List of abbreviations

Anti A	*Monospecific Antisera Brucella abortus*
Anti M	*Monospecific Antisera Brucella melitensis*
B. abortus	*Brucella abortus*
B. melitensis	*Brucella melitensis*
bp	*Base Pair*
BSA	*Bovine Serum Albumin*
CFU	*Colony Forming Unit*
DNase	*Deoxyribonuclease*
EDTA	*Ethyelene-Diamine-Tetra acetic Acid*
ELISA	*Enzyme Linked Immunosorbant Assay*
Mol. W.	*Molecular Weight*
MRT	*Milk Ring Test*
O.D.	*Optical Density*
PBS	*Phosphate Buffer Saline*
PBST	*Phosphate Buffer Saline + Tween 80*
RBPT	*Rose Bengal Plate Test*
RT	*Rivanol Test*
S 19	*Brucella abortus strain 19*
S/B	*Spleen weigh / Body weight*
SAT	*Standard Tube Agglutination Test*
RB51	*Brucella abortus strain RB51*
TBS	*Tris + Buffer Saline*
TBST	*TBS + Tween*

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Summary

Serum and milk samples collected from recently and chronically infected cattle herds with brucellosis that have not been vaccinated and the reactor results with RBPT was 45.8 % in acutely infected herds and 29.3% in chronically infected one and the percentage of MRT were 36.0% and 16.0% respectively. While bacteriological studies revealed that isolation of *B. melitensis* biovar 3, from 4 herds.

In vaccinated herds with S. 19 and non infected with Brucella the percentage of blood reactors by RBPT and SAT was 2.7% and 1.3% respectively and this due to persistent antibody titer due to vaccination, and only 4.6 % of milk samples were positive to MRT.

In infected vaccinated herds with S 19 the results of RBPT, SAT and MRT were 15.4%, 24.75% and 28.47%, respectively and *B. melitensis* biovar 3 was isolated from infected milk and aborted foeti. While *B.abortus biovar 1* was also isolated from infected milk and aborted foeti from one herd.

In cattle herd that vaccinated at calf hood with S 19 then re-vaccinated in adult with RB51 and become infected with Brucella, the results of RBPT, SAT and MRT were 14.9%, 29.82% and 24.35%

respectively and *B. melitensis* biovar 3 was isolated bacteriologically from infected milk, lymph nodes and aborted foeti.

On the other hand, challenge test on groups of Guinea pigs each group contains 10 adult male Guinea pigs free from brucellosis.

- Group 1 Guinea pigs vaccinated with 2.3 x 10^8 CFU RB51 and serologically studied for 16 weeks. There is no any evidence of serological responses to RB51, average body size 551.1 g and spleen size 0.865 g and according to **Keppie and Smith 1972** equilibration spleen weight/body weight percent was 0.157% and this considered slightly elevated figure and isolation of RB51 from spleen of 3 animals and in heavy culture.
- Group 2 Guinea pigs not vaccinated with any vicinal strain and then infected with 1 x 10^5 CFU *B. melitensis* field strain. *B. melitensis* was isolated from spleen of most animals and spleen weight/body weight % was
- Group 3 Guinea pigs vaccinated with 1 x 10^7 CFU S.19 and challenged after 8 weeks with 1 x 10^5 CFU *B. abortus* field strain and according to **Keppie and Smith 1972,** S.19 could protect 60% Guinea pigs when challenged with *B. abortus* as the spleen weight/body weight was 0.51%.
- Group 4 Guinea pigs vaccinated with 1 x 10^8 CFU RB51 then challenged with 1 x 10^5 CFU *B. abortus* field strain isolated from spleen of 5 animals and according to **Keppie and Smith 1972**, 0.186% that the spleen weight/body weight was 0.51%.

- Group 5 Guinea pigs vaccinated with 1 x 10^7 CFU S.19 then challenged with 1 x 10^5 CFU *B. melitensis* field strain. *B. melitensis* were isolated from 4 animals (40%) and the spleen weight/body weight was 0.172% and according to **Keppie and Smith 1972**, S 19 has a degree of protection to *B. melitensis*.
- Group 6 Guinea pigs vaccinated with 2.3 x 10^8 CFU RB51 then challenged with 1 x 10^5 CFU *B. melitensis* field strain. It is clear that there is no protection due to no clearance of organism from animal spleen and isolated from spleen of all animals in heavy culture and the spleen weight/body weight was 0.198% and this mean very weak protection.
- Group 7 Guinea pigs vaccinated with 2.3 x 10^8 CFU RB51 then after 8 weeks re-vaccinated with 1 x 10^5 CFU RB51 (reduced dose) then challenged with 1 x 10^5 CFU *B. melitensis* field strain. It is found that in most cases there is no clearance of organisms (field strain) from animal spleen and the spleen weight/body weight was 0.199% and according to **Keppie and Smith 1972**. the protection level was very low and in 8 animals *B. melitensis* biovar 3 was isolated and total bacterial count was > 50 CFU/gm spleen.
- In Group 8 Guinea pigs vaccinated with 1 x 10^7 CFU S.19 then revaccinated 6 weeks later with 1 x 10^7 CFU RB51 (reduced dose) it is clear that this group showed the highest protection level among all groups due to .
- Group 9 Guinea pigs vaccinated with 2.3 x 10^8 CFU RB51 then boostered with 1 x 10^7 CFU S.19 6 weeks later and then challenged with 1 x 10^5 CFU *B. melitensis* field strain.

Bacteriologically, *B. melitensis* was isolated from the spleen of 20% of animals in heavy culture and the spleen weight/body weight was 0.159% and this mean good protection.

AMOS PCR Amplification used for Diagnosis and differentiates between Brucella vaccinal strains (S 19 & SRB51) and local field isolates (Brucella melitensis biovar 3 & Brucella abortus biovar 1)

The analysis of AMOS PCR revealed that there was no significant difference between the two vaccines strain 19(bp 543) and RB51 (bp 535), there were a clear difference between Brucella abortus (bp 517) and Brucella melitensis local strains (bp 792)

Also AMOS PCR was applied on vicinal strains (S 19 & RB51) and on local field strains *B. melitensis* & *B. abortus* showed homogeneity between all abortus strains (S 19, RB51and *B.abortus* reference strain), also showed minute genetic variation between reference strains and local field strains.