



LOS ANIMALES DE LABORATORIO EN EL DESARROLLO DE LAS VACUNAS DE COVID-19 EN CUBA.

Fuentes Dasha, Sosa Iliana, Castillo Rebeca, Fernández Nidia, Pérez Lency.

Centro Nacional para la Producción de Animales de Laboratorio (CENPALAB), La Habana, Cuba.
Email: dasha.fuentes@cenpalab.cu

INTRODUCCION



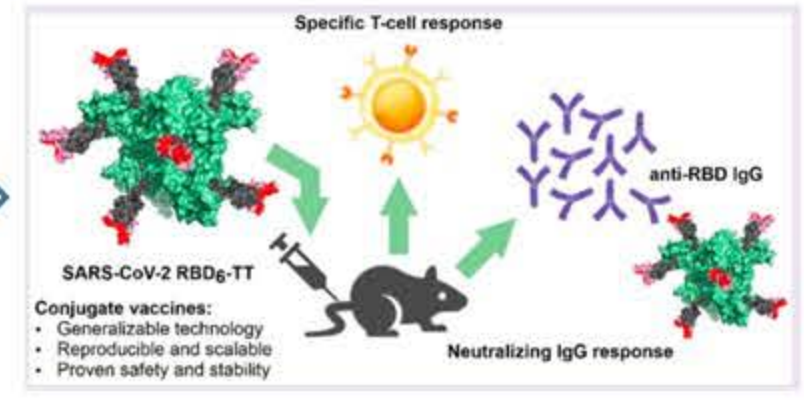
Enfermedad por coronavirus de 2019 (COVID-19), enfermedad infecciosa causada por virus SARS-CoV-2.

Declarada pandemia, OMS: marzo de 2020



Mapa del número verificado de infectados per cápita por la COVID-19 al 20 de noviembre de 2022. Tasa por cada 1.000 habitantes: > 100 casos, 30 – 100 casos, 10 – 30 casos, 3 – 10 casos, 1 – 3 casos, 0,3 – 1 casos, > 0 – 0,3 casos.

Prevención: Desarrollo de vacunas eficaces



Grupos de investigadores de todo el mundo, incluyendo Cuba, comenzaron a trabajar para la obtención de vacunas que frenaran la propagación de la enfermedad.

Objetivo: exponer el empleo de los animales de laboratorio en el desarrollo de las vacunas de COVID-19: Abdala y Soberana en Cuba.

