

# Strangles in horses can be caused by vaccination with Pinnacle I.N.

Use of whole genome sequencing in  
solving a diagnostic dilemma



Ray Cursons  
Olivia Patty  
Heng Zhang



Karen Steward  
Andrew Waller

# Disease Management

- Prevention is the singularly most important aspect
  - Vaccinate regularly with your veterinarian's advice
  - Keep horse areas cleaned up and sanitized
  - Proper feeds and feeding management
- Control is invaluable once a disease is diagnosed
  - Separate ill horses (have an isolation area)
  - Disinfecting is a cornerstone of many control measures
- Cure is the goal once a disease is diagnosed
  - Get treatment early to avoid additional complications
  - Rigorously follow the prescribed treatment guidelines

# Why Is Vaccination Important

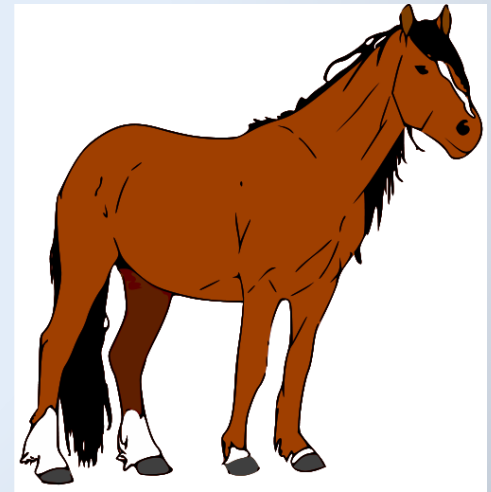
Vaccines control diseases and can protect individual animals from illness and death.

Target specific diseases



Stop epidemics

Protect vaccinated animals



# Vaccination

## Does not guarantee disease prevention

- Intramuscular route
  - High rate of vaccine reactions
  - Approximately 60% effective
- Intranasal route
  - Initial 2 doses, 2-3weeks apart-protected 1 month after second dose
  - Annual booster
- Not recommended during an outbreak
- Recovered horses often develop immunity to the infecting strain for up to 5 years

