Veterinary Microbiology 162 (2013) 663–669

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Diversity of seM in *Streptococcus equi* subsp. *equi* isolated from strangles outbreaks

Felipe Libardoni [a], Andréia Vielmo [a], Luana Farias [a], Letícia Beatriz Matter [a], Luciana Pötter [b], Fernando Rosado Spilki [c], Agueda Castagna de Vargas [a,*]

[a] *Laboratório de Bacteriologia, Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria, Programa de Pós-Graduação em Medicina Veterinária, 97105-900 Santa Maria, RS, Brazil*

[b] *Programa Pós-Graduação em Zootecnia, Universidade Federal de Santa Maria – UFSM, RS/Departamento de Zootecnia, 97105-900 Santa Maria, RS, Brazil*

[c] *Laboratório de Microbiologia Molecular, Instituto de Ciências da Saúde, Programa de Pós-Graduação em Qualidade Ambiental, Universidade Feevale, 93352-000 Novo Hamburgo, RS, Brazil*

ARTICLE INFO

Article history:
Received 15 June 2012
Received in revised form 6 September 2012
Accepted 8 September 2012

Keywords:
Strangles
seM alleles
Equine
PubMLST-seM

ABSTRACT

Strangles is the main upper respiratory tract disease of horses. There are currently no studies on the changes in alleles of the M protein gene (seM) in Brazilian isolates of *Streptococcus equi* ssp. *equi* (*S. equi*). This study aimed to analyze and differentiate molecularly *S. equi* isolates from equine clinical specimens from southern Brazil, between 1994 and 2010. seM alleles were analyzed in 47 isolates of *S. equi* obtained from clinical cases of strangles (15 Thoroughbred horses, 29 Crioulo breed horses and three *Brasileiro de Hipismo* – BH). seM alleles characterization was performed by comparing variable region sequences of the seM gene. The alleles were also phylogenetically grouped by Neighbor-joining analysis, which demonstrated the geographic distribution of those in properties from southern Brazil. Fifteen alleles of the gene seM were found among the 47 *S. equi* isolates analyzed. Among these, only one allele (seM-61), which was identified in seven isolates (14.9%), was found in the database PubMLST-seM. Within the new alleles, allele seM-115 was the most prevalent, having been found in 13 isolates (27.7%), followed by allele seM-117 in 10 isolates (21.3%). In the Brazilian horse population studied, there is greater diversity of M protein alleles in *S. equi* isolates compared to worldwide data deposited in PubMLST-seM. Among the 15 seM alleles identified, only one allele sequence was previously published. The alleles identification is important to control the disease by guiding selection of strains for the manufacture of commercial and autogenous vaccines.

1. Introduction

Strangles is the main upper respiratory tract disease of horses (Slater, 2007). The primary etiology is attributed to *Streptococcus equi* subspecies *equi* (*S. equi*), a β-hemolytic bacteria which belongs to Lancefield group C. *S. equi* is closely related to *Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*), a commensal bacterium of the equine respiratory tract that can cause opportunistic infections (Holden et al., 2009).

M protein (SeM) is a surface antigen produced by *S. equi* that increases its virulence relative to *S. zooepidemicus*. SeM binds to fibrinogen and immunoglobulin G (IgG), inhibiting the deposition of C3b complement on the bacterial surface, resulting in inhibition of phagocytosis (Boschwitz and Timoney, 1994; Meehan et al., 2001). The use of recombinant SeM-targeted vaccination has been successful in experiments with mice (Meehan et al., 1998), but the same results were not replicated with horses. This fact led to the hypothesis that structural variations of this

* Corresponding author at: Universidade Federal de Santa Maria (UFSM), Av. Roraima 1000, prédio 44, sala 5137, 97105-900 Santa Maria, RS, Brazil. Tel.: +55 55 32208107; fax: +55 55 32208257.
E-mail address: agueda.vargas@gmail.com (A.C. de Vargas).

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http://dx.doi.org/10.1016/j.vetmic.2012.09.010

protein may exist (Sheoran et al., 2002), contradicting the previous proposition of uniformity (Galan and Timoney, 1988).

Molecular studies demonstrated differences (mutations) in the gene sequence encoding the M protein, located at the N-terminal end (Chanter et al., 2000; Anzai et al., 2005). The seM gene region has been used to differentiate isolates (Anzai et al., 2005) and to identify sources of outbreaks (Kelly et al., 2006). In addition, allele frequencies were observed to change among strains in a given period, suggesting selection pressure in these strains (Kelly et al., 2006; Ivens et al., 2011). Given the importance of greater knowledge of strangles and its etiological agent, a study on the variations of the M protein alleles in Brazilian isolates was warranted. This study aimed to analyze and differentiate isolates of *S. equi* obtained from equine strangles clinical samples from Rio Grande, Brazil, using phylogenetic analysis and differentiation of alleles based on sequencing the 5′ end of the seM gene (encoding the N-terminal end M protein).