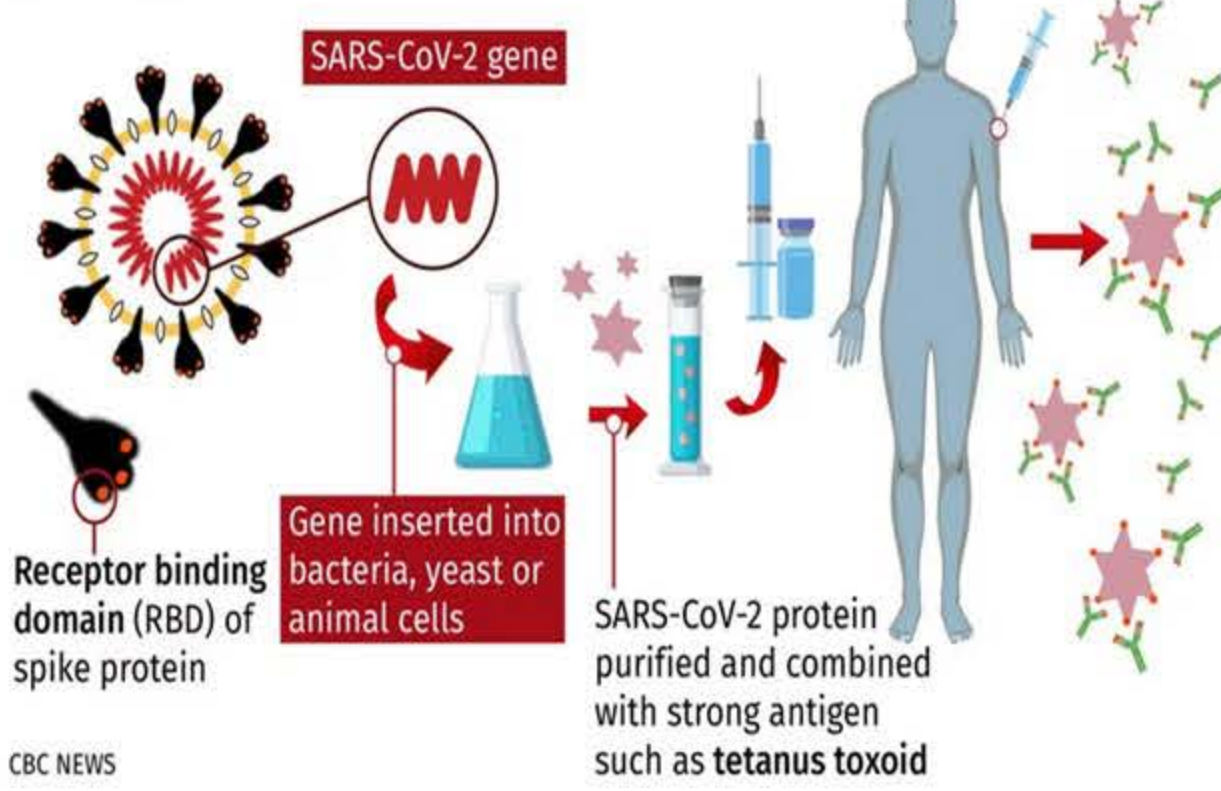
MATERIALES Y MÉTODOS

Diseño de vacunas conjugadas a partir de subunidades virales (RBD) obtenido por vía recombinante: SOBERANA01, SOBERANA02 y ABDALA

Pruebas de concepto y desarrollo de las vacunas
Inmunogenicidad

Toxicología regulatoria
Tolerancia local Inmunotoxicología
Dosis repetida

SARS-CoV-2 virus



ELISA: Titulación de Ac, seroneutralización
Respuesta celular
Transferencia pasiva de esplenocitos de ratones inmunizados

Protección frente al reto viral

Respuesta protectora ante inoculación con virus SARS-CoV-2
Modelo idóneo para la reproducción de la enfermedad



