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Potential Coverage of a Multivalent M Protein-Based Group A Streptococcal Vaccine

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Abstract

Background—The greatest burden of Group A streptococcal (GAS) disease worldwide is due to acute rheumatic fever (ARF) and rheumatic heart disease (RHD). Safe, effective and affordable vaccines designed to prevent GAS infections that trigger ARF could reduce the overall global morbidity and mortality from RHD. The current study evaluated the potential coverage of a new 30-valent M protein-based vaccine using GAS isolates from school children in Bamako, Mali, a population at high risk for the development of RHD.

Methods—The bactericidal activity of rabbit antisera against the 30-valent vaccine was assessed using a collection of GAS isolates recovered during a study of the epidemiology of pharyngitis in Bamako.

Results—Single isolates representing 42 of 67 *emm*-types, accounting for 85% of the GAS infections during the study, were evaluated. All (14/14) of the vaccine *emm*-types in the collection were opsonized (bactericidal killing >50%) and 26/28 non-vaccine types were opsonized.

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Authors Contributions

J.B.D. was the primary author of the manuscript, supervised the laboratory-based studies at the University of Tennessee and was responsible for the overall content of the manuscript. T.A.P. performed and supervised the performance of the bactericidal tests, performed and analyzed the *emm* sequence designations, and contributed to the writing and editing of the manuscript.
B.T. was responsible for the isolation and identification of GAS isolates obtained from subjects enrolled in the clinical study in Bamako and the isolation of genomic DNA and preparation of PCR fragments for sequencing.
S.O.S., M.T. and K.L.K. were responsible for the conduct of the clinical study in Bamako and contributed to the writing of the manuscript.
J.P.N. was responsible for *emm* sequencing and initial assignments of *emm*-types in the collection.

Disclosure Statement

Conflicts of Interests

J.B.D. is the inventor of certain technologies related to the development of GAS vaccines. The University of Tennessee Research Corporation has licensed the technology to Vaxent, LLC.
J.B.D. serves as the Chief Scientific Officer of Vaxent.
All other authors disclose no financial or personal potential conflicts.

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Bactericidal activity was observed against 60% of the total *emm*-types recovered in Bamako, which accounted for 81% of all infections.

Conclusions—Multivalent vaccines comprised of N-terminal M peptides elicit bactericidal antibodies against a broad range of GAS serotypes, indicating that their efficacy may extend beyond the *emm*-types included in the vaccine.

Keywords

group A streptococcal vaccine; M protein; bactericidal activity; vaccine coverage

1. Introduction

Group A streptococci (GAS) cause a broad spectrum of diseases worldwide that ranges from uncomplicated pharyngitis and skin infections to life-threatening invasive infections that include pneumonia, bacteremia, necrotizing fasciitis, streptococcal toxic shock syndrome, as well as the immune-mediated nonsuppurative sequelae of acute rheumatic fever (ARF), rheumatic heart disease (RHD), and glomerulonephritis. The greatest burden of GAS disease, as defined by morbidity and mortality, is due to RHD in low- and middle-income countries. Minimum estimates suggest that RHD affects 15 million individuals resulting in ~230,000 deaths each year [1]. However, as more data become available from low-income countries [2-4], the true burden of RHD is becoming clearer and it appears that the disease burden outlined previously is considerably underestimated.

Current strategies for the prevention of ARF and RHD rely on antibiotic treatment of symptomatic pharyngitis (primary prevention) or continuous administration of antibiotics to individuals with documented ARF (secondary prevention). These prevention strategies are costly and, for the most part, are ineffective in resource-poor countries where 90% of the disease burden exists [5]. The development of a safe, effective and affordable vaccine designed to prevent the GAS infections that trigger ARF/RHD has long been considered an attractive approach. Vaccine development has faced obstacles related to the complex epidemiology of GAS infections, especially in developing countries [6], and safety concerns based on the theoretical possibility that the vaccines may elicit autoimmune responses that could mimic ARF or other sequelae of GAS infections [7].