# Vaccination Terms

**Modified Live (attenuated) Vaccine:** Vaccines that are alive but have lost their disease-causing ability

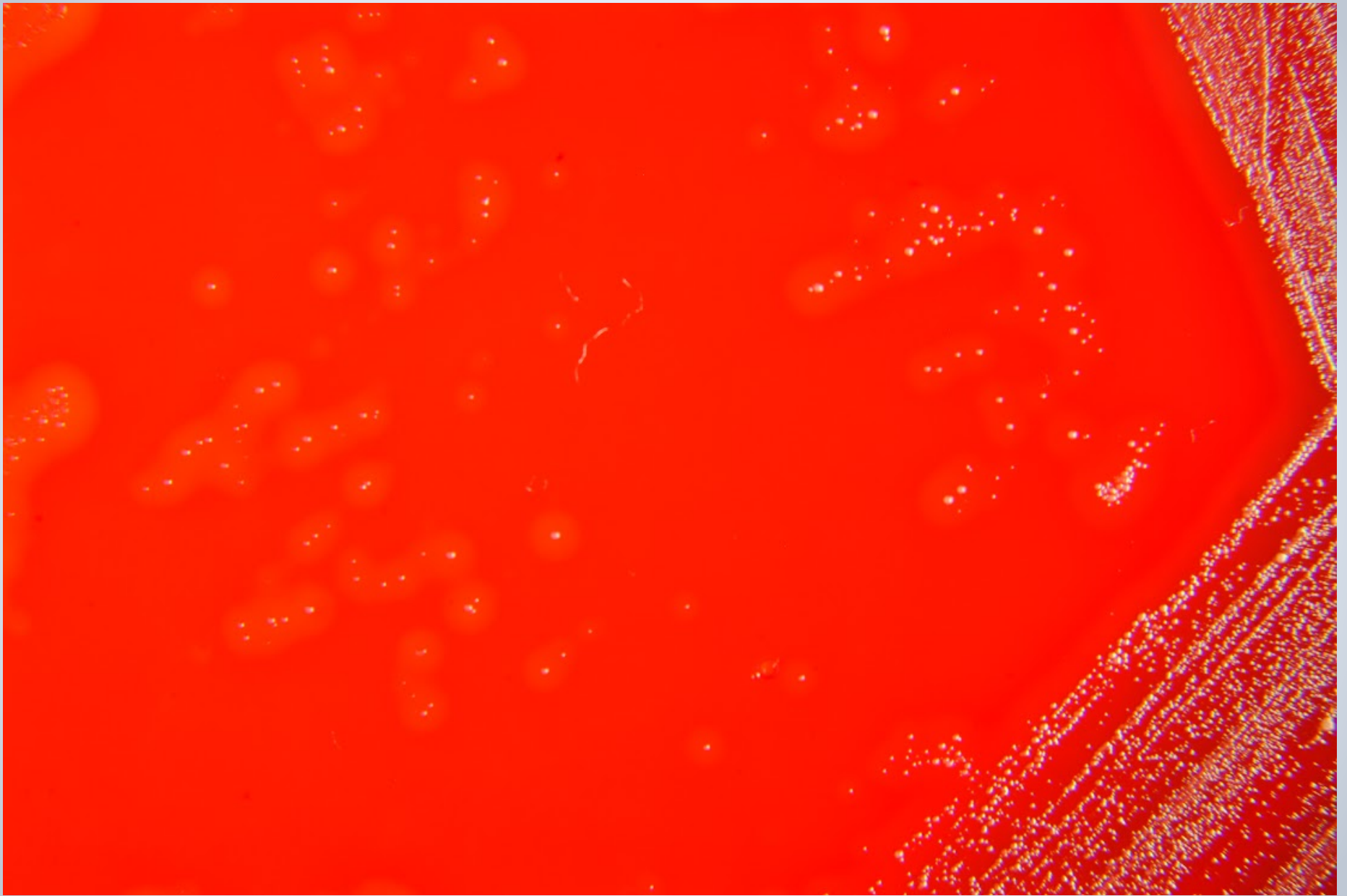
**Killed Vaccine:** Vaccines that don't contain live pathogens, but still achieve an immune response