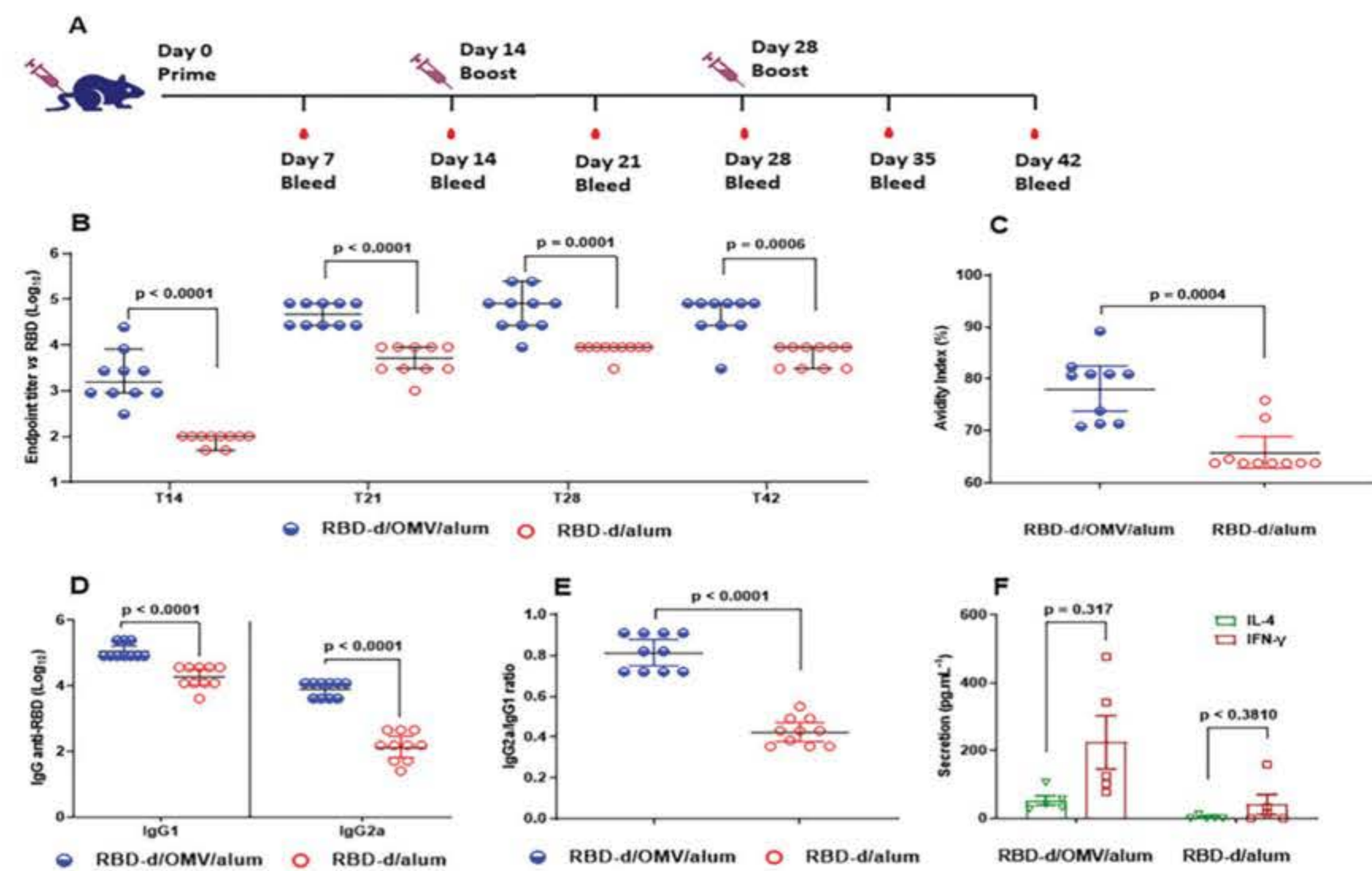
Group	Animals n	Administration hours	Dose/Vol./Route	Animals euthanized (n) at x days after 3 rd dose
Finlay-FR-02*	15	0, 24, 72	RBD 12.5 µg + TT 11.5 µg + Al(OH) ₃ 250 µg/0.25 mL/i.m	5 5 5
Placebo	15	0, 24, 72	Al(OH) ₃ 250 µg/0.25 mL + AS/i.m	5 5 5
Control	15	0, 24, 72	PSS 0.9%/0.2 mL/L.m	5 5 5

Legend: n- Number; PSS- Physiological saline solution; AS- Auxiliary substances: Na CL, NaH₂PO₄, Na₂HPO₄, Thiomersal and injection water; i.m: Intramuscularly; * - Human dose RBD 25 µg + 20 µg TT + 500 µg Al(OH)₃ in 0.5 mL.

