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Short communication

Evidence of lateral gene transfer among strains of *Streptococcus zooepidemicus* in weanling horses with respiratory disease

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ABSTRACT

Streptococcus zooepidemicus (Sz) is a tonsillar commensal of healthy horses but with potential to opportunistically invade the lower respiratory tract. Sz is genetically variable and recombinogenic based on analysis of gene sequences including *szp*, *szm* and MLST data. Although a variety of serovars of the protective SzP are commonly harbored in the tonsils of the same horse, lower respiratory infections usually involve a single clone. Nevertheless, isolation of specific clones from epizootics of respiratory disease has been recently reported in horses and dogs in N. America, Europe and Asia. In this report, we provide evidence suggestive of lateral gene exchange and recombination between strains of Sz from cases of respiratory disease secondary to experimental equine herpes 1 virus infection in an isolated group of weanling horses and ponies. Nasal swabs of 13 of 18 weanlings with respiratory disease yielded mucoid colonies of Sz following culture. Comparison of *arcC*, *nrdE*, *proS*, *spi*, *tdk*, *tpi* and *yqiL* of these Sz revealed 3 Clades. Clade-1 (ST-212) and 2 (ST-24) were composed of 7 and 3 isolates, respectively. ST-24 and 212 differed in all 7 housekeeping as well as *szp* and *szm* alleles. Two isolates of Clade-1 were assigned to ST-308, a single locus variant of ST-212 that contained the *proS*-16 allele sequenced in ST-24. One isolate of ST-308 contained *szm*-2, the same allele sequenced in Clade 2 isolates; the other was positive for the *szp*-N2HV2 allele of Clade 2. These observations are consistent with gene transfer between Sz in the natural host and may explain formation of novel clones that invade the lower respiratory tract or cause epizootics of respiratory disease in dogs and horses.

1. Introduction

Streptococcus equi subsp. *zooepidemicus* (Sz) of Lancefield group C is a tonsillar commensal of healthy horses but with the potential to opportunistically cause disease secondary to infection with influenza or herpes virus (Timoney, 2004). Most horses harbor multiple serovars of Sz in their lingual and palatine tonsils (Anzai et al., 2000). However, opportunist lung infections usually result from invasion by a single founder serovar that varies from horse to horse in a group (Anzai et al., 2000). In addition to its role as an opportunist invader, the recent identification of specific strains of Sz responsible for clonal epizootics of respiratory disease in horses and shelter dogs suggests emergence of genetic variants with enhanced transmissibility and virulence (Pesavento et al., 2008; Björnsdóttir et al., 2012; Lindahl et al., 2013; Velineni and Timoney, 2013a).

Sz of equine origin are genetically variable as evidenced by restriction fragment profiles, SzP analysis, pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) (Walker and Timoney, 1998; Webb et al., 2008). At the time of writing, 319 different sequence types (22 November 2013) were listed in the Sz pubMLST database (http://pubmlst.org/szooepidemicus/). The surface anchored immuno-protective protein SzP is mosaic in character being formed by at least two N-terminal and 5 hypervariable sequences combined with a variable number of C-terminal PEPK repeats. Similarly, the fibrinogen and plasminogen binding SzM is a mosaic of sequences that varies by strain (Velineni and Timoney, 2013b). Diversity in sequence of other surface anchored proteins is also evident from comparison of genome sequences, which, with MLST data, suggests the Sz population is highly recombinogenic (Webb et al., 2008).

This study was designed to investigate the genetic and phenotypic characteristics of Sz isolated from cases of respiratory disease secondary to experimental infection with equine herpes virus (EHV-1 A183) infection in a group of 20 weanling horses and ponies maintained in isolation.

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2. Materials and methods

2.1. Sources of isolates

Twelve weanlings (4, 27, 53, 59, 912, 9802, 9803, 9807, 9809, 9810, 9811 and 9812) were vaccinated with commercial EHV vaccines (Rhinomune, Pneumocort K) on Days 0 and 49 as described elsewhere (Breathnach et al., 2001). Another group of 6 (49, 54, 56, 9801, 9804 and 9809) housed separately were inoculated with virulent army EHV-1 A183 strain. Two weanlings (77 and 9808) also housed separately were uninoculated controls. The primary aim of these treatments was to determine the effects of vaccination or previous EHV-1 A183 infection on virus specific mucosal IgA/IgGa, b, M and (T) responses and on subsequent resistance to challenge with virulent virus. All weanlings including controls were housed together and challenged intranasally with EHV-1 A183 on Day 91. Over the following 42 days, clinical signs of respiratory infection including purulent nasal discharge, cough and pneumonia developed and persisted in all but 2 weanlings. Two affected weanlings died. Nasal swabs from all surviving weanlings collected 42 days after challenge were cultured on CNA blood agar. Single mucoid beta hemolytic colonies were saved from cultures of 13 weanlings and identified as Sz by fermentation reactions in lactose, sorbitol and trehalose.

2.2. Immunoblot analysis

Surface proteins in mutanolysin extracts of all Sz isolates from overnight culture in Todd–Hewitt Broth (THB) at 37 °C (Timoney et al., 1995) were immunoblotted with antiserum specific for recombinant SzPW60 following SDS–PAGE and transferred to nitrocellulose (Walker and Timoney, 1998; Anzai et al., 2000). The SzPW60 specific antiserum contains antibody to cross reactive epitopes of SzP and reacts with the mature protein and its fragments.