2. Material and methods

2.1. Isolates characterization

Forty-seven bacterial isolates identified as *S. equi* from specimens submitted to the Laboratory of Bacteriology (LABAC/UFSM) were used in this study. Isolates came from nasal secretion samples and lymph node aspirates of horses (three *Brasileiro de Hipismo* breed – BH, 15

Table 1
Characterization of origin (properties) of strangles outbreaks and isolates of *Streptococcus equi* subspecies *equi* for 21 equine properties. Properties were of the following types: Thoroughbred (PSC), Crioulo, and *Brasileiro de Hipismo* (BH) located in Rio Grande do Sul – Brazil.

Properties	Outbreak	Allele	Breed	Isolate	GenBank accession
A	20/02/2008	seM-115	Crioulo	37/08-1	JX494355
A	20/02/2008	seM-115	Crioulo	37/08-V	JX476945
A	20/02/2008	seM-115	Crioulo	41/08-NV	JX494356
B	16/07/2008	seM-116	Crioulo	53/08-2	JX494359
B	16/07/2008	seM-116	Crioulo	53/08-3	JX494360
B	14/12/2010	seM-61	Crioulo	178/10	JX502616
C	30/07/2008	seM-117	Crioulo	152/08-1	JX494362
C	30/07/2008	seM-117	Crioulo	152/08-2	JX494363
C	30/07/2008	seM-117	Crioulo	152/08-3	JX494364
C	04/04/2009	seM-117	Crioulo	90/09-2	JX497740
C	04/04/2009	seM-118	Crioulo	90/09-1	JX494368
C	04/04/2009	seM-119	Crioulo	90/09-3	JX497741
D	07/05/2007	seM-120	Crioulo	171/07	JX476943
D	20/11/2009	seM-117	Crioulo	261/09-1	JX502605
D	20/11/2009	seM-117	Crioulo	261/09-4	JX502606
E	08/08/2010	seM-115	Crioulo	105/10-A	JX502615
E	08/08/2010	seM-115	Crioulo	105/10-C	JX502613
E	08/08/2010	seM-115	Crioulo	105/10-D	JX502614
E	08/08/2010	seM-115	Crioulo	105/10-AA	JX502610
E	08/08/2010	seM-115	Crioulo	105/10-AC	JX502612
F	07/03/2003	seM-115	Crioulo	78/03	JX402752
F	07/05/2003	seM-115	Crioulo	366/03	JX476938
F	23/11/2005	seM-120	Crioulo	566/05	JX476939
G	19/08/2008	seM-115	Crioulo	162/08-B	JX494365
G	04/09/2009	seM-121	Crioulo	62/09	JX494367
H	24/11/1994	seM-115	Crioulo	291/94	JX470315
I	21/05/2010	seM-122	Crioulo	77/10	JX502611
J	20/04/2009	seM-120	Crioulo	94/09-P	JX497744
K	22/05/2009	seM-117	Crioulo	113/09	JX497746
L	21/05/2002	seM-124	Crioulo	204/02	JX470316
M	06/03/2008	seM-61	PSC	47/08-2	JX494357
M	06/03/2008	seM-61	PSC	47/08-4	JX494358
M	10/12/2009	seM-61	PSC	280/09	JX502607
N	29/04/2009	seM-117	PSC	97/09-C	JX497745
N	13/05/2010	seM-123	PSC	68/10-C	JX502609
N	13/05/2010	seM-123	PSC	68/10-F	JX502608
O	04/07/1994	seM-119	PSC	99/94	JX470314
O	01/12/2006	seM-61	PSC	702/06	JX476941
O	01/12/2006	seM-61	PSC	732/06	JX476942
P	07/05/2003	seM-115	PSC	166/03	JX470318
Q	25/06/2008	seM-61	PSC	128/08-N	JX494361
Q	06/11/2006	seM-125	PSC	628/06	JX476940
R	16/07/2007	seM-126	PSC	246/07	JX476944
S	05/12/2008	seM-127	PSC	272/08-J	JX494366
T	29/04/2009	seM-117	BH	91/09-H	JX497743
T	29/04/2009	seM-117	BH	91/09-Q	JX497742
U	05/05/2003	seM-128	BH	126/03	JX470317