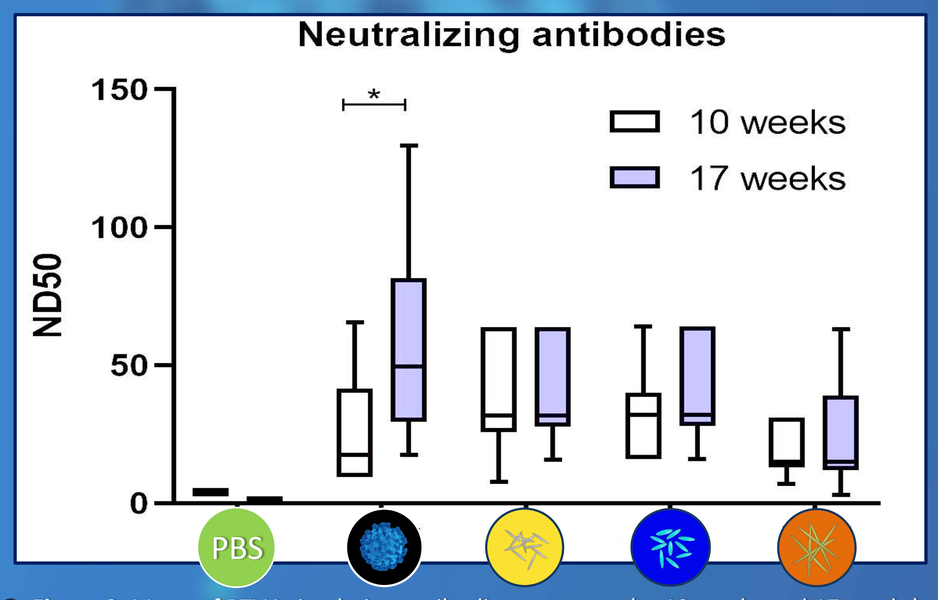


Figure 2. Mean of BTV4 circulating antibodies per group (at 10 weeks and 17 weeks) Wilcoxon signed range test only revealed a statistical significance for NAV group between week 10 and 17 (*asterisk*; $p<0.05$).