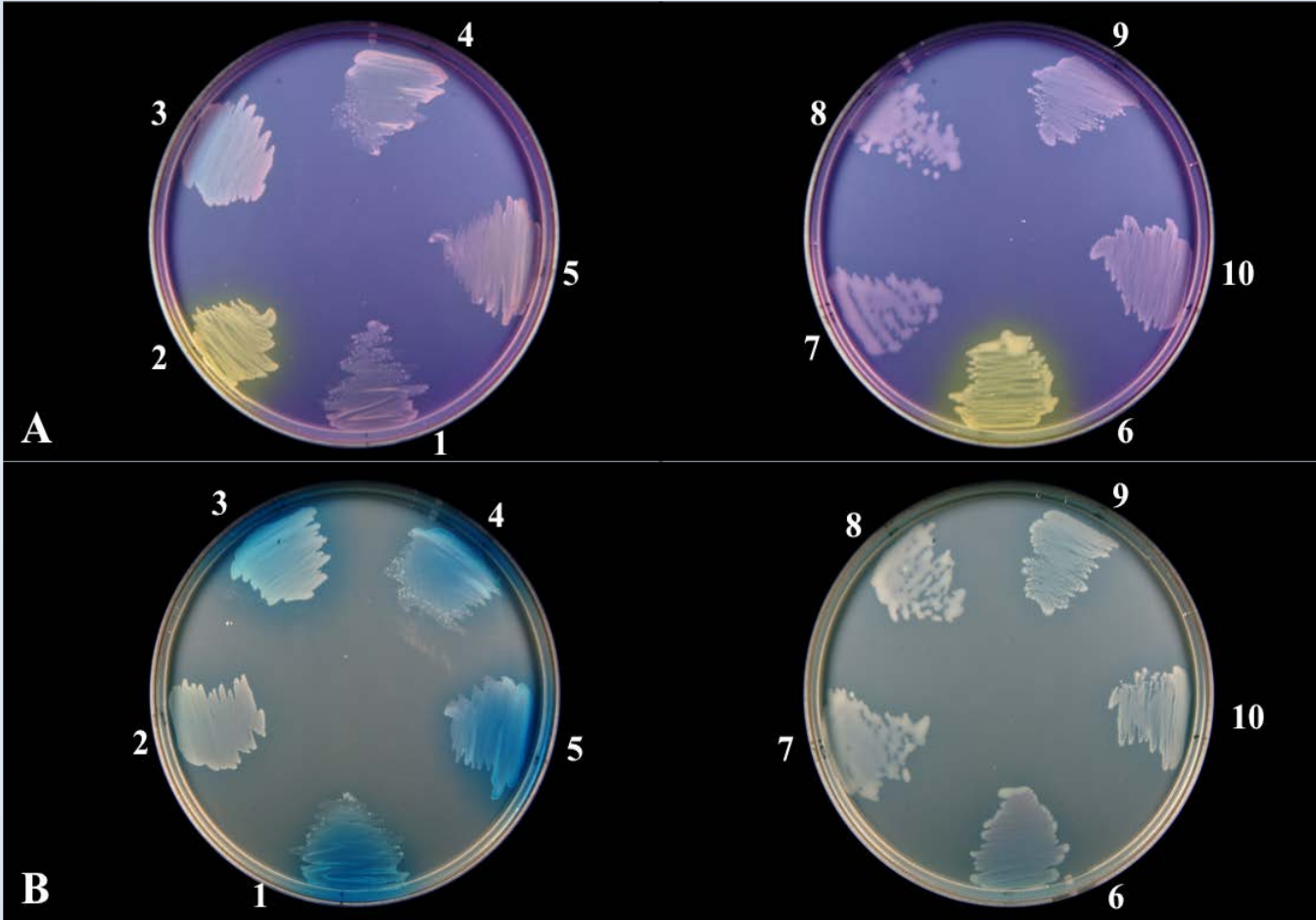
# Case 1

- Sample 136 was obtained from a female yearling Standardbred
- Swollen retropharyngeal abscess was observed 10 days after the first Pinnacle vaccination (batch# 139192C).
- A bacteriological swab taken from the abscess grew small dry colonies of  $\beta$  haemolytic streptococcus
- Single sub colonies were negative for sorbitol and trehalose fermentation but positive for seM PCR

# Case 2

- Sample 142 was obtained from a 10 month thoroughbred filly.
- She was observed to have both mandibular lymph nodes swollen after administration of the second dose of Pinnacle vaccine (14 days after the first dose)
- On examination she was bright and afebrile
- Swab grew small dry colonies of  $\beta$  haemolytic streptococci which were negative for sorbitol and trehalose fermentation but positive for seM PCR.





# Diagnostic Dilemma

- Were the infections due solely to vaccine strains?
- Were the infections due to reversion of the vaccine strain ?
- Were the infections due to some recombination event between wild type *S equi* and the vaccine strain
- Were the infections due to an acapsular wild type strain?





# Whole Genome Sequencing

- Next generation ( or massively parallel )sequencing allow the examination of complete bacterial genomes
- WGS can theoretically resolve bacterial strains that differ by a single DNA base pair.
- 3 or fewer variants **genomically indistinguishable**
- Up to 12 variants **closely related**
- 13 or more variants **unrelated**

# NGS of *S. equi* isolates Se136 and SE142

- The core genome sequences of Se136 and Se142 each contained 124 of the 126 SNPs common to SeCF32 and both Pinnacle strains.
- All five SNPs in the SeM gene were shared, confirming that all of these isolates contain the SeM-2 allele.
- Se136 and Se142 shared 67 of the 68 mutations identified in the core genome of the sequenced Pinnacle variants.
- The previously reported deletion of a single guanine residue in the promoter region of SzPSe in the Pinnacle strain was also identified in Se136 and Se142 at position 912,150 bp relative to the Se4047 reference genome.

