Supplementary materials for the article:
Zafar H. et al. Evaluation of a *Salmonella* Strain Isolated from Honeybee Gut as a Potential Live Oral Vaccine Against Lethal Infection of *Salmonella* Typhimurium
Pol J Microbiol. 2019, Vol. 68, No 2, 173–183.

Table SI
The details of the safety evaluation test.

Group SR1

Antigen	Post vaccination Hours	Results based on the average of readings of five rabbits					
		Physical abnormalities	Average body temperature	Daily food intake	Water intake	Weight	Recovery of HB1 antigen CFU/ml
HB1 live antigen	24	None	102.9	Normal	Normal	Normal	1×10^3
	48	-	102.5	Dropped	Dropped	Normal	1×10^4
	72	-	103.1	Normal	Normal	Normal	1×10^4
	96	-	102.5	Normal	Normal	Normal	1×10^5
	120	-	103.1	Normal	Normal	Normal	1×10^5
	144	-	101.6	Normal	Normal	Normal	1×10^4
	168	-	102.2	Normal	Normal	Normal	1×10^4

Group SR2

Antigen	Post vaccination hours	Results based on the average of readings of five rabbits					
		Physical abnormalities	Average body Temperature	Food intake	Water Intake	Changes in weight	Recovery of HB1 antigen CFU/ml (Average)
HB1 live antigen	24	None	103.2	Normal	Normal	Normal	1×10^5
	48	-	102.9	Normal	Normal	Normal	1×10^8
	72	-	102.5	Normal	Normal	Normal	1×10^8
	96	-	102.7	Normal	Normal	Normal	1×10^7
	120	-	102.1	Normal	Normal	Normal	1×10^7
	144	-	101.9	Normal	Normal	Normal	1×10^6
	168	-	102.5	Normal	Normal	Normal	1×10^7

Group SR3 (control)

Antigen	Post vaccination Hours	Results based on the average of readings obtained from of the five rabbits					
		Physical abnormalities	Average body Temperature	Food intake	Water Intake	Changes in weight	Recovery of HB1 antigen CFU/ml
HB1 live antigen	24	None	100.7	Normal	Normal	Normal	-
	48	-	102.0	Normal	Normal	Normal	-
	72	-	102.1	Normal	Normal	Normal	-
	96	-	102.5	Normal	Normal	Normal	-
	120	-	102.3	Normal	Normal	Normal	-
	144	-	102.4	Normal	Normal	Normal	-
	168	-	102.3	Normal	Normal	Normal	-

Table SII
Table for determination of LD50 of *S.* Typhimuirum (ATCC 14028).

Groups	Dilution of bacteria	Dose cells	Observed values		Accumulative values		Rates	
			Dead	Alive	Total dead	Total alive	Fractional ratio	Percent ratio
1	10^{-2}	1×10^{6}	6/6	0/6	15	0	15/15	100%
2	10^{-3}	1×10^{5}	4/6	2/6	9	2	9/11	80%
3	10^{-4}	1×10^{4}	3/6	3/6	5	5	5/10	50%
4	10^{-5}	1×10^{3}	1/6	5/6	2	10	2/12	16%
5	10^{-6}	1×10^{2}	1/6	5/6	1	15	1/16	6%
6	10^{-7}	1×10^{1}	0/6	6/6	0	21	0/21	0
7	PBS		0/6	6/6	0	27	0/27	0

No of rabbits in each group was 6, while total rabbits were 42.

The percentage of mortality was calculated according to the method of Reed and Munch, (1938). Proportional distance = % mortality above 50% - 50% / % mortality above 50% - mortality below 50% . The LD50 of S. Typhimuirum (ATCC14028) was considered to be 1×10^{4} cfu/ml.

Table SIII
Results of Challenge/Protection test against *S.* Typhimurium applied at 70 days post immunization.

Vaccinated groups	No. of animals challenged	Morbidity	Survival	Percentage protection
R1	10	3	7/10	70
R2	10	0	10/10	100
R3	10	2	2/10	80
R4	10	0	10/10	10

Appendix A

The recovery percentage of isolates from the human, animal and insect enteric sources.

Type of sample	Species	No. of samples	Growth in TTB	*Salmonella* colonies on SSA/BSA	Total *Salmonella* isolates
Stool/ feces/ dropping/ insect gut contents	Human TY(1-15)	15	4(26.6%)	3(20%)	3(20%) TY1, TY2 TY3
	Cat CT (1-15)	15	3(20%)	1(6.6%)	1(6.6%) CT7
	Sheep SP (1-15)	15	3(20%)	1(6.6%)	1(6.6%) SP6
	Honey bee HB (1-15)	15	5(33.3%)	2(13.3%)	2(13.3%) HB1, HB11
	Poultry birds PB (1-15)	15	4(26.6%)	2(13.3%)	2(13.3%) PB2, PB9
	Horse HE (1-15)	15	1(6.6%)	1(6.6%)	1(6.6%) HE13
Total		90	20(22.2%)	10(11.1%)	10(11.1%)

TTB = Tetrathionate broth,
SSA = *Salmonella-Shigella* agar
BSA =Bismuth-Sulphite agar

Appendix B

Colony morphology of *Salmonella* isolates.