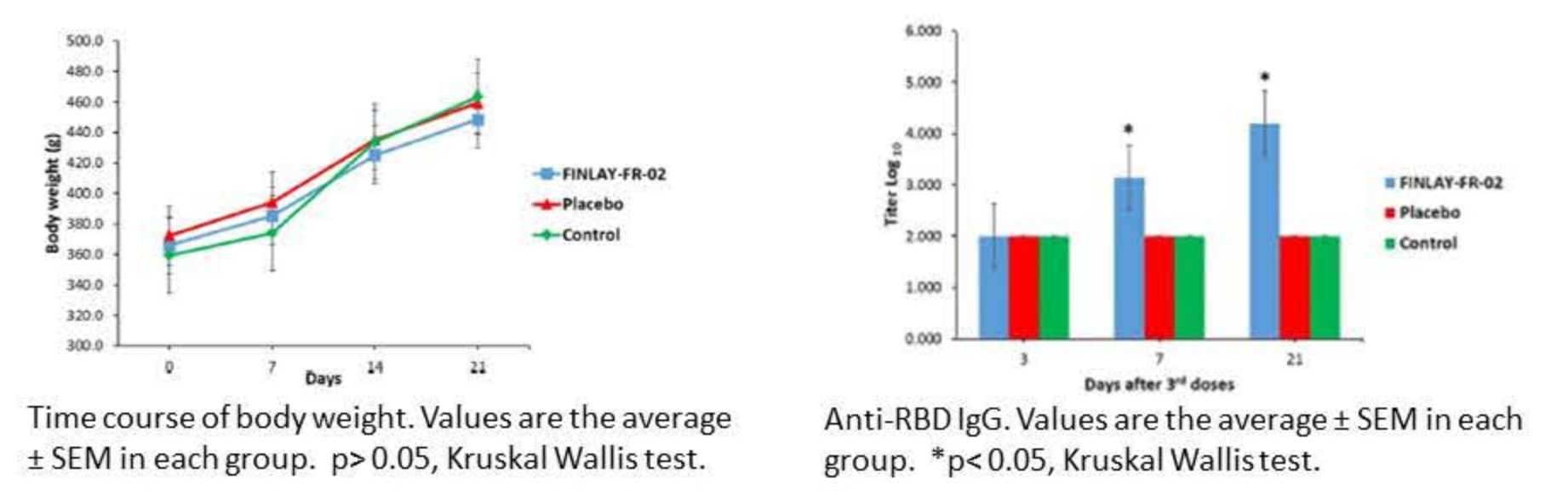
RESULTADOS

Las vacunas inducen una potente respuesta inmune en animales de laboratorio, predominantemente IgG con alta especificidad y proliferación de células B de memoria de larga duración.

Las vacunas resultaron bien toleradas, no se detectaron muertes, cambios fisiológicos de interés, efectos locales adversos ni toxicidad sistémica (Toxicology 2022, 247, 153161).



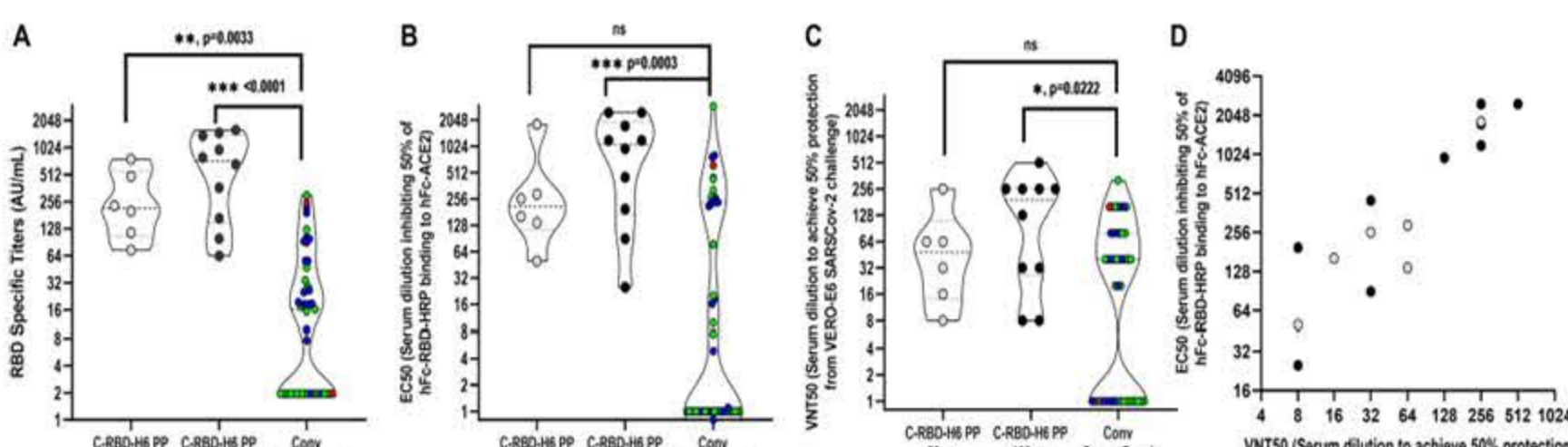
Preclinical immunogenicity evaluation. Immunization of BALB/c mice with RBD-d/OMV/alum (blue) and RBD-d/alum (red). (A) Immunization protocol. (B) Anti-RBD IgG at days 14, 21, 28 and 42. (C) Avidity index of anti-RBD IgG elicited on day 42. (D) Anti-RBD IgG1 and IgG2a titers. (E) Anti-RBD IgG2a/IgG1 ratio. (F) Cytokine secretion (IL-4 and IFN-g) after in vitro stimulation with RBD-d. RSC Chem. Biol. 2022, 3, 242.