## Snps of pinnacle shared with SeCF32

Position in Se4047	CDS name	Product	Mutation	Se 4047	Se CF32	Pinnacle H+	Pinnacle H-	Se136	Se142
15345	ftsH	putative cell division protease FtsH	N	A	G	G	G	G	G
35948	purF	putative amidophosphoribosyltransferase precursor	N	A	G	G	G	G	G
55146	adhE	aldehyde-alcohol dehydrogenase 2 [includes: alcohol dehydrogenase; acetaldehyde dehydrogenase]	S	C	T	T	T	T	T
68486	rpsJ	30S ribosomal protein S10	N	C	T	T	T	T	T
86184	SEQ_0085	putative membrane protein (pseudogene)	-	C	T	T	T	T	T
99943	SEQ_0094	ABC transporter ATP-binding protein	N	C	T	T	T	T	T
129228	Intergenic	-	-	T	C	C	C	C	C
222627	Intergenic	-	-	T	C	C	C	C	C
237583	SEQ_0256	putative cell surface-anchored protein	N	C	A	A	A	A	A
246505	SEQ_0260	putative collagen-like cell surface-anchored protein SclH	S	T	G	G	G	G	G
271709	gcaD	bifunctional GlmU protein [includes: UDP-N-acetylglucosamine pyrophosphorylase; glucosamine-1-phosphate N-acetyltransferase]	S	T	C	C	C	C	C
271724	gcaD	bifunctional GlmU protein [includes: UDP-N-acetylglucosamine pyrophosphorylase; glucosamine-1-phosphate N-acetyltransferase]	S	G	A	A	A	A	A
271745	gcaD	bifunctional GlmU protein [includes: UDP-N-acetylglucosamine pyrophosphorylase; glucosamine-1-phosphate N-acetyltransferase]	S	C	T	T	T	T	T
281366	sms	putative DNA repair protein	N	T	C	C	C	C	C
305027	SEQ_0321	extracellular solute-binding lipoprotein	N	A	G	G	G	G	G
326762	plr	glyceraldehyde-3-phosphate dehydrogenase	N	G	T	T	T	T	T

Position in Se4047	CDS name	Product	Mutation	Se4047	SeCF32	Pinnacle H+	Pinnacle H-	Se136	Se142
384203	SEQ_0402	putative cell surface-anchored protein	N	C	T	T	T	T	T
410026	SEQ_0432	endoglycosidase (EndoS)	N	G	A	A	A	A	A
411742	SEQ_0432	endoglycosidase (EndoS)	N	G	T	T	T	T	T
435708	SEQ_0451	putative membrane protein	S	C	T	T	T	T	T
493653	SEQ_0514	conserved hypothetical protein (fragment)	-	C	T	T	T	T	T
503929	Intergenic	-	-	A	G	G	G	G	G
532993	sagI	ABC-2 type transporter protein	N	T	C	C	C	C	C
561705	SEQ_0577	hypothetical protein	N	G	A	A	A	A	A
574596	SEQ_0591	sensor histidine kinase	N	C	T	T	T	T	T
587942	asnA	aspartate--ammonia ligase	Nonsense	C	T	T	T	T	T
599783	murD	UDP-N-acetylmuramoylalanine--D-glutamate ligase	N	C	T	T	T	T	T
614234	clpE	putative ATP-dependent Clp protease ATP-binding subunit	N	A	G	G	G	G	G
621349	SEQ_0637	putative phosphoglucomutase	N	G	A	A	A	A	A
764549	SEQ_0771	putative membrane protein	S	C	A	A	A	A	A
770851	ppc	putative phosphoenolpyruvate carboxylase	N	C	T	T	T	T	T
776501	SEQ_0781	putative ABC transporter, ATP-binding/permease protein	N	T	G	G	G	G	G
827960	asnS	asparaginyl-tRNA synthetase	S	A	G	G	G	G	G
829040	SEQ_0855	putative collagen-like surface-anchored protein ScIF	N	C	T	T	T	T	.

