



VETERINARIA

RIVISTA DI
SANITÀ PUBBLICA
VETERINARIA

ITALIANA

Supplementary material



	BRUCELLA VACCINE	TEST		
		Rose Bengal Test	Lateral Flow Assay	Brucellin Skin Test
week post vaccination		Synbiotics Bengatest	Anigen Bionote B. <i>Brucella</i> rapid test	Synbiotics Brucellergen OCB
0	CZV Rev1, 153295 CZV Ocurev, 142477	15BGT80	2301058	162058
2		15BGT80	2301058	not done
4		15BGT80	2301058	10001
12		15BGT80	missing	162058
30		missing	missing	162058
74		missing	not done	missing
184		18ZBAB011	not done	920115

Table SM I. Supplementary material 1: injection and testing schedule. The commercial names are provided, and the vaccines and tests' batch numbers are indicated. "Missing" is indicated whenever the information is not available.

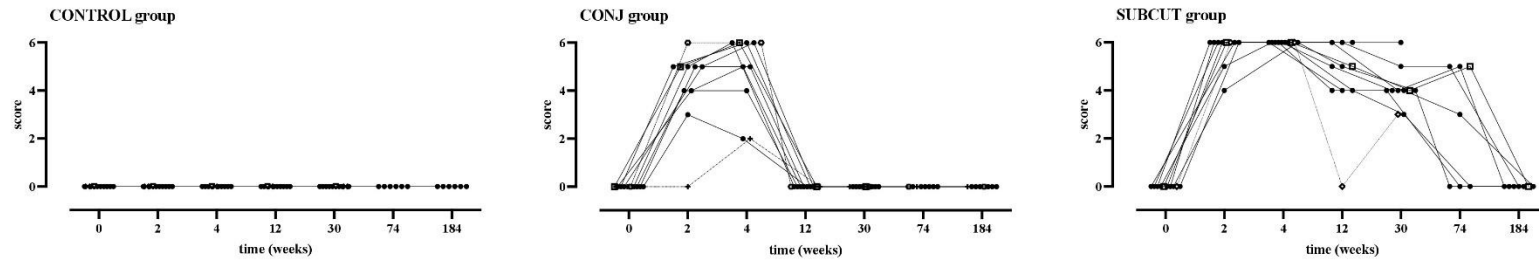
vaccination route	AGE (in days)		SEX		sampling size
	mean	standard deviation	male	female	
none	148,6	51,18	5	5	10
conjunctival	176,0	94,72	4	6	10
subcutaneous	186,7	97,11	5	5	10

Table SM II. Supplementary material 2: age and sex distribution in each experimental group.

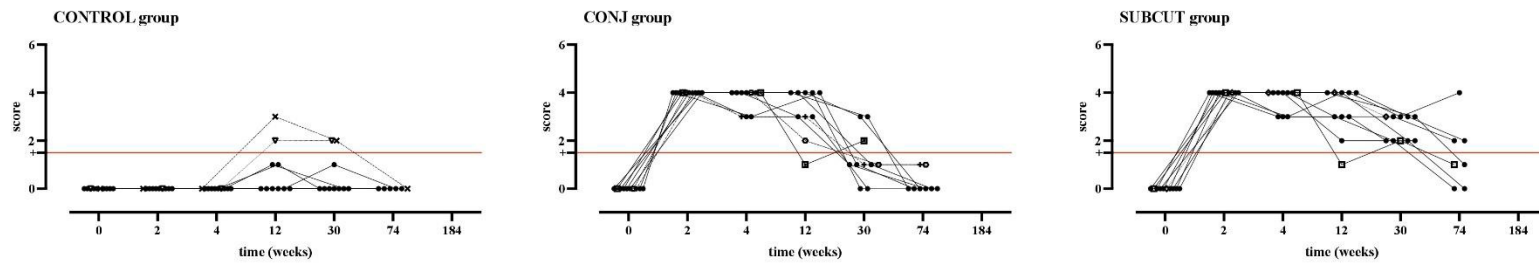
serological test	parameters for interpretation	category	score
RBT	no agglutination seen after 4 minutes + longer agitation + careful examination under bright light. Viscosity change is sometime observed.	negative	0/6
	very small aggregates that require longer agitation and careful inspection under bright light	positive_very_weak	2/6
	agglutination requires longer agitation and careful inspection under bright light	positive_weak	3/6
	clear agglutination at reading at 4 minutes with gentle agitation	positive	4/6
	clear agglutination within 4 minutes without further agitation	positive_strong	5/6
	clear agglutination as soon as both serum and reagent are mixed together	positive_very_strong	6/6
LFA	no line at test band when read is done 20min after sample deposit. Control line positive	negative	0/6
	very faint line visible at test band under certain light condition and under certain angle. Some people won't see it. Won't show on a photography. Control line positive	positive_very_very_weak	1/6
	line visible at test band under certain light condition and under certain angle. Usually seen by several people. Won't show on a photography. Control line positive	positive_very_weak	2/6
	not so contrasty line appears at test band when reading is done 20 minutes after sample deposit. Control line positive	positive_weak	3/6
	clear and contrasty line appears at test band when reading is done 20 minutes after sample deposit. Control line positive	positive	4/6
	clear and strong line appears before the liquid reaches the control band, control line positive	positive_strong	5/6

Appendix 1. score chart for the rose Bengal test (RBT) and the lateral flow assay (LFA) Anigen rapid B. Brucella test.