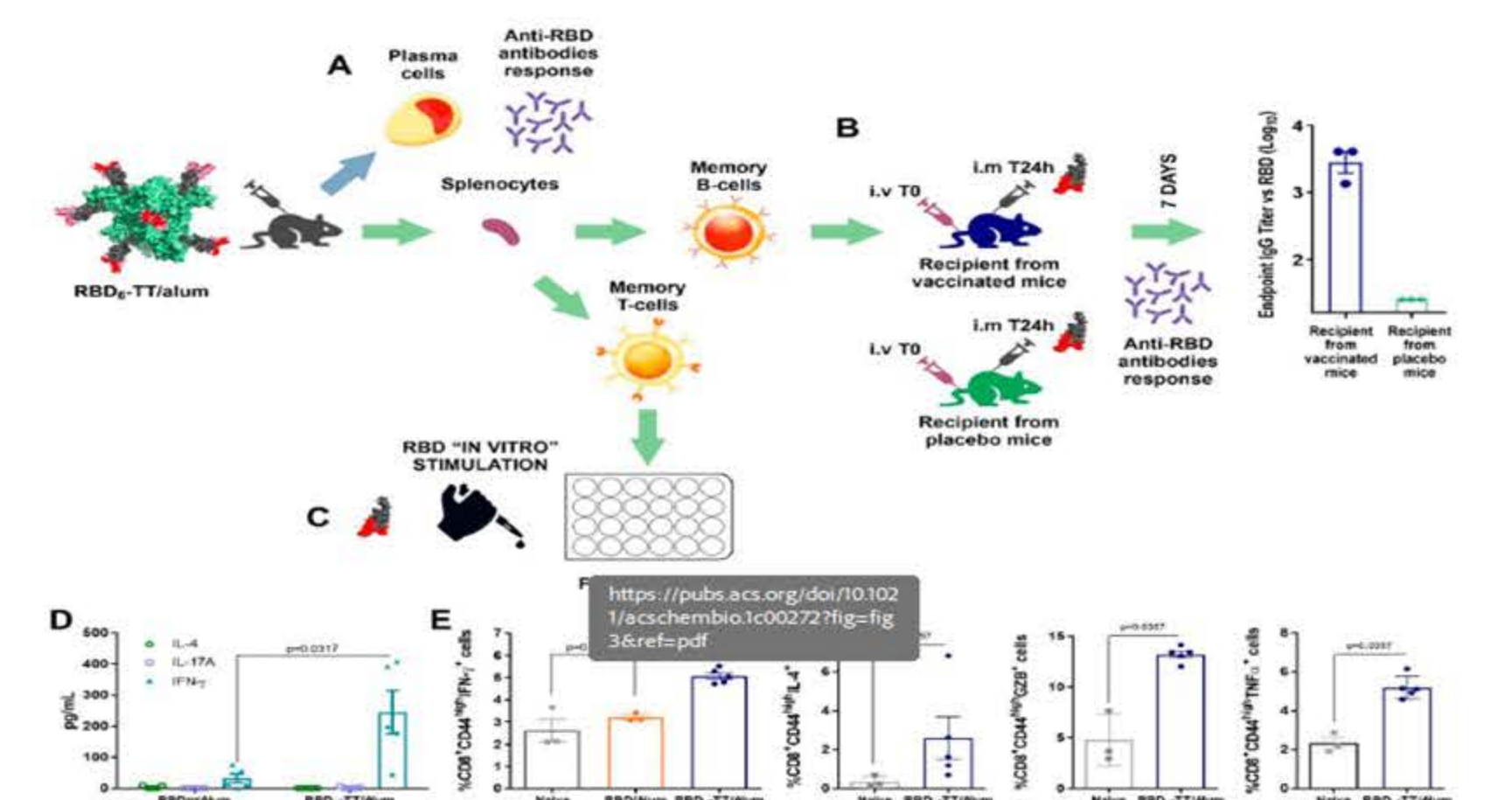
Time course of body weight. Values are the average ± SEM in each group. p > 0.05, Kruskal Wallis test. Anti-RBD IgG. Values are the average ± SEM in each group. *p < 0.05, Kruskal Wallis test.