Position in Se4047	CDS name	Product	Mutation	Se4047	SeCF32	Pinnacle H+	Pinnacle H-	Se136	Se142
843198	SEQ_0866	putative beta-galactosidase precursor (pseudogene)	-	A	G	G	G	G	G
845718	SEQ_0872	sugar phosphotransferase system (PTS), mannose/fructose/sorbose family, IID component	N	A	G	G	G	G	G
871397	SEQ_0896	putative exported protein	N	A	G	G	G	G	G
877535	SEQ_0904	endonuclease/exonuclease/phosphatase family surface anchored protein	N	T	C	C	C	C	C
879585	Intergenic	-	-	C	T	T	T	T	T
893251	glgP	glycogen phosphorylase	S	C	T	T	T	T	T
915905	SEQ_0935	collagen binding, putative ancillary pilus subunit Cne	N	A	G	G	G	G	G
918629	Intergenic	-	-	G	T	T	T	T	T
923906	SEQ_0944	putative cell surface-anchored pullulanase	N	A	G	G	G	G	G
959663	SEQ_0971	putative exopolysaccharide biosynthesis protein	N	A	G	G	G	G	G
1018120	SEQ_1031	ABC transporter permease protein	N	G	A	A	A	A	A
1019562	SEQ_1031	ABC transporter permease protein	S	C	A	A	A	A	A
1039061	ciaR	response regulator protein	N	C	T	T	T	T	T
1048466	SEQ_1061	putative lipoprotein	S	T	C	C	C	C	C
1065100	SEQ_1077	putative cell surface-anchored C5A peptidase precursor (pseudogene)	-	G	A	A	A	A	A
1082651	SEQ_1093	conserved hypothetical protein	N	C	T	T	T	T	T
1093041	mvaD	mevalonate diphosphate decarboxylase	N	G	A	A	A	A	A
1108279	Intergenic	-	-	G	A	A	A	A	A

Position in Se4047	CDS name	Product	Mutation	Se4047	SeCF32	Pinnacle H+	Pinnacle H-	Se136	Se142
1151452	nox	NADH oxidase	N	A	G	G	G	G	G
1169048	Intergenic	-	-	C	T	T	T	T	T
1170265	Intergenic	-	-	T	C	C	C	C	C
1188884	citG	putative 2-(5''-triphosphoribosyl)-3'-dephosphocoenzyme-A synthase	N	A	C	C	C	C	C
1197999	topA	DNA topoisomerase I	S	A	G	G	G	G	G
1204800	SEQ_1226	putative cell surface-anchored thioredoxin (fragment)	-	T	C	C	C	C	C
1294595	SEQ_1298	mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase family protein	N	A	G	G	G	G	G
1301187	SEQ_1303	putative membrane protein	S	T	C	C	C	C	C
1352514	Intergenic	-	-	C	T	T	T	T	T
1358309	SEQ_1357	conserved hypothetical protein	N	T	C	C	C	C	C
1365549	pepV	putative Xaa-His dipeptidase	S	A	G	G	G	G	G
1369498	SEQ_1369	extracellular solute-binding protein	N	A	G	G	G	G	G
1382434	Intergenic	-	-	T	C	C	C	C	C
1393718	SEQ_1397	putative phosphoglucosamine mutase	N	T	A	A	A	A	A
1410544	SEQ_1409	metallo-beta-lactamase superfamily protein	S	G	A	A	A	A	A
1416703	SEQ_1414	conserved hypothetical protein	S	G	A	A	A	A	A
1420131	rmIB	putative dTDP-glucose-4,6-dehydratase	N	G	T	T	T	T	T
1434510	SEQ_1434	phosphoribulokinase/uridine kinase family protein	N	C	T	T	T	T	T