2.3. Multi-locus sequence, szp and szm typing

Multi-locus sequence typing (MLST) (*arcC*, *nrdE*, *proS*, *spi*, *tdk*, *tpi* and *yqiL*) was performed (Webb et al., 2008) on all Sz isolates and ST's (sequence types) assigned (http://pubmlst.org/szooepidemicus/). The *szp* (Moore and Bryans typing antigen) and *szm* sequences were also amplified using forward and reverse primers IGSzP-F and R (Ijaz et al., 2011) and SzMF-5′ ATA AAG AAG TTC CTG TCA TTA 3′ and SzMR-5′ CAA CAG ACA GGA GAC TGT TGC 3′. Their amplicons (1212 and 1929 bp, respectively) were then sequenced (Eurofin MWG Operon, Huntsville, Alabama) and amino acid sequences predicted (Table 1).

3. Results

All 18 surviving weanling horses had purulent nasal discharge on Day 42 after virulent EHV-1 A183 challenge. Cultures of nasal swabs from 13 weanlings yielded heavy growths of mucoid Sz as confirmed by acid production in lactose and sorbitol broths but not in trehalose. SzP blot patterns were similar for isolates from 8 weanlings (49, 54, 56, 912, 9801, 9803, 9804 and 9809) (Fig. 1). A second blot pattern was evident for isolates from 4 weanlings (4, 77, 9802 and 9812). The pattern of Sz59 was unlike that of other isolates. The immunoblot patterns suggested dominance of 2 similar Sz clones in the group of weanlings.

MLST analysis based on 7 different housekeeping alleles (*arcC*, *nrdE*, *proS*, *spi*, *tdk*, *tpi*, and *yqiL*) revealed dominance of 2 Clades. Clades 1 (ST-212) and 2 (ST-24) were composed of isolates 49, 54, 56, 9801, 9803, 9804, 9809 and 4, 77, 9802, respectively.

Table 1
Sequence type of mucoid Sz isolated from groups of previously infected (EHV-1 A183), vaccinated and non-vaccinated equine weanlings that developed clinical signs of respiratory disease following experimental challenge with virulent army EHV-1 A183 strain. Isolates were from nasal swabs collected 42 days after challenge.

Group	Horse/pony	Sz	Clade	Sequence type (ST)
I	Pony 9801	9801	1	212
I	Horse 54	54	1	212
V	Pony 912	912	1	308
C	Horse 77	77	2	24
V	Horse 4	4	2	24
I	Horse 56	56	1	212
V	Horse 59	59	3	8
V	Pony 9803	9803	1	212
I	Pony 9804	9804	1	212
V	Pony 9809	9809	1	212
V	Pony 9812	9812	1	308
I	Horse 49	49	1	212
V	Pony 9802	9802	2	24

Group I ($n = 6$) weanlings were infected with virulent army EHV-1 A183 on Day 0. Groups V ($n = 12$) were vaccinated with commercial EHV vaccines on Days 0 and 49. Group C ($n = 2$) were uninfected and unvaccinated.

Sz912 and 9812 were assigned to the novel ST-308, based on presence of the *proS*-16 instead of *proS*-22 (Fig. 2). Clade 3 isolate Sz59 was typed as ST-8.

Predicted SzP amino acid sequences of Clade 1 isolates except that of Sz9812 were identical consisting of N2, HV4 variable regions and 17 PEPK repeats (Walker and Timoney, 1998). Predicted SzP amino acid sequences of Clades 2 and 3 isolates consisted of N2, HV2, 17 PEPK repeats and N1, HV1, 20 PEPK repeats, respectively. *szm* sequences of isolates from each Clade were designated *szm*-1, 2 and 3 (Fig. 2). All Clade 1 isolates had *szm*-1 sequence except Sz912 which had *szm*-2 sequence. A combination of the data for *szp*, *szm* and housekeeping alleles, suggests the possibility that Sz9812 was formed by horizontal transfer of *proS*-16 and *szp*-N2HV2. Sz912 was possibly formed by horizontal transfer of *proS*-16 and *szm*-2 allelic loci from Sz of Clade 2. However, since a limited number of isolates collected at a single point in time were available, it is impossible to be certain as to the direction, source and number of transfer events that resulted in the genotypes of these Clades. GenBank accession numbers for *szp* nucleotide sequences are KF032041 (Sz9801), KF032045 (Sz54), KF032049 (Sz912), KF032048 (Sz77), KF032042 (Sz4), KF032046 (Sz56), KF032047 (Sz59), KF032050 (Sz9803), KF032051 (Sz9804), KF032052 (Sz9809), KF032053 (Sz9812), KF032044 (Sz49), KF032054 (Sz9802) and for *szm* nucleotide sequences are KF142636 (Sz9801), KF142637 (Sz54), KF142638 (Sz912), KF142639 (Sz77), KF142640 (Sz4), KF142642 (Sz56), KF142643 (Sz59) and KF142644 (Sz9812).

4. Discussion

Equine herpes-1, 4 and 2 are among the viral agents that can predispose weanling foals to secondary invasion by Sz (Wilson, 1992). Weanling foals in the present study made virus specific mucosal IgA responses to virulent virus but not to commercial attenuated or inactivated virus (Breathnach et al., 2001). However, although vaccination or prior infection with live virulent virus resulted in reduced viremia and associated pyrexia following challenge with virulent virus, susceptibility to secondary invasion by Sz was not reduced. Bronchopneumonia associated with Sz was observed in vaccinated weanlings (4, 59, 912, 9801, 9802, 9803, 9809 and 9812), in weanlings previously infected with EHV-1 (49, 54, 56 and 9804) and in one control (77) weanling.

Although multiple serovars of Sz commonly colonize the tonsillar crypts, secondary invasion usually involves a single serovar that varies from foal to foal in a group (Anzai et al., 2000). However,