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Short communication

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

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ABSTRACT

The differentiation of live attenuated vaccine strains from their progenitor and wild-type counterparts is important for ongoing surveillance of product safety and improved guidelines on their use. We utilised a genome sequencing approach to confirm that two cases of strangles in previously healthy horses that had received the Pinnacle I. N. vaccine (Zoetis) were caused by the vaccine strain. Our data shed new light on the safety of this vaccine and suggest that factors beyond the maturity of the animal's immune system influence the development of adverse reactions.

1. Introduction

Strangles, one of the most prevalent infectious diseases of horses worldwide, is caused by the equine-restricted bacterium *Streptococcus equi* subspecies *equi* (*S. equi*) [10]. Prevention of this highly contagious disease is managed by quarantine, diagnostic testing for the presence of *S. equi* and vaccination [1]. The Pinnacle I. N. (Pinnacle) vaccine (Zoetis), which is available for use in several countries including the USA, Canada and New Zealand, is based on a live attenuated, non-encapsulated SeM-2 strain of *S. equi* [2]. The vaccine strain was attenuated via N-methyl-N′-nitro-N-nitrosoguanidine (NTG) treatment of *S. equi* strain CF32 (*Se*CF32), which was originally isolated from a horse with strangles in New York during 1981 [3] and typically displays high and low capsule phenotypes [4].

The genomes of *Se*CF32 together with high and low capsule variants of Pinnacle share 126 single nucleotide polymorphisms (SNPs) with *Se*CF32 relative to the core reference genome of *S. equi* strain 4047 (*Se*4047), in agreement with the known origins of this vaccine [5]. A further 68 unique mutations were identified that were present in both of the Pinnacle phenotypes, but absent from *Se*CF32 and 222 other isolates of *S. equi* [5]. As a consequence of its derivation through treatment with NTG, Pinnacle-specific mutations were significantly enriched for C → T and G → A, but deficient in A → G and T → C transitions [6] when compared to DNA sequence data from 223 other strains of *S. equi*, including *Se*CF32 [5]. It is not known which of the 68 mutations in Pinnacle contribute to the attenuation of this strain. One of the mutations is predicted to lead to the truncation of SEQ_1295, a putative membrane protein, whilst 41 mutations are predicted to lead to non-synonymous changes in the amino acid sequences of the encoded proteins [5]. However, very few of these mutations may contribute to the attenuation of the vaccine strain, raising questions regarding its potential to revert to virulence. The reversion of attenuating mutations in Pinnacle would provide one explanation for the occurrence of cases of disease in horses shortly after vaccination with this product. In this report, we utilised genome sequencing to identify the cause of two cases of strangles in horses in New Zealand shortly after vaccination with Pinnacle.

2. Materials and methods

2.1. Clinical history

Isolates were recovered from two cases of strangles in recently vaccinated horses. The first horse, a Standardbred yearling filly developed a retropharyngeal lymph node abscess 10 days after the first vaccination with Pinnacle, from which *S. equi* strain 136 (*Se*136) was recovered on the 27th June 2011 [2]. The second horse, a Thoroughbred filly, was vaccinated with an initial dose of Pinnacle

Abbreviations: NTG, N-methyl-N′-nitro-N-nitrosoguanidine; Pinnacle, Pinnacle I. N. vaccine (Zoetis); *S. equi*, *Streptococcus equi* subspecies *equi*; *Se*136, *Streptococcus equi* subspecies *equi* strain 136; *Se*142, *Streptococcus equi* subspecies *equi* strain 142; *Se*4047, *Streptococcus equi* subspecies *equi* strain 4047; *Se*CF32, *Streptococcus equi* subspecies *equi* strain CF32; SNPs, single nucleotide polymorphisms; TE, Tris-EDTA buffer.

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Table 1
Specific single nucleotide polymorphisms of Pinnacle vaccine strains relative to the *Se*4047 reference and *Se*CF32 genomes.

Position in *Se*4047	CDS name	Product	Mutation	Reference base	*Se*CF32	Pinnacle H+	Pinnacle H−	*Se*136	*Se*142
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
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