Position in Se4047	CDS name	Product	Mutation	Se4047	SeCF32	Pinnacle H+	Pinnacle H-	Se136	Se142
1437575	malM	putative 4-alpha-glucanotransferase	S	T	G	G	G	G	G
1453066	malX	putative maltose/maltodextrin-binding protein precursor	N	T	C	C	C	C	C
1470134	SEQ_1461	putative regulatory protein	N	A	T	T	T	T	T
1476310	pepS	aminopeptidase PepS (pseudogene)	-	C	T	T	T	T	T
1515816	SEQ_1505	putative exported protein	N	A	G	G	G	G	G
1547148	phoR	putative alkaline phosphatase synthesis sensor protein	S	C	T	T	T	T	T
1558867	Intergenic	-	-	A	G	G	G	G	G
1565188	smc	putative chromosome partition protein	N	C	T	T	T	T	T
1580714	vicK	sensor histidine kinase	S	T	A	A	A	A	A
1590904	SEQ_1587	two-component response regulator	N	T	G	G	G	G	G
1592663	Intergenic	-	-	C	T	T	T	T	T
1613700	SEQ_1606	putative collagen-binding collagen-like cell surface-anchored protein FneC	N	A	T	T	T	T	T
1616621	fpg	formamidopyrimidine-DNA glycosylase	S	T	C	C	C	C	C
1628273	Intergenic	-	-	A	C	C	C	.	C
1628522	SEQ_1619	putative repressor protein	N	G	T	T	T	T	T
1642901	era	GTP-binding protein Era homolog	N	T	G	G	G	G	G
1645614	SEQ_1637	uracil DNA glycosylase superfamily protein	N	G	A	A	A	A	A
1658038	rplA	50S ribosomal protein L1	N	C	T	T	T	T	T

Position in Se4047	CDS name	Product	Mutation	Se4047	SeCF32	Pinnacle H+	Pinnacle H-	Se136	Se142
1679353	valS	valyl-tRNA synthetase	S	G	A	A	A	A	A
1706846	SEQ_1694	GntR family regulatory protein	S	T	C	C	C	C	C
1710546	SEQ_1696	glycosyl hydrolases family protein	S	T	C	C	C	C	C
1722028	SEQ_1709	bacteriocin transport/processing ATP-binding protein	N	C	T	T	T	T	T
1724304	SEQ_1711	sensor histidine kinase	S	A	G	G	G	G	G
1780065	SEQ_1773	putative membrane protein	N	T	C	C	C	C	C
1787944	rpoZ	DNA-directed RNA polymerase omega chain	N	T	C	C	C	C	C
1801845	nadE	NH(3)-dependent NAD(+) synthetase	S	G	A	A	A	A	A
1805955	SEQ_1795	putative thioredoxin reductase	S	A	G	G	G	G	G
1827750	sclB	collagen-like surface-anchored protein SclI	N	C	G	G	G	.	.
1830866	SEQ_1819	probable transaldolase	N	G	C	C	C	C	C
1887682	SEQ_1887	replication initiation and membrane attachment protein	S	T	C	C	C	C	C
1891918	SEQ_1891	conserved hypothetical protein	N	A	C	C	C	C	C
1905135	Intergenic	-	-	C	T	T	T	T	T
1952038	SEQ_1944	putative exported protein	S	A	G	G	G	G	G
1952095	SEQ_1944	putative exported protein	S	A	G	G	G	G	G
1958402	Intergenic	-	-	C	T	T	T	T	T
1978783	SEQ_1957	Streptococcal histidine triad protein	N	C	T	T	T	T	T
2017200	SEQ_1958	putative exported protein	N	C	G	G	G	G	G

Position in Se4047	CDS name	Product	Mutation	Se4047	SeCF32	Pinnacle H+	Pinnacle H-	Se136	Se142
2023629	polC	DNA polymerase III PolC-type	S	G	A	A	A	A	A
2043737	fbp	antiphagocytic cell surface-anchored fibrinogen- and IgG Fc-binding protein SeM	N	A	G	G	G	G	G
2044537	fbp	antiphagocytic cell surface-anchored fibrinogen- and IgG Fc-binding protein SeM	N	A	C	C	C	C	C
2044647	fbp	antiphagocytic cell surface-anchored fibrinogen- and IgG Fc-binding protein SeM	N	C	T	T	T	T	T
2044779	fbp	antiphagocytic cell surface-anchored fibrinogen- and IgG Fc-binding protein SeM	N	T	C	C	C	C	C
2044792	fbp	antiphagocytic cell surface-anchored fibrinogen- and IgG Fc-binding protein SeM	S/N	T	A	A	A	A	A
2046937	SEQ_2019	putative NrdI-like protein	N	C	T	T	T	T	T
2141022	SEQ_2126	transport system permease protein	N	T	C	C	C	C	C
2147594	pepA	putative glutamyl-aminopeptidase	N	G	C	C	C	C	C
2154656	SEQ_2145	conserved hypothetical protein	S	T	C	C	C	C	C
2186008	SEQ_2173	putative two-component regulatory system, sensor kinase protein	S	C	A	A	A	A	A
2203798	SEQ_2190	putative cell surface-anchored protein	N	G	A	A	A	A	A