Groups	Blood chemistry and hemoglobin analysis of rats vaccinated with FINLAY-FR-02 to COVID-19.						
	Glucose (mmol/L)	Cholesterol (mmol/L)	Triglycerides (mmol/L)	TP (g/dL)	Urate (µmol/L)	Alb (g/L)	Creatinine (µmol/L)
3 days after 3 rd doses							
FINLAY-FR-02	9.46 ± 0.81	1.62 ± 0.08	1.42 ± 0.42	5.08 ± 0.30	56.46 ± 10.00	36.66 ± 1.39	58.02 ± 10.10
Placebo	10.16 ± 1.47	1.24 ± 0.32	1.24 ± 0.32	5.71 ± 0.17	60.61 ± 20.93	37.02 ± 1.03	58.55 ± 15.67
Control	10.70 ± 0.32	1.93 ± 0.23	1.49 ± 0.45	5.03 ± 0.32	61.02 ± 17.95	37.08 ± 2.12	57.10 ± 15.67
7 days after 3 rd doses							
FINLAY-FR-02	6.91 ± 0.78	1.50 ± 0.21	0.96 ± 0.65	5.01 ± 0.28	56.30 ± 20.5	35.36 ± 1.27	21.72 ± 17.44
Placebo	7.44 ± 0.62	1.40 ± 0.19	1.14 ± 0.27	5.74 ± 0.24	48.52 ± 11.94	35.42 ± 1.01	22.53 ± 10.27
Control	7.83 ± 1.15	1.82 ± 0.10	0.92 ± 0.19	5.91 ± 0.33	47.60 ± 16.53	35.60 ± 1.52	18.50 ± 0.34
21 days after 3 rd doses							
FINLAY-FR-02	8.39 ± 1.58	1.42 ± 0.07	0.69 ± 0.14	6.00 ± 0.31	50.26 ± 16.24	38.67 ± 1.90	48.60 ± 11.99
Placebo	8.79 ± 1.05	1.37 ± 0.38	0.77 ± 0.31	6.01 ± 0.21	56.37 ± 24.07	38.62 ± 1.12	40.93 ± 7.42
Control	8.65 ± 1.06	1.51 ± 0.18	0.70 ± 0.39	5.94 ± 0.43	52.14 ± 26.33	39.10 ± 1.66	43.14 ± 16.70
Groups	AST (U/L)	CPK (U/L)	LDH (U/L)	ALP (U/L)	ALT (U/L)	Urea (mmol/L)	HB
3 days after 3 rd doses							
FINLAY-FR-02	43.53 ± 10.66	272.66 ± 101.03	183.21 ± 41.44	333.40 ± 66.76	26.07 ± 5.10	6.61 ± 0.57	12.62 ± 0.76
Placebo	56.34 ± 14.94	327.60 ± 51.94	204.55 ± 16.15	330.43 ± 23.77	25.26 ± 4.59	7.32 ± 1.17	11.99 ± 1.06
Control	52.85 ± 15.54	273.21 ± 61.19	182.55 ± 25.20	300.12 ± 65.63	30.26 ± 6.57	6.99 ± 1.66	12.02 ± 0.30
7 days after 3 rd doses							
FINLAY-FR-02	55.99 ± 5.57	472.14 ± 106.35	-	252.63 ± 82.51	23.05 ± 4.97	8.79 ± 1.43	13.54 ± 1.13
Placebo	59.01 ± 9.25	637.95 ± 66.95	-	259.05 ± 82.37	20.25 ± 3.90	7.46 ± 0.83	12.91 ± 0.48
Control	57.50 ± 10.98	695.74 ± 288.47	-	224.22 ± 26.56	19.21 ± 3.47	7.40 ± 1.53	13.33 ± 1.59
21 days after 3 rd doses							
FINLAY-FR-02	60.10 ± 0.59	506.95 ± 255.05	107.37 ± 5.40	192.68 ± 23.17	28.98 ± 6.83	8.05 ± 0.35	12.30 ± 0.54
Placebo	76.01 ± 11.21	669.80 ± 420.52	115.25 ± 14.84	206.43 ± 38.07	30.55 ± 0.76	6.83 ± 0.31	12.40 ± 1.16
Control	72.75 ± 16.57	611.30 ± 361.37	112.62 ± 10.42	224.77 ± 44.56	32.56 ± 0.57	7.35 ± 1.25	11.00 ± 1.16