Significant progress has been made recently in the design and clinical development of complex multivalent M protein-based vaccines [8-10]. The surface M protein is a major virulence determinant of GAS that contains opsonic epitopes [11]. M protein antibodies that are produced following natural infection [12] or vaccination [9] are bactericidal in human blood. Bactericidal antibodies have been associated with protection against infection with the homologous serotype of GAS [13]. A number of structure-function studies have revealed that the N-terminal peptides of M proteins contain epitopes that evoke antibodies with the greatest bactericidal activity and are least likely to cross-react with human tissue antigens [7]. This observation has been the basis for the design of complex recombinant hybrid vaccine proteins containing opsonic epitopes representing M proteins from multiple serotypes of GAS [8, 10].

We have previously shown that a 26-valent vaccine containing M peptides from serotypes of GAS representing the vast majority of infections in the USA and Canada [14] was safe and immunogenic in adult volunteers [9]. In pre-clinical and clinical studies, the vaccine evoked bactericidal antibodies against all of the vaccine serotypes of GAS [9]. Recently, we constructed a 30-valent vaccine whose composition was based on more extensive surveillance data from the USA and Europe [15-17]. In animal studies, the 30-valent vaccine evoked bactericidal antibodies against all of the vaccine serotypes of GAS [10]. In addition,

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significant bactericidal activity was observed against a number of laboratory strains representing non-vaccine serotypes of GAS, suggesting that the potential efficacy of this vaccine may extend beyond those serotypes represented in the vaccine [10]. This observation is potentially significant because the predicted coverage of the previous 26-valent vaccine in resource-poor countries is low compared to economically developed countries [6].

In the current study, we have evaluated the bactericidal antibody activity of the 30-valent vaccine antisera raised in rabbits using a collection of pharyngeal isolates of GAS obtained from symptomatic school children in Bamako, Mali [18]. The overall goal was to assess the potential coverage of the new vaccine in a sub-Saharan African population at high risk for developing ARF and RHD and where the epidemiology and *emm*-type prevalence of GAS infections is very different from that observed in the USA, Canada or Europe [15-17].

2. Materials and Methods

Immunization of rabbits with the 30-valent vaccine

Three New Zealand white rabbits were immunized with 800μg doses of the 30-valent vaccine proteins adsorbed to alum at time 0, 4 weeks and 8 weeks, as previously reported [10]. Serum was obtained prior to the first injection and at 2 weeks following the last injection. The antibody titers against the M peptides contained in the vaccine and functional bactericidal activity of the antisera against all of the vaccine serotypes of GAS have previously been published [10]. Animal protocols were approved by the Institutional Animal Care and Use Committees of the University of Tennessee Health Science Center and the Veterans Affairs Medical Center, Memphis, TN.

Clinical isolates of GAS from school children in Bamako

The clinical isolates of GAS used in this report were obtained during a comprehensive assessment of the molecular epidemiology of symptomatic pharyngitis in school children in Bamako, Mali conducted during 2006-2009 [18]. Briefly, children ranging from five to 16 years of age attending any of four participating schools in Djikoroni-para and Sebenikoro urban neighborhoods in Commune 4 of Bamako were eligible.

Students who presented to the school infirmary or school-associated health center complaining of sore throat were enrolled. Upon presentation, study personnel confirmed that the student was experiencing throat pain, then symptoms of pharyngitis were obtained and a brief focal examination was performed. Throat culture swabs were placed in Amies charcoal media (CultureSwab™, Becton Dickinson) and transported to the microbiology laboratory at the Center for Vaccine Development-Mali at ambient temperature within six hours. Upon arrival in the laboratory, swabs were streaked onto 5% sheep's blood agar, a bacitracin disk (0.04 U) was placed, and the plate was incubated at 35-37°C. GAS were beta-hemolytic, catalase-negative, bacitracin-sensitive, Gram-positive cocci in pairs and chains that tested positive for group A streptococcal antigen on agglutination test. Bacterial isolates were stored at −80°C in trypticase soy broth with 15% glycerol. All human subjects protocols were approved by the Ethics Committee of the Faculte de Medecine, Pharmacie et Odontostomatologie in Bamako, Mali, and the respective Institutional Review Boards of the University of Maryland School of Medicine, the University of Tennessee Health Science Center, and the Memphis Veterans Affairs Medical Center.