# Pinnacle specific snps

Position in Se4047	CDS name	Product	Mutation	Reference base	SeCF32	Pinnacle H+	Pinnacle H-	Se136	Se142
70401	rplW	50S ribosomal protein L23	S	C	.	T	T	T	T
75140	rpsN	30S ribosomal protein S14	N	C	.	T	T	T	T
141893	ahpC	alkyl hydroperoxide reductase subunit C	N	G	.	A	A	A	A
187907	SEQ_0204	conserved hypothetical protein	N	G	.	A	A	A	A
200494	Intergenic	-	-	C	.	T	T	T	T
264006	SEQ_0281	putative membrane protein	N	G	.	A	A	A	A
264915	Intergenic	-	-	C	.	T	T	T	T
290826	SEQ_0305	membrane protein OxaA 1 precursor	S	G	.	A	A	A	A
296807	SEQ_0311	hypothetical protein	N	G	.	A	A	A	A
296904	SEQ_0311	hypothetical protein	S	G	.	A	A	A	A
314994	engC	probable GTPase EngC	N	G	.	A	A	A	A
377115	spi	putative signal peptidase I	N	C	.	T	T	T	T
377136	spi	putative signal peptidase I	S	T	.	G	G	G	G
379647	mutS2	putative DNA mismatch repair protein	N	C	.	T	T	T	T
412238	Intergenic	-	-	C	.	T	T	T	T
423883	SEQ_0442	putative amidase	N	C	.	A	A	A	A
433041	SEQ_0449	ABC transporter ATP-binding membrane protein	N	C	.	T	T	T	T
433209	SEQ_0449	ABC transporter ATP-binding membrane protein	N	C	.	T	T	T	T
479326	manM	putative mannose-specific phosphotransferase system (PTS), IIC component	N	C	.	T	T	T	T
512781	SEQ_0535	TetR family regulatory protein	N	C	.	T	T	T	T
520587	SEQ_0542	isoprenylcysteine carboxyl methyltransferase (ICMT) family protein	S	C	.	T	T	T	T
551092	Intergenic	-	-	C	.	T	T	T	T
558306	nrdE	ribonucleoside-diphosphate reductase alpha subunit	N	C	.	T	T	T	T
637656	Intergenic	-	-	C	.	T	T	T	T
704512	dgoA	putative 2-dehydro-3-deoxy-6-phosphogalactonate aldolase	S	G	.	A	A	A	A
709838	SEQ_0720	glycosyl hydrolase family protein	N	G	.	A	A	A	A
714042	SEQ_0723	putative DNA-binding protein	N	G	.	A	A	A	A
775341	SEQ_0781	putative ABC transporter, ATP-binding/permease protein	N	C	.	T	T	T	T
776727	Intergenic	-	-	C	.	T	T	T	T
835191	adcA	zinc-binding protein AdcA precursor	N	G	.	A	A	A	A
855905	SEQ_0885	voltage gated chloride channel	S	G	.	A	A	A	A
866774	SEQ_0893	putative haloacid dehalogenase-like hydrolase	N	G	.	A	A	A	A
879265	SEQ_0904	endonuclease/exonuclease/phosphatase family surface anchored protein	N	G	.	A	A	A	A
1045149	SEQ_1057	putative methyltransferase	N	G	.	A	A	A	A
1081948	SEQ_1092	putative methyltransferase	N	G	.	A	A	A	A