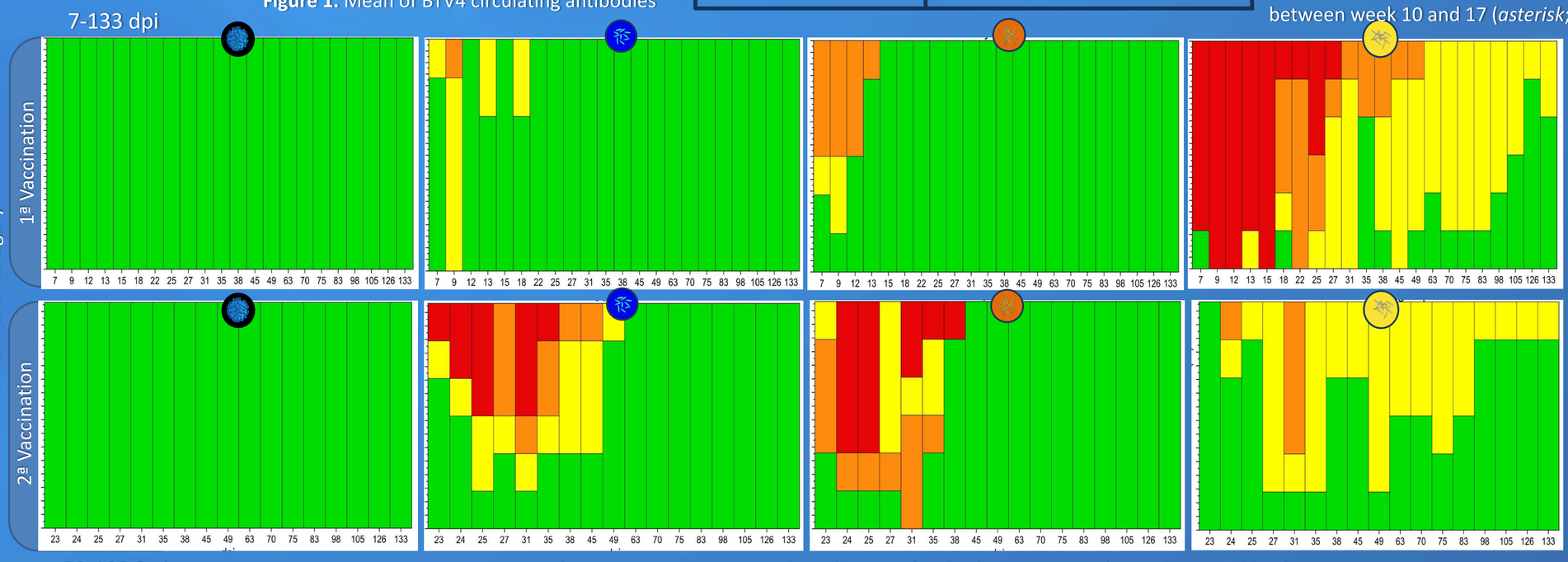


Figure 3. Frequency of IS assessment categories by palpation after both vaccinations (according to Table 1).

Percentage of IS lesions found at post-mortem examination

%	75	0	0	0

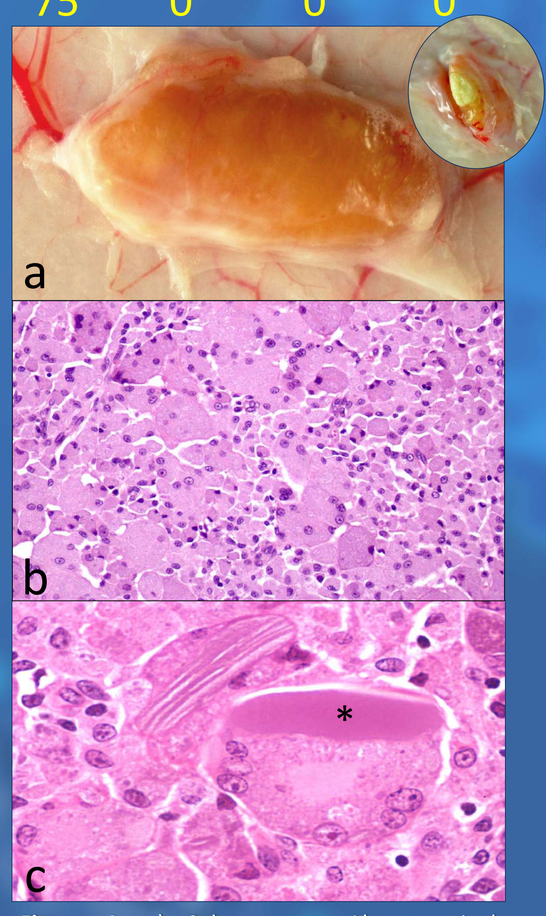


Figure 4. a) Subcutaneous Al-postvaccinal granuloma with a caseous core b) & c) Multiple macrophages with intracytoplasmic vacuoles with Al and Al crystalloid bodies (*).

CONCLUSIONS

- Vaccines with CPNP, MCT and NAV induced circulating BTV4-antibody levels higher or similar to those induced by vaccines with Al.
- The titer of BTV4 neutralizing antibodies did not differ significantly between groups and between weeks 10 and 17, except for NAV group.
- Vaccines with CPNP and MCT induced moderate and time-limited tissue reactions in the IS, whereas inocula with Al induced moderate to severe reactions that persisted until the end of the experiment.
- The NAV vaccine generated an immune response comparable to Al, with a statistically significant elevation in neutralizing antibody titer between week 10 and week 17 and with no detectable subcutaneous reactions in the IS.
- **The use of biodegradable adjuvants or even non-adjuvanted inactivated viruses may be alternatives to Al, since they induce a similar immunological response in the absence of associated persistent IS reactions.**

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