Emm-typing

DNA from the GAS isolates was extracted and the 5′ portion of the *emm* gene was amplified using standard methodology developed at the Centers for Disease Control,

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Atlanta, GA, USA [19]. The amplified DNA product was sent to the University of Maryland Biopolymer Laboratory for sequencing. The nucleotide sequence was then compared using a standard FASTA alignment algorithm against known *emm* gene sequences on the CDC website and an *emm*-type and sub-type were assigned.

Indirect bactericidal tests

Bactericidal assays were performed as previously described [20]. Briefly, 0.05 ml of Todd-Hewitt broth containing bacteria (~50-200 CFU) was added to 0.1 ml of preimmune or immune serum and 0.35 ml of lightly heparinized non-immune human blood and the mixture was rotated for three hours at 37!C. Then 0·1 ml of this mixture was diluted 10- and 100-fold and 0.1 ml aliquots were added to melted sheep's blood agar. Pour plates were prepared and viable organisms (CFU) were counted after overnight incubation at 37!C. The results were expressed as percent killing, which was calculated using the following formula: [(CFU after three hours growth with preimmune serum – CFU after three hours growth with immune serum) ! CFU after three hours growth with preimmune serum] × 100. For each assay, the CFU added to the test mixtures was determined and only those assays that resulted in growth of the test strain to at least five generations in the presence of preimmune serum were used to express percent killing in the presence of immune serum.

Emm sequence analyses

Primary structural homologies among *emm* sequences contained in the 30-valent vaccine and the *emm* sequences of non-vaccine types were identified using the Swiss Institute of Bioinformatics SIM alignment program [21]. The translated sequences corresponding to the N-terminal 50 amino acid residues of the mature M proteins [22] were individually aligned with the complete sequences of the 30-valent vaccine proteins using the comparison matrix PAM40, a gap open penalty of 12 and a gap extension penalty of 4. The alignments resulting in the top three scores were analyzed for their position in the 30-valent vaccine proteins and percent identity was recorded.

3. Results

During the epidemiologic study in Bamako, a total of 372 GAS were recovered from throat cultures of enrolled subjects, of which 305 were available for analysis in this report. A complete description of the incidence of GAS pharyngitis, the molecular epidemiology of the infections and the prevalence of specific *emm*-types and sub-types recovered is published elsewhere [18]. The goal of this study was to determine the potential efficacy of a new 30-valent M protein-based vaccine by assessing the functional activity of vaccine antiserum using isolates of GAS recovered from infected children in Mali. The GAS isolates analyzed in this study contained a total of 67 *emm*-types, 18 of which were *emm*-types included in the vaccine (VTs) and 49 that were non-vaccine *emm*-types (NVTs) (Table 1). Single clinical isolates of GAS representing 42 of the most common *emm*-types recovered (14 VTs and 28 NVTs) were obtained from the collection in Bamako to use in bactericidal assays.

Indirect bactericidal assays were performed using a single rabbit antiserum raised against the 30-valent vaccine [10]. Bactericidal activity was demonstrated against all14 VTs tested (mean, 87%; range 65-100%) (Fig. 1). Of the NVTs tested, 26 of 28 (93%) were killed at a level >50% (overall mean 81%, range 24-99%). The total number of *emm*-types killed among the total number tested was 40/42 (95%) (Table 1). Among the 42 *emm*-types present in the isolates studied in bactericidal assays, 10 were represented by 2 *emm* alleles (subtypes) and 1 was represented by 3 alleles. The remainder contained *emm* genes of the same allele (data not shown). Of the 14 VTs tested, 7 of the isolates contained *emm* genes

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that differed in sequence from the subtype represented in the vaccine and all were opsonized by the vaccine antisera (Fig. 1).

The potential (theoretical) efficacy of the 30-valent vaccine in a specific population can be estimated using the prevalence of specific *emm*-types within a relatively large sample of GAS isolated from symptomatic children in combination with the results of functional in vitro assays that have been associated with protection in animal models [23] or humans [13]. Therefore, we analyzed the results of bactericidal assays in relation to the *emm*-types present in the collection of 305 GAS isolates (Fig. 2). The vaccine antisera resulted in >50% bactericidal killing of *emm*-types accounting for 84% of the infections in this cohort of children. Included in this assessment are all 18 of the VTs, four of which were not available from the Mali collection but were tested previously [10], and the 26 NVTs that were opsonized by the antisera. These results, which are based on bactericidal killing of NVTs, is in distinct contrast to the potential of preventing 37% of infections caused by only those *emm*-types represented in the vaccine (Table 1).

