

Wade B, et al. Two putative zinc metalloproteases contribute to the virulence of *Clostridium perfringens* strains that cause avian necrotic enteritis

Supplementary Table 1. Oligonucleotide primers.

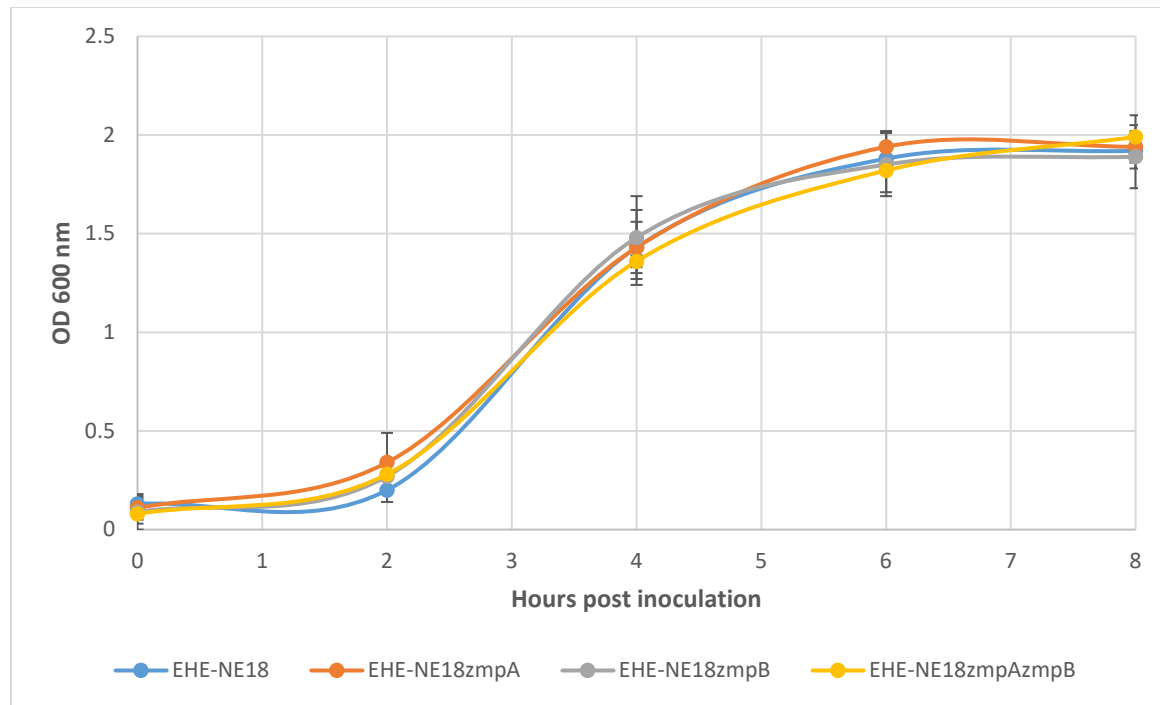
Combination	Target	Product size (bp)	Sequence (5'–3')	Source
1	<i>zmpB</i> (EHE-NE18_1611)	107	F: CTTTCTGCTACAACAATTGATG R: TTACTTACCCTTGCTTGATG	Current study
2	<i>zmpA</i> (EHE-NE18_3281)	377	F: AGGTTCCGGTAGCGTAAG R: TAGCGTTTGCCCAGTCAG	Current study
3	<i>zmpA</i> downstream flanking region	2,168	F: CCGACTAGTATTATCAAGAAGGCAGATGG R: CCGACTAGTGTGTCAGCATATTCACAGAAC	Current study
4	<i>zmpA</i> upstream flanking region	2,586	F: ATCAGCTCATACTGGTCTTAC R: GCTAGCCCAACATTGCTCTTAAGTTTC	Current study
5	Upstream and downstream of <i>zmpA</i>	9,456 (wild-type); 5,917 (mutant)	F: AGGTGTTGGAGTTATAGATG R: TTTCCTACAAC TTAAATTC	Current study
6	Upstream of <i>zmpA</i> and within <i>catP</i>	No product (wild-type); 3,306 (mutant)	F: AGGTGTTGGAGTTATAGATG R: CGCCATTCAGAGTTTAGGAC	Current study
7	Downstream of <i>zmpA</i> and within <i>catP</i>	No product (wild-type); 3,021 (mutant)	F: CGGCAAATGTGAAATCCG R: TTTCCTACAAC TTAAATTC	Current study
8	<i>zmpB</i> TargeTron Construct	Final product 353	IBS: AAAAAAGCTTATAATTATCCTTACATCACC ATGACGTGCGCCAGATAGGGTG EBS2: TGAACGCAAGTTTCTAATTTTCGGTTTGATGT CGATAGAGGAAAAGTGTCT EBSU: CGAAATTAGAACTTGCGTTCAGTAAAC EBS1D: CAGATTGTACAAATGTGGTGATAACAGATA AGTCCATGACTATAACTTACCTTTCTTTGT	11

9	flanking <i>ermB</i> insertion site in <i>zmpB</i> mutant	366 (wild-type); ~2,200 (mutant)	F: AGATGAGGGAGATGATTTTAATTTAG R: GCCTTAGTAGGATTCTCAGAAGTTTC	12
10	Upstream of <i>cpe2358</i>	648	F: CGCATCAGATATTATTATTCC R: CTATTTTCAGAAGCCTTATTAAC	Current study
11	Partial coding sequence of <i>cpe2358</i>	585	F: TGATTTACTCCATGTATTC R: CTATTTACTAAGCCAACCTC	Current study
12	Partial coding sequence of <i>cpe2358</i>	641	F: GGTATTGTTGTTATAGATATAG R: TTCTTGTTTCAGATTTATTTAG	Current study
13	Partial coding sequence of <i>cpe2358</i>	697	F: AAACGGTATAAATGTTATAAG R: GATTAATACCTAAGACATTAAG	Current study
14	Partial coding sequence of <i>cpe2358</i>	822	F: AATGCAAGTTAACTCCTTAG R: CAATACAAGAGCAATTAATAG	Current study

Supplementary Table 2. Single-nucleotide polymorphisms present in EHE-NE18*zmpA* and EHE-NE18*zmpB*.

Gene affected	Strain 13 equivalent	Nature of change	Position (aa)
EHE-NE18 <i>zmpA</i>			
Methyltransferase, UbiE/COQ5 family	<i>CPE0887</i>	Substitution (L to I)	110
Alcohol dehydrogenase, short-chain family	<i>CPE2074</i>	Substitution (D to E)	302
EHE-NE18 <i>zmpB</i>			
Queuine tRNA-ribosyltransferase	<i>CPE1945</i>	Substitution (A to S)	15
Sensory box protein/sigma-54 dependent transcriptional regulator	<i>CPE2358</i>	Substitution (A to S)	54

aa = amino acid.



Supplementary Figure 1. Growth curves of EHE-NE18 and selected isogenic mutants. Strains were grown in liquid brain–heart infusion with optical density (OD) recorded at 600 nm every 2 h. Growth curves were repeated in duplicate. One-way ANOVA showed no significant difference in growth at any time.