Position in Se4047	CDS name	Product	Mutation	Reference base	SeCF32	Pinnacle H+	Pinnacle H-	Se136	Se142
1131643	SEQ_1143	conserved hypothetical protein	N	G	.	A	A	A	A
1145206	prfA	peptide chain release factor 1	S	C	.	T	T	T	T
1186985	SEQ_1206	putative Mg2+/citrate complex transporter	N	C	.	T	T	T	T
1201432	rnh	ribonuclease HII	N	C	.	T	T	T	T
1281023	SEQ_1281	putative ABC transporter, ATP-binding/permease protein (pseudogene)	-	C	.	T	T	T	T
1292817	SEQ_1295	putative membrane protein	Nonsense	C	.	T	T	T	T
1319788	lspA	lipoprotein signal peptidase	N	C	.	T	T	T	T
1404022	pdhB	putative pyruvate dehydrogenase E1 component, beta subunit	S	C	.	T	T	T	T
1423796	SEQ_1421	NIF3 (NGG1p interacting factor 3) family protein	N	C	.	T	T	T	T
1443533	malD	putative maltose/maltodextrin ABC transport system permease protein	N	C	.	T	T	T	T
1458644	dltA	D-alanine-poly(phosphoribitol) ligase subunit 1	S	C	.	T	T	T	T
1513627	SEQ_1505	putative exported protein	S	C	.	T	T	T	T
1516582	SEQ_1507	putative membrane protein (pseudogene)	-	C	.	T	T	T	T
1553733	SEQ_1554	beta-glucoside-specific phosphotransferase system (PTS), IIABC component	N	G	.	A	A	A	A
1592195	SEQ_1588	putative lantibiotic leader peptide processing serine protease	S	C	.	T	T	T	T
1613302	SEQ_1606	putative collagen-binding collagen-like cell surface-anchored protein FneC	N	G	.	A	A	.	.
1614104	SEQ_1607	putative collagen-binding surface-anchored protein FneD (pseudogene)	-	G	.	A	A	A	A
1624578	SEQ_1613	sugar phosphotransferase system (PTS), IIC component (pseudogene)	-	G	.	A	A	A	A
1634730	atoA	acetate CoA-transferase beta subunit	N	G	.	A	A	A	A
1640322	SEQ_1630	ABC transporter, ATP-binding protein	N	G	.	A	A	A	A
1780549	stk1	serine/threonine-protein kinase	S	G	.	A	A	A	A
1794496	SEQ_1786	putative RNA methylase family protein	N	G	.	A	A	A	A
1805291	SEQ_1794	amino acid permease	N	G	.	A	A	A	A
1811984	mraW	putative helicase	N	C	.	T	T	T	T
1838198	holB	putative DNA polymerase III, delta' subunit	N	G	.	A	A	A	A
1856339	SEQ_1848	PAP2 superfamily protein	N	G	.	A	A	A	A
1865773	xerD	tyrosine recombinase XerD	S	G	.	A	A	A	A
1883354	SEQ_1884	putative helicase	S	G	.	A	A	A	A
1890206	csrS	sensor histidine kinase	N	G	.	A	A	A	A
1890358	csrS	sensor histidine kinase	N	A	.	G	.	.	.
1905579	SEQ_1903	extracellular solute-binding protein	Nonsense	G	.	.	A	.	.
1937188	sufD	FeS assembly protein SufD	N	G	.	A	A	A	A
2062011	SEQ_2032	putative ATPase	N	C	.	T	.	.	.
2176769	SEQ_2165	putative antibiotic resistance transporter, ATP-binding protein	N	G	.	A	A	A	A
2195898	SEQ_2182	putative response regulator	N	G	.	A	A	A	A
2233125	Intergenic	-	-	G	.	A	A	A	A

# Factors affecting vaccine efficacy

- **Host Factors**
- Age
- Comorbidity
- Prior exposure
- Time since vaccination
- **Vaccine Characteristics**
- Live vs inactivated
- Mode of delivery
- Vaccine Composition
- **Match to circulating strains**

# acknowledgements

- New Zealand Equine Research Foundation
- Veterinarians
- University of Waikato
- The Animal Health Trust