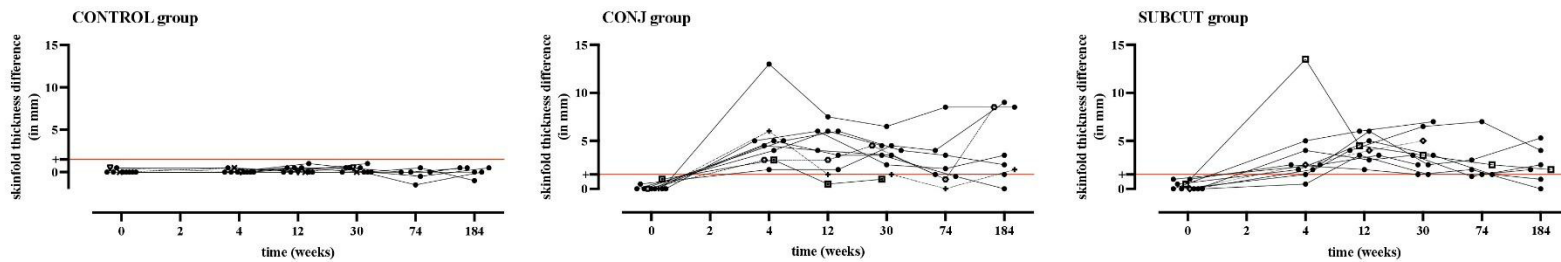
A - RBT



B - LFA



C - BST



Appendix 2. Individual temporal evolutions of test outcomes in each experimental group. Each row (A, B and C) presents the evolution of score according to time for each test: rose Bengal test (RBT), lateral flow assay (LFA) and brucellin skin test (BST). Graphs on the left present the results in the control group, in the middle are the results obtained after conjunctival administration of the *B. melitensis* Rev1. vaccine, and on the right the results after subcutaneous vaccination. The results from individuals that reacted oddly appear with different symbols.

pairs compared	sampling size in both groups		RBT		LFA		BST	
			percent of positivity	mean score	percent of positivity	mean score	percent of positivity	mean thickness difference
Week 0								
CONTROL vs. CONJ	10	10	=	=	=	=	=	>0.9999
CONTROL vs. SUBCUT	10	10	=	=	=	=	=	>0.9999
CONJ vs. SUBCUT	10	10	=	=	=	=	=	>0.9999
Week 2								
CONTROL vs. CONJ	10	10	<0.0001	<0.0001	<0.0001	<0.0001	x	x
CONTROL vs. SUBCUT	10	10	<0.0001	<0.0001	<0.0001	<0.0001	x	x
CONJ vs. SUBCUT	10	10	0,5951	0,0558	0,5951	identical	x	x
Week 4								
CONTROL vs. CONJ	10	10	<0.0001	<0.0001	0,0005	<0.0001	<0.0001	<0.0001
CONTROL vs. SUBCUT	10	10	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0,0002
CONJ vs. SUBCUT	10	10	identical	0,1002	0,3358	>0.9999	0,5951	0,2671
Week 12								
CONTROL vs. CONJ	10	10	=	=	0,0136	0,0001	<0.0001	<0.0001
CONTROL vs. SUBCUT	10	10	<0.0001	<0.0001	0,0017	0,0001	<0.0001	<0.0001
CONJ vs. SUBCUT	10	10	<0.0001	<0.0001	0,822	>0.9999	0,5951	>0.9999
Week 30								
CONTROL vs. CONJ	10	10	=	=	0,8754	0,1796	<0.0001	0,0004
CONTROL vs. SUBCUT	10	10	<0.0001	<0.0001	0,0005	<0.0001	<0.0001	0,0003
CONJ vs. SUBCUT	10	10	<0.0001	<0.0001	0,0034	0,0173	0,5951	>0.9999
Week 74								
CONTROL vs. CONJ	6	8	=	=	=	0,3358	0,0062	0,014
CONTROL vs. SUBCUT	6	7	0,0674	0,0778	0,1655	0,0787	<0.0001	0,0126
CONJ vs. SUBCUT	8	7	0,0674	0,0778	0,1655	0,1513	0,3358	>0.9999
Week 184								
CONTROL vs. CONJ	6	8	=	=	x	x	0,0005	0,0001
CONTROL vs. SUBCUT	6	7	=	=	x	x	0,0193	0,0673
CONJ vs. SUBCUT	8	7	=	=	x	x	0,7564	0,1634

CONTROL was the unvaccinated group; CONJ and SUBCUT were the groups vaccinated by conjunctival or subcutaneous route, respectively

mixed statistical model in Prism

= : identical value in both groups

Appendix 3. values for each pairwise comparison performed at each time point for the percentage of positivity and for the mean score (or mean skinfold thickness increase) obtained with rose Bengal test (RBT), lateral flow assay (LFA) and brucellin skin test (BST)