Isolate	*SSA*		BSA	
	Morphology	Diameter range	Morphology	Diameter range
TY1	Straw colored with blackish center	0.9-1.5 mm	Black colored	0.8-1.5 mm
TY2	Straw colored with blackish center	0.8-1.5mm	Black colored	0.75- 1mm
TY3	Straw colored with blackish center	0.75-1 mm	Black colored	0.75-1.5 mm
CT 7	Straw colored with blackish center	0.5-0.75 mm	Black colored	0.5-1mm
SP 6	Straw colored with blackish center	0.9-1 mm	Circular Straw color	0.5-1.5 mm
HB 1	Straw colored with blackish center	0.8-1mm	Circular Straw colored	0.75-1.5 mm
HB 11	Straw colored with blackish center	0.7- 1mm	Circular Straw colored	0.9-1 mm
PB 2	Straw colored with blackish center	0.8-1 mm	Circular Straw colored	0.8- 1.5mm
PB 9	Straw colored with blackish center	0.6-0.75 mm	Circular Straw colored	0.5-1 mm
HE 13	Straw colored with blackish center	Up to 1mm	Circular Straw colored	0.9-1.5 mm

Appendix C

Fasta sequence of TY1 (Accession# MH985341) alongwith top 4 hits from BLAST.

>TY1
CTCGAGCTACCATGCACTCAACGGTAACAGGAAGCACTTGCTGCTTTGCT
GACGAGTGGCGGACGGGTGAGTAGTGTCTGGGAAACTGCCTGATGGAGGG
GGATAACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAA
AGAGGGGGACCTTCGGGCCTCTTGCCATCATATGTGCCCATATGGGATTA
TCTTGTTGGTGAGGTAACGGCTCACCTGGGCGACGATCCCTATCTGGTCT
GAGAGGATGACCACCCACACTGGAACTGAGACACGGTCCACACTCCTACG
GGAGGCAGCAGTGGGGAATATTGCACAGTGGGCGCAAGCCTGATGCACCC
ATGCCGCGTGTATGAAAAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGG
GAAGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGCAAAA
GAAGCACCGGCTAACTCCGTGCCAGCACCCGCGGTAATACGGAGGGTGCA
AGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCACGCGGTCTGTCAA
GTCAGATGTGATATCCCCGGTCTCAACCTGAGAACTGCATTCGAAACTGG
CAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCGGGTGTAGCGGTGATA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAGCGCGGCCCCTGCACAAG
ACTGACGCTCATGTGCGAAGCGTGGAGAGCAACAGGATTAGATCCTCTGA
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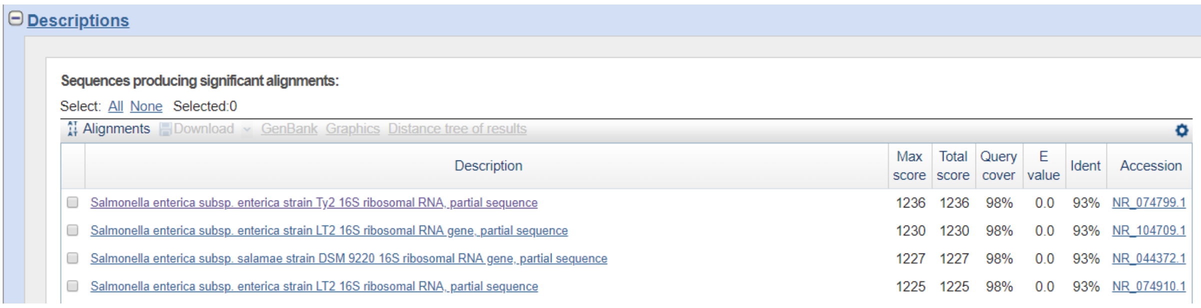

Sequences producing significant alignments:

☐	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Salmonella enterica subsp. enterica strain Ty2 16S ribosomal RNA, partial sequence	1236	1236	98%	0.0	93%	NR_074799.1
☐	Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA gene, partial sequence	1230	1230	98%	0.0	93%	NR_104709.1
☐	Salmonella enterica subsp. salamae strain DSM 9220 16S ribosomal RNA gene, partial sequence	1227	1227	98%	0.0	93%	NR_044372.1
☐	Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA, partial sequence	1225	1225	98%	0.0	93%	NR_074910.1

FASTA sequence of TY2 (Accession# MH985355) alongwith top 4 hits from BLAST.