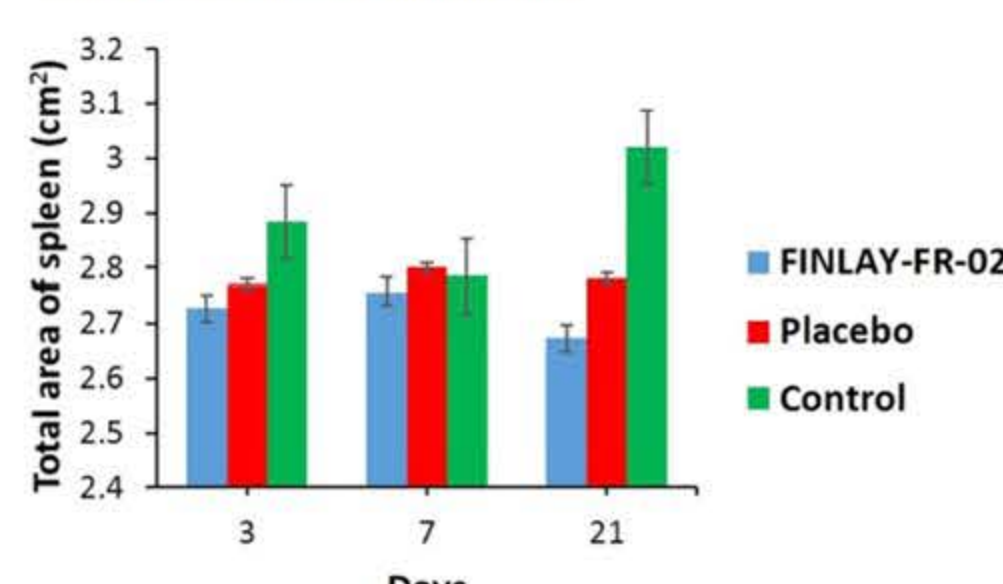
Legend: Values represent the mean ± SEM of the 5 animals in each group per time-point. No statistical differences were found between the groups for any parameter. (a) TP: total Protein, ALB: albumin, (b) AST: aspartate aminotransferase, CPK: creatine phosphokinase, LDH: lactate dehydrogenase, ALP: Alkaline phosphatase, ALT: alanine aminotransferase, HB: hemoglobin.