One explanation for the degree of bactericidal activity observed with the 30-vaccine antisera against NVTs is the potential presence of shared opsonic epitopes among M proteins of GAS from different *emm*-types. To assess this possibility, we performed sequence alignments of the translated sequences of the four proteins contained in the 30-valent vaccine and the N-terminal 50 amino acids of the mature M proteins from each NVT that was cross-opsonized (Table 2). In many cases there appeared to be significant sequence identity with one or more of the vaccine M peptide sequences, which could account for the cross-reactive bactericidal activity observed against some NVTs. However, in some cases the sequence identity was lower than might be predicted to result in cross-reactive antibody binding to M epitopes.

4. Discussion

GAS diseases, especially ARF and RHD, cause significant morbidity and mortality throughout the world [1]. Vaccine prevention of the infections that trigger rheumatic fever would represent a new strategy that would not require the additional community health infrastructure necessary to implement and maintain programs using antibiotic prevention. Although there are several GAS vaccine candidates in various stages of clinical and pre-clinical development [24], the best evidence developed over the longest period of time supports the use of M protein peptides to elicit protective immunity [11]. The major practical obstacle in the development of a single vaccine that could be deployed to all geographic regions has been the complexity of the epidemiology of GAS infections in low- and moderate-income countries [6]. Using straightforward analyses of potential vaccine coverage based only on the M serotypes included in the vaccines compared to the serotypes of GAS reported from various geographic locations, the multivalent vaccines have been declared sub-optimal in many parts of the world [6]. In the present study we have demonstrated that the 30-valent vaccine evoked bactericidal antibodies against a significant number of NVTs of GAS. If vaccine serotypes only were used to predict the utility of the 30-valent vaccine in Bamako, the coverage would be approximately 37% of all pharyngeal infections. However, when functional bactericidal activity was included in the analysis, the potential coverage increased to 84% of infections and 66% of all *emm*-types isolated. Of note is that not all isolates from Bamako were assessed in functional assays because we selected those that were recovered most frequently for this study. Of the NVTs that were evaluated, bactericidal killing of >50% was observed with 93% of the isolates.

The degree of bactericidal activity against NVTs of GAS observed in this study was not totally unanticipated considering sequence similarities in the N-terminal regions of the >200

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emm-types, which excludes additional unverified sequence types, now listed by the Streptococcal Laboratory of the Centers for Disease Control [22]. The N-terminal regions of the M proteins have historically been shown to contain "type-specific" epitopes, as originally defined by Lancefield [11]. M typing antisera were used for decades in immunoprecipitation reactions to define the serotype of the M protein expressed by a particular isolate of GAS. The typing antisera were absorbed with various heterologous serotypes of GAS to ensure type-specific precipitation of the unknown M protein. The current practice of assigning an *emm*-type based on the 5′ sequence of the *emm* gene effectively differentiates one isolate from another in epidemiologic studies but none of the new *emm*-types has been studied to differentiate them based on serologic similarities or differences. We believe that the structural similarities among M proteins, especially the newer *emm*-types, may at least partially account for the range of cross-reactive bactericidal antibodies observed in this study.

5. Conclusion

Taken together with our previous report [10], the 30-valent vaccine evoked antibodies with bactericidal activity against a total of 72 *emm*-types of GAS, which includes the 30 VTs and 42 NVTs. These results suggest that vaccine coverage against pharyngitis isolates of GAS could extend well beyond the *emm*-types contained in the vaccine. Future studies to determine the overall potential coverage of the vaccine will require functional assays using a comprehensive collection of GAS from diverse regions of the world. The true efficacy of any GAS vaccine will ultimately be defined by carefully designed clinical trials in different geographic locations. The results of such trials will indicate whether a multivalent M protein-based vaccine can be designed for high-income, as well as low- and moderate-income countries that will have a significant impact on the overall incidence of GAS infections and their sequelae.

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Highlights

- !! Multivalent group A streptococcal vaccine
- !! Extended vaccine coverage to include non-vaccine serotypes of group A streptococci
- !! Potential protection in a population at high risk for RHD
- !! Potential for vaccine prevention of GAS infections that may trigger acute rheumatic fever

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