>TY2
CTCGAGCTACCATGCACTCAACGGTAACAGGAAGCACTTGCTGCTTTGCT
GACGAGTGGCGGACGGGTGAGTAGTGTCTGGGAAACTGCCTGATGGAGGG
GGATAACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAA
AGAGGGGGACCTTCGGGCCTCTTGCCATCATATGTGCCCATATGGGATTA
TCTTGTTGGTGAGGTAACGGCTCACCAGGGCGACGATCCCTATCTGGTCT
GAGAGGATGACCACCCACACTGGAACTGAGACACGGTCCACACTCCTACG
GGAGGCAGCAGTGGGGAATATTGCACAGTGGGCGCAAGCCTGATGCACCC
ATGCCGCGTGTATGAAAAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGG
GAAGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGCAAAA
GAAGCACCGGCTAACTCCGTGCCAGCACCCGCGGTAATACGGAGGGTGCA
AGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCACGCGGTCTGTCAA
GTCAGATGTGATATCCCCGGTCTCAACCTGAGAACTGCATTCGAAACTGG
CAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCGGGTGTAGCGGTGATA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAGCGCGGCCCCTGCACAAG
ACTGACGCTCATGTGCGAAGCGTGGAGAGCAACAGGATTAGATCCTCTGA
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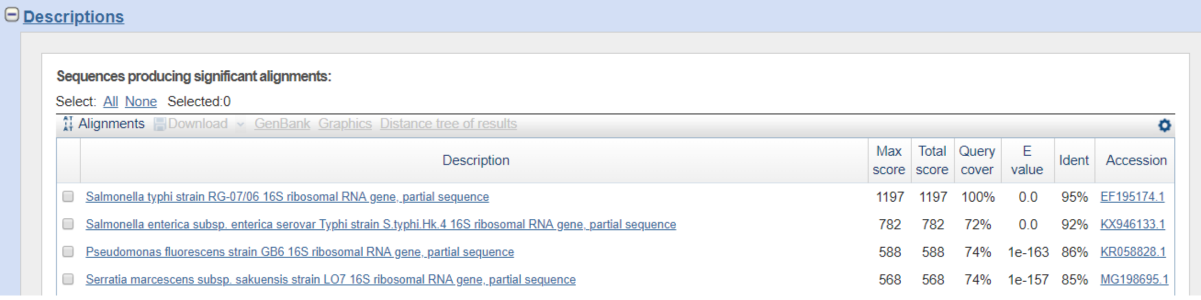

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☐	Salmonella enterica subsp. enterica serovar Typhi strain S.typhi.Hk.4 16S ribosomal RNA gene, partial sequence	782	782	72%	0.0	92%	KX946133.1
☐	Pseudomonas fluorescens strain GB6 16S ribosomal RNA gene, partial sequence	588	588	74%	1e-163	86%	KR058828.1
☐	Serratia marcescens subsp. sakuensis strain LO7 16S ribosomal RNA gene, partial sequence	568	568	74%	1e-157	85%	MG198695.1

FASTA sequence of TY3 (Accession# MH989533) alongwith top 4 hits from BLAST.

>TY3
TCTCGAGCTCCATGCACTCAACGGTAACAGGAAGCACTTGCTGCTTTGCT
GACGAGTGGCGGACGGGTGAGTAGTGTCTGGGAATCTGCCTGATGGAGGG
GGATAACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAA
AGAGGGGGACCTTCGGGCCTCTTGCCCTCATATGTGCCCATATGGGATTA
TCTTGTTGGTGAGGTAACGGCTCACCAGGGCGACTATCCCTATCTGGTCT
GAGAGGATGACCACCCACTCTGGAACTGAGACACGGTCCACACTCCTACG
GGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCACCC
ATGCCGCGTGTATGAAAAAGGCCTTCGGGTTGTAAAGTACTTTCCGCGGG
GAGGAAGGTGTTGTGTTTAATAACCGCAGCATTTGACGTTACCCGCAAAA
AAAACACCCGCTATCTCCGTGCCAGCACCCGCGGAAATACAGAGGGTGCA
AGCGTTTATCAGAATTTCTGGGGCGTAAAGCGCACGCACGCGCTCTGTCA
AGTCAGATGTGAAATCCCCCGGGCCTCCCCCTGGGGAACTGCATTCTAAA
CTGGCAGGCTTGAGTCTTGTAAAGGGGGGGTAAAAATTCCATGTGTAGCG
GTGAAATGCGTAGAGATCTGGAAGAATACCGGTGGCGAAAGCGGCCCCCT
GCGACAAAGACTGACGCTCCTGTGCGAAAGCGTGAGAGCACACAAGTATA
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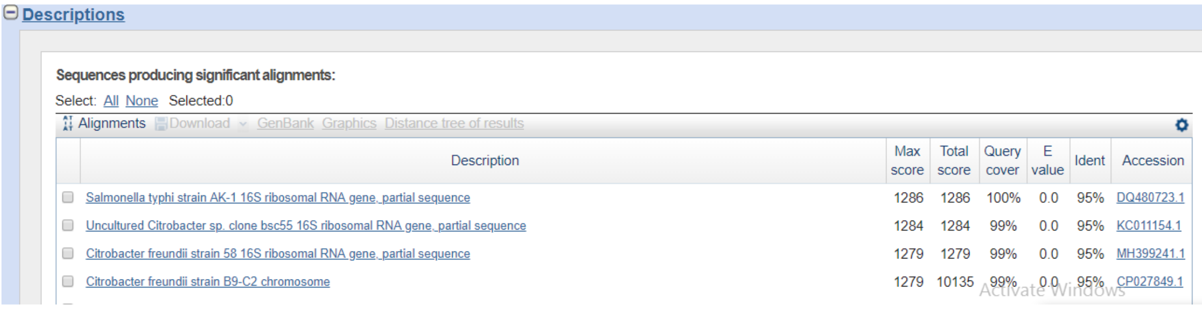

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☐	Uncultured Citrobacter sp. clone bsc55 16S ribosomal RNA gene, partial sequence	1284	1284	99%	0.0	95%	KC011154.1
☐	Citrobacter freundii strain 58 16S ribosomal RNA gene, partial sequence	1279	1279	99%	0.0	95%	MH399241.1
☐	Citrobacter freundii strain B9-C2 chromosome	1279	10135	99%	0.0	95%	CP027849.1

Fasta sequence of HB1 (Accession #MH985334) alongwith top 4 hits from BLAST.