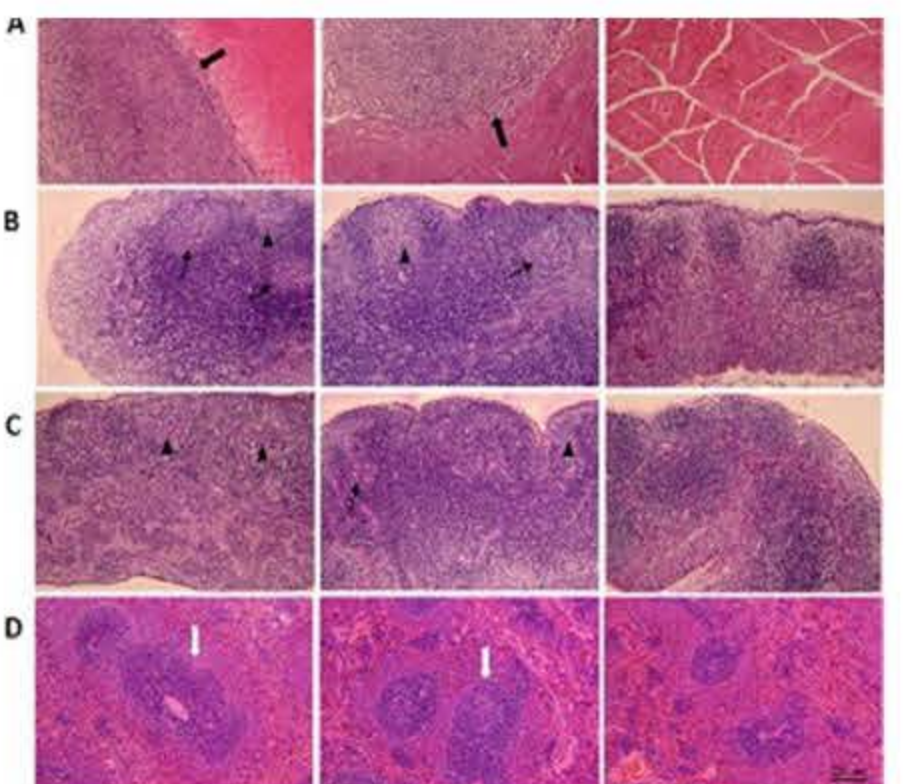
Immunogenicity of C-RBD-H6 PP in NHP. (A) Evaluation of RBD specific IgG in NHP immunized with doses of 50 µg (6 animals) or 100 µg (10 animals) of C-RBD-H6 PP, 14 days after the end of a 0–14–28 days intramuscular schedule. (B) Evaluation in NHP of EC50 for ACE2 binding inhibition. (C) Evaluation in NHP of EC50 for the PRNT in the microneutralization assay. (D) Association/correlation analysis of the ACE2 binding inhibition and microneutralization tests in NHP (Spearman, r = 0.8994, p < 0.0001). New BIOTECHNOLOGY, 2022, 72, 11–21.



Memory B and T cells induced by RBD6-TT. (A) Representation of the primary immune response to RBD6-TT/alum (blue arrow). (B) Classical passive transfer of splenocytes from RBD6-TT/alum-immunized BALB/c mice (up) and naive mice (control, down) and stimulated with RBD/alum (up, strong secondary response on day 7 after immunization). (C) T-cell stimulation with RBD. (D) Cytokine secretion after in vitro RBD stimulation. (E) % RBD-specific memory T CD8+CD44 highIFN+; (F) % RBD-specific memory T CD8+CD44 highIL-4+; % RBD-specific memory T CD8+CD44 high Granzyme+; % RBD-specific memory T CD8+CD44 high TNF+. ACS Chem. Biol. 2021, 16, 1223–1233



Time course of morphometric macroscopic area of spleen of male SD rats. Values are the average ± SEM of the 5 animals in each group. Kruskal-Wallis test p ≥ 0.05.



Histopathological findings in organs related to the immune system. I- FINLAY-FR-02, II-Placebo, III- Control; A- Muscle, B- Popliteal lymph node, C- Deep inguinal lymph node, D- Spleen. HE: hematoxylin-eosin, rod: 200 µm.



Aprobación por la entidad regulatoria de Cuba, actualmente registradas en:

- Cuba
- México
- Venezuela
- Viet Nam
- Nicaragua
- Irán

CONCLUSIONES: La metodología aplicada permitió realizar las pruebas no clínicas de los candidatos a vacunas, así como los ensayos que brindaron la confianza necesaria a los reguladores para autorizar su uso en humanos. Por esta razón podría servir de base para el proceso de obtención de nuevas vacunas para entidades infecciosas que afecten al hombre y/o los animales.