>HB1
CCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCATGTCGAAC
GGTAACAGGAAGCAGCTTGCCTCTTCGCTGACGAGTGGCGGACGGGTGAG
TAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTA
GCTAATACAGCATAATGTCGCAGGACCAAAGAGGGGGACCTTCGGGCCTC
TTGCCATCAGATGTGCCTAGATGGGATTAGCTTGTTGGTGAGGTAACGGC
TCACCAAGGCGACGATCCTTAGCTGGTCTGAGATGATGACCAGCCACACT
GGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGCCCAATAT
TCTACTTATTTTTCAACCCTCATCCAGGGATGCAGCGTGTATGAAGAAGG
CCTTCGGGTCGGATTGTACTGGCAGCGCCGTGGTTGGGGATTTGGCTTAT

AACCTTGTTCATTGACGATGGGCGCAGAAGAAGCACCGGCTAACTCAGTG
CCAGCAGCCGCGGTAATACGGAAGGTGCAAGCGTTAATCGGAATTACTGG
GCGTAAAGCGCACGCCGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGG
CTCAACCTGGGAACTGCATTCGAAACTGGCGGGCTGGAGTCTTGTAGAGG
GGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAAT
ACCGGTGGCGAAGGCGGCCCCCTGGACTTAGACTGACGCTCAGGTGCGAA
AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC
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CGTTAAGTAGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATG
AATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGC
AACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAAGTTTGCAGAGAT
GCGAATGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCA
GCTCGTGTTGTGAAATGTTGGGTTAAGTCGGGCAACGAGCGCAACCCTTA
TCCTTTGTTGCCAGCGGTCCCCCCGGGGACACGGGGATGACGGCCTGACT
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CAGAGCTACACACGTGCGACAATGGCGCATACAAAGAGAAGCGACCTCGC
GAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCTGGACTGGAGTCTGC
AACTCGACTCCATGAAGTCGGAATCGCCCGGAATTGTGCATCAGGGTGCC
AGGGAGATTAGGAACCGGGGCCTTGTAGACAGGTCCTGTCACAGGATGGG
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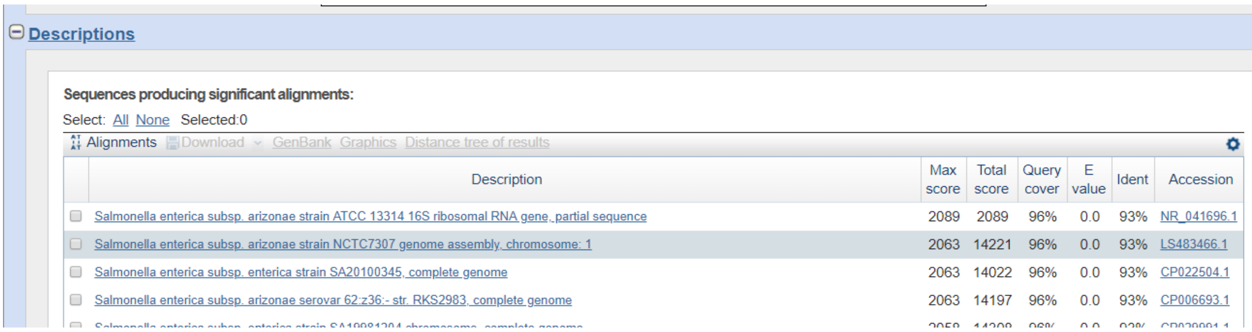

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☐	Salmonella enterica subsp. arizonae strain NCTC7307 genome assembly, chromosome: 1	2063	14221	96%	0.0	93%	LS483466.1
☐	Salmonella enterica subsp. enterica strain SA20100345, complete genome	2063	14022	96%	0.0	93%	CP022504.1
☐	Salmonella enterica subsp. arizonae serovar 62:z36:- str. RKS2983, complete genome	2063	14197	96%	0.0	93%	CP006693.1

Fasta sequence of HB11 (Accession# MH985335) alongwith top 4 hits from BLAST.

>HB11
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GCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGGGATCAGTTGTGCCCT
GATGGGGTTAGCTGGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCC
TAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTGGA
GACACCTACGGGAGGCAGCAGTGGGGAATGTTGCACAATGGGCGCAAGCC
TGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAACTAA
GGGCAACAGAGAGGAAGGTGTTGTGGTTAATTTCCGCAGCAATTGACGTT
ACCCGCAGAAGTTGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATAC
GGGGGGTGCAAGCGTTAATCGGGGGTACTGGGCGTAAAGCGCACGCAGGC
GGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCAT
TCGAAACTGGCAGGCTTGAGTCTTGTGGAGGGGGGTAGAATTCCAGGTGT
AGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGGG
CCCTGGACAAAGACTGACGCTCAGGTGCGTTTGCGTGGGGAGCAAACAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCTACTTGGAGGCTG
TGCCCTTGAGCCGTGGCTTCCGGAGCTAACGCGTTAAGTAGACCGCCTGG

GGAGTACGGCCGCAAGGTTAAAACTCGGGTGGGTTGACGGCCCCCGCAG
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GGTCTTGACATCCACGGAAGGTCTCAGAGATGAGACTGTGCCTTCGGGAG
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CGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGG
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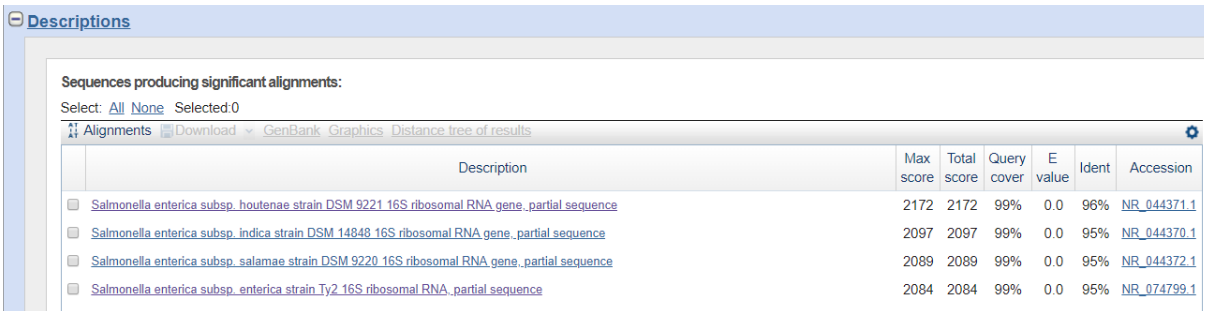

⊖ Descriptions

Sequences producing significant alignments:

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☐	Salmonella enterica subsp. indica strain DSM 14848 16S ribosomal RNA gene, partial sequence	2097	2097	99%	0.0	95%	NR_044370.1
☐	Salmonella enterica subsp. salamae strain DSM 9220 16S ribosomal RNA gene, partial sequence	2089	2089	99%	0.0	95%	NR_044372.1
☐	Salmonella enterica subsp. enterica strain Ty2 16S ribosomal RNA, partial sequence	2084	2084	99%	0.0	95%	NR_074799.1

FASTA sequence of CT7 (Accession# MH985332) alongwith top 4 hits from BLAST.

> CT7
GGCCTCTTGCCTTCAAATGTGCCCAGATGGGATTAGCTAGTTGGTGTGGTAACGG
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GACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCAGCGT
GTATGAAGAAGGCCTT
CGGGTTGTAAAGTACTTTCAGTGGGGTGGAAGGTGTTGTGGTTAATAACAGCAGC
AATTGACGTTACCAG
CAGAAGAAGCACCGGCTAACTCCGTGCCTGCAGCCGCGGTAATACGGAGGGTGC
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TACTGGGCGTAAAGCGCCCGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGG
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AGCGGTGAAATGCGT
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TTGGAGGTTGTGCCC
TTGAGGTGTGGCTTCCGGAGCTAACGTGTTAAGTAGACCGCCTGGGGAGTACGGC
CGCAAGGTTAAAACT
CAAATGAATTGACGGGGGCCCGCCCAAGTGGTGGAGCATGTGGTTTAATTCGATG
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TGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTTATCCT
TTGTTGCCTGTGATTAGGTCGGGAACTCAAAGGAAACTGCCAGTGATAAACTGGA
GGAAGGTGGGGATGA

CGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCA
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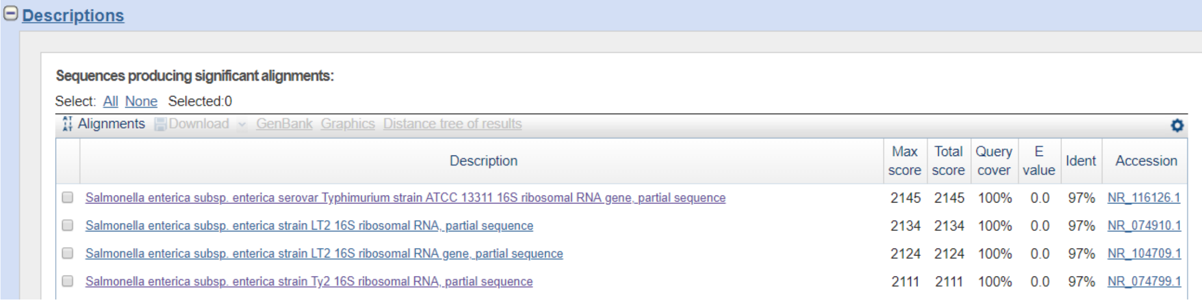

Sequences producing significant alignments:

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☐	Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA, partial sequence	2134	2134	100%	0.0	97%	NR_074910.1
☐	Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA gene, partial sequence	2124	2124	100%	0.0	97%	NR_104709.1
☐	Salmonella enterica subsp. enterica strain Ty2 16S ribosomal RNA, partial sequence	2111	2111	100%	0.0	97%	NR_074799.1

FASTA sequence of SP6 (Accession #MH985331) alongwith top 4 hits from BLAST.

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GGGATAACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCA
AAGAGGGGGACCTTCGGGCCTCTTGCCATCATATGTGCCCATATGGGATT
ATCTTGTTGGTGAGGTAACGGCTCACCAGGGCGACGATCCCTATCTGGTC
TGAGAGGATGACCACCCACACTGGAACTGACACACGGTCCACACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAGTGGGCGCAAGCCTGATGCACC
CATGCCGCGTGTATGAAAAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGG
GGAAGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGCAAA
AAAAGCACCGGCTAACTCCGTGCCAGCACCCGCGGTAATACAGAGGGTGC
AAGCGTTAATCGGAATTACTGGGCGTAGAGCGCACGCACGCGGTCTGTCA
AGTCAGATGTGAAATCCCCGGGCTCAACCTGAGAACTGCATTCGAAACTG
GCTGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCGGGTGTAGCGGTGAA
ATGCGTAGAGATCTGGAAGAATACCGGTGGCGAGCGCGGCCCCCCTGCAC
AAGACTGACGCTCATGTGCGAAGCGTGGAGAGCAACGGGATTAGATACCC
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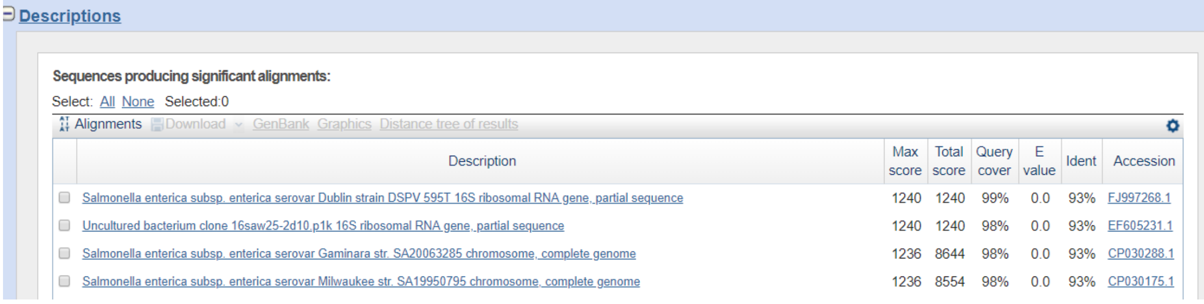

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	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Salmonella enterica subsp. enterica serovar Dublin strain DSPV 595T 16S ribosomal RNA gene, partial sequence	1240	1240	99%	0.0	93%	FJ997268.1
☐	Uncultured bacterium clone 16saw25-2d10.p1k 16S ribosomal RNA gene, partial sequence	1240	1240	98%	0.0	93%	EF605231.1
☐	Salmonella enterica subsp. enterica serovar Gaminara str. SA20063285 chromosome, complete genome	1236	8644	98%	0.0	93%	CP030288.1
☐	Salmonella enterica subsp. enterica serovar Milwaukee str. SA19950795 chromosome, complete genome	1236	8554	98%	0.0	93%	CP030175.1

Fasta sequence of PB2 (Accession# MH985338) alongwith top 4 hits from BLAST.

>PB2
AAATTGAAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTA
ACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACG
AGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGAT

AACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAAAGAG
GGGGACCTTCCCGCCACAAGCCAGCACATCTCGGCTGATCCCATTACCAA
GTTGGTGAGGTAACGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGA
GGATGACCAGCCACACTGGTTCTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATTGGGGGAAAAATGATGCAGGGATGG
GGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGG
AAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGCAGAAGAAG
CACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCG
TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCG
GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGG
CTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG
TAGAGATCTGGAGGAATACCGGTGGGGCGAAGGCGGCCCCCTGGACAAAG
ACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT
GGTAGTCCACGCCGTAAACGATGTCTACTTGGAGGTTGTGCCCTTGAGAG
CGTGGCTTCCGGAGCTAACGCGTTAAGTAGACCGCCATGACAGTACGGCC
GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAG
CATGTGATCCGATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACAT
CCACGGAAGTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAG
TGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTTCC
GCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACT
CAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGAATCGCAGGTA
C


Sequences producing significant alignments:

Select: All None Selected:0

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Salmonella gallinarum 16S ribosomal RNA gene, partial sequence	2004	2004	99%	0.0	97%	AF057360.1
☐	Salmonella enterica subsp. enterica serovar Anatum strain R16.0676 chromosome, complete genome	1958	13318	99%	0.0	96%	CP029800.1
☐	Salmonella enterica subsp. enterica serovar Anatum str. USDA-ARS-USMARC-1765, complete genome	1958	13377	99%	0.0	96%	CP014659.2
☐	Salmonella enterica subsp. enterica serovar Anatum str. USDA-ARS-USMARC-1781, complete genome	1958	13294	99%	0.0	96%	CP014666.2

Fasta sequence of PB9 (Accession# MH985340) alongwith top 4 hits from BLAST.
>PB9
AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTA
ACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACG
AGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGACGCGGAT
AACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAAAGAG
GGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTT
GTTGGTGAGGTAACGGCTCACCAAGGCGACGATCCCTTCCTGGTCTGAGA
GGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC
CGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGG
AAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGCAGAAGAAG
CACCGGCAAACGCCGTGCCTGCAGCCGCGGTAATACGGAGGGTGCAAGCG
TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCG
GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGG
CTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG
TAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGAC
TGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGG
TAGTCCACGCCGTAAACGATGTCTACTTGGAGGTTGTGCCCTTGAGGCGT
GGCTTCCGGAGCTAACGCGTTAAGTAGACCGCCTGGGGAGTACGGCCGCA

AGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCA
CGGAAGTTTTCAGAGATGAGAATGGTGCCTTCGGGAACCGTGAGACAGGT
GCTGCATGGCTGTCGTCAGCTCGTGTTGTGGGGTGTTCCCTTAAGTGGGG
CTTCGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTC
AAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGT
CATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATAC
AAAGAGAAGCGCCCACGCGTGTGCGAGGGGAGGTCATGGGGTACGACGCA
GGCCGGAAAGGGGTCGGCAACTCGACTCCAGGAAGTCGGAATCGCTAGTA
ATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACAC
CGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACC
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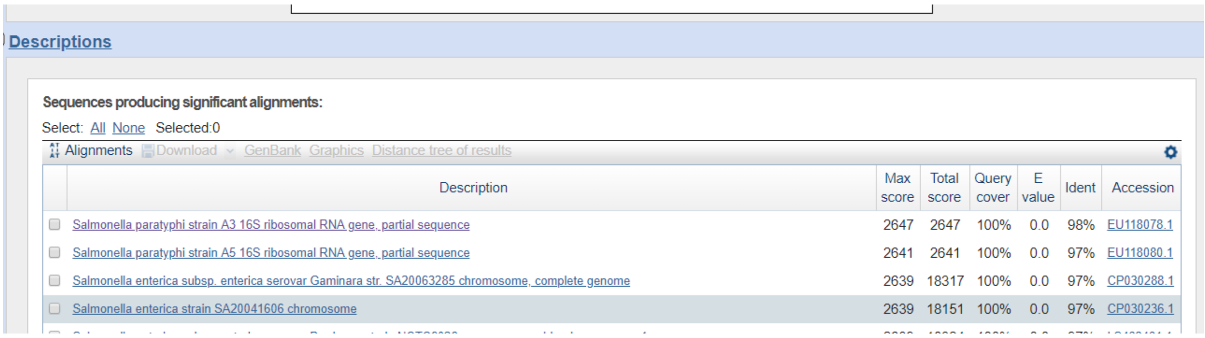

Descriptions

Sequences producing significant alignments:

Select: All None Selected:0

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Salmonella paratyphi strain A3 16S ribosomal RNA gene, partial sequence	2647	2647	100%	0.0	98%	EU118078.1
☐	Salmonella paratyphi strain A5 16S ribosomal RNA gene, partial sequence	2641	2641	100%	0.0	97%	EU118080.1
☐	Salmonella enterica subsp. enterica serovar Gaminara str. SA20063285 chromosome, complete genome	2639	18317	100%	0.0	97%	CP030288.1
☐	Salmonella enterica strain SA20041606 chromosome	2639	18151	100%	0.0	97%	CP030236.1

Fasta sequence of HE13 (Accession# MH985333) alongiwth top 4 hits from BLAST.

>HE13
ATTGAACGCTGGTGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGA
AACAGCTTGCTGCTTCGCTGACGAGTGGCGACCGGGTGAGTAATGTCTGG
GAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTGGCTAATACAG
CATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAG
ATGTGCCCAGATGGGATTAGCTTGTTGGTGAGGTAACGGCTCACCAAGGC
GACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGA
CACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG
GCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTT
GTAAAGTACTTTCAGCGGGGAGCAAGGTGTTGTGGTTAATAACCGCAGCA
ATTGAAGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAACAACAG
CGGTAATAAGGAGGGTGCAATTGTTAATCGGAATTACTGGGCGTAAAGCG
CACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGG
GAACTGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGAGGGGGGTAGAAT
TCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG
AAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCAAGCCGTAAACGATGTCTACT
TGCAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTAG
ACCGCCTGGGCAGTACGGCAGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCAGCACAATGGTGCAGCATGTGGTTTAATTCGATGCAAAGCGAAGA
ACCTTACCTGGTCTTGACATCCACAGAANNNTCCAGAGATGAATTNGTGC
CTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAACTCGTGTTG
TGAAATGTTGGGTTAAGTCCAACAAAGAGCGCAACCCTTATCCTTTGTTG
CCAACGATTAGGTGGGGAACTCAAAGGAGACTGCCAGTGATAAACTGCAG
CAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTAAGACCAGGGCTACA
CAAGTGCTACAATGGTGCATACAAAGAGAATTGACCTCGCGAGAGCAAGC

GCACCTCATAAAGTGCGTCGTAGTCCGCATTGGAGTCTGCAACTCGACTC
CATGAAGTAGGAATCGCTAGTAATCGTGCATCAGGGTGCCAAGGTGAATA
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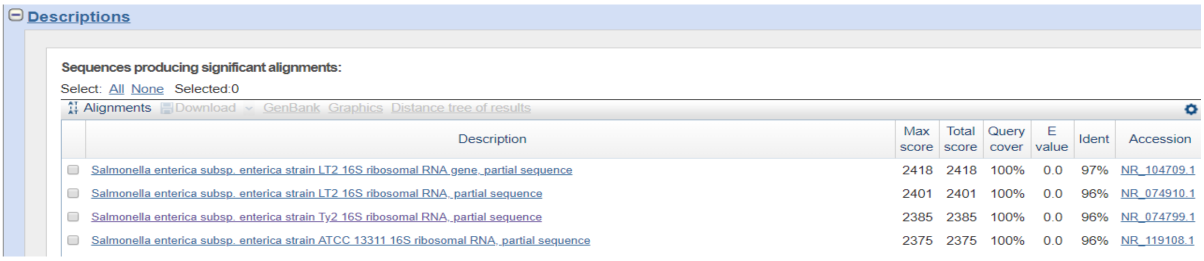

Sequences producing significant alignments:

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA gene, partial sequence	2418	2418	100%	0.0	97%	NR_104709.1
☐	Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA, partial sequence	2401	2401	100%	0.0	96%	NR_074910.1
☐	Salmonella enterica subsp. enterica strain Ty2 16S ribosomal RNA, partial sequence	2385	2385	100%	0.0	96%	NR_074799.1
☐	Salmonella enterica subsp. enterica strain ATCC 13311 16S ribosomal RNA, partial sequence	2375	2375	100%	0.0	96%	NR_119108.1

Appendix E

Results of safety test for HB1 antigen (0.5 ml) in Group SR1, 7 days post oral immunization.

Type of antigen	Post immunization Hours	Results based on the average of readings of 5 rabbits					
		Physical abnormalities	Average Body temperature	Daily food intake	Water intake	Weight	Recovery of HB1 antigen CFU/ml
HB1 live antigen	24	None	102.9	Normal	Normal	Normal	1×10^{3}
	48	-	102.5	Dropped	Dropped	Normal	1×10^{4}
	72	-	103.1	Normal	Normal	Normal	1×10^{4}
	96	-	102.5	Normal	Normal	Normal	1×10^{5}
	120	-	103.1	Normal	Normal	Normal	1×10^{5}
	144	-	101.6	Normal	Normal	Normal	1×10^{4}
	168	-	102.2	Normal	Normal	Normal	1×10^{4}

Results of Safety test for HB1 antigen (1ml) in Group SR2 7 days post oral immunization.

Type of antigen	Post immunization hours	Results based on the average of readings of 5 rabbits					
		Physical abnormalities	Average Body Temperature	Food intake	Water Intake	Changes in weight	Recovery of HB1 antigen CFU/ml
HB1 live antigen	24	None	103.2	Normal	Normal	Normal	1×10^{6}
	48	-	102.9	Normal	Normal	Normal	1×10^{8}
	72	-	102.5	Normal	Normal	Normal	1×10^{8}
	96	-	102.7	Normal	Normal	Normal	1×10^{7}
	120	-	102.1	Normal	Normal	Normal	1×10^{7}
	144	-	101.9	Normal	Normal	Normal	1×10^{6}
	168	-	102.5	Normal	Normal	Normal	1×10^{7}

Results of Safety test for control group of rabbits given normal saline 0.5ml.

Type of antigen	Post vaccination Hours	Results based on the average of readings obtained from of the 5 rabbits					
		Physical abnormalities	Average Body Temperature	Food intake	Water Intake	Changes in weight	Recovery of HB1 antigen CFU/ml
HB1 live antigen	24	None	100.7	Normal	Normal	Normal	-
	48	-	102.0	Normal	Normal	Normal	-
	72	-	102.1	Normal	Normal	Normal	-
	96	-	102.5	Normal	Normal	Normal	-
	120	-	102.3	Normal	Normal	Normal	-
	144	-	102.4	Normal	Normal	Normal	-
	168	-	102.3	Normal	Normal	Normal	-

Appendix F

Results of IHA antibody titers in the four groups of immunized rabbits.

Sampling post vaccination days	Indirect hemagglutination titer in the four group of rabbits			
	R1	R2	R3	R4
Day 10	1:16	1:8	1:8	-
	1:4	1:16	1:16	-
	1:8	1:16	1:16	-
	1:8	1:32	1:32	-
	1:16	1:8	1:8	-
	1:16	1:8	1:8	-
	1:8	1:8	1:8	-
	1:8	1:8	1:8	-
	1:4	1:16	1:16	-
	1:16	1:16	1:16	-
Day 20	1:32	1:64	1:32	-
	1:16	1:32	1:32	-
	1:8	1:32	1:32	-
	1:16	1:32	1:32	-
	1:16	1:64	1:16	-
	1:32	1:32	1:32	-
	1:32	1:32	1:32	-
	1:16	1:16	1:32	-
	1:8	1:128	1:16	-
	1:16	1:64	1:32	-
Day 30	1:64	1:128	1:16	-
	1:64	1:64	1:32	-
	1:16	1:64	1:64	-
	1:8	1:64	1:64	-
	1:32	1:256	1:32	-
	1:64	1:128	1:32	-
	1:32	1:64	1:64	-
	1:32	1:64	1:64	-
	1:32	1:128	1:64	-
	1:64	1:64	1:64	-
Day 40	1:128	1:512	1:64	-
	1:64	1:256	1:128	-
	1:32	1:64	1:256	-
	1:32	1:64	1:128	-
	1:64	1:256	1:32	-
	1:64	1:64	1:64	-
	1:128	1:64	1:128	-
	1:64	1:64	1:256	-
	1:32	1:256	1:256	-
	1:128	1:64	1:128	-
Day 50	1:256	1:64	1:128	-
	1:128	1:64	1:64	-
	1:64	1:32	1:128	-
	1:32	1:32	1:64	-
	1:64	1:64	1:64	-
	1:32	1:32	1:64	-
	1:128	1:64	1:128	-
	1:64	1:32	1:128	-

	1:32	1:128	1:128	-
	1:128	1:32	1:16	-
Day 60	1:64	1:32	1:8	-
	1:32	1:64	1:4	-
	1:16	1:16	1:8	-
	1:32	1:64	1:16	-
	1:8	1:32	1:16	-
	1:16	1:16	1:4	-
	1:64	1:16	1:4	-
	1:16	1:8	1:16	-
	1:32	1:64	1:32	-
	1:128	1:32	1:8	-
Day 70	1:4	1:16	1:4	-
	1:4	1:4	1:2	-
	1:16	1:8	1:4	-
	1:8	1:4	1:4	-
	1:2	1:8	1:8	-
	1:4	1:2	-	-
	1:8	-	1:4	-
	-	1:4	1:8	-
	1:4	1:4	-	-
	1:32	1:32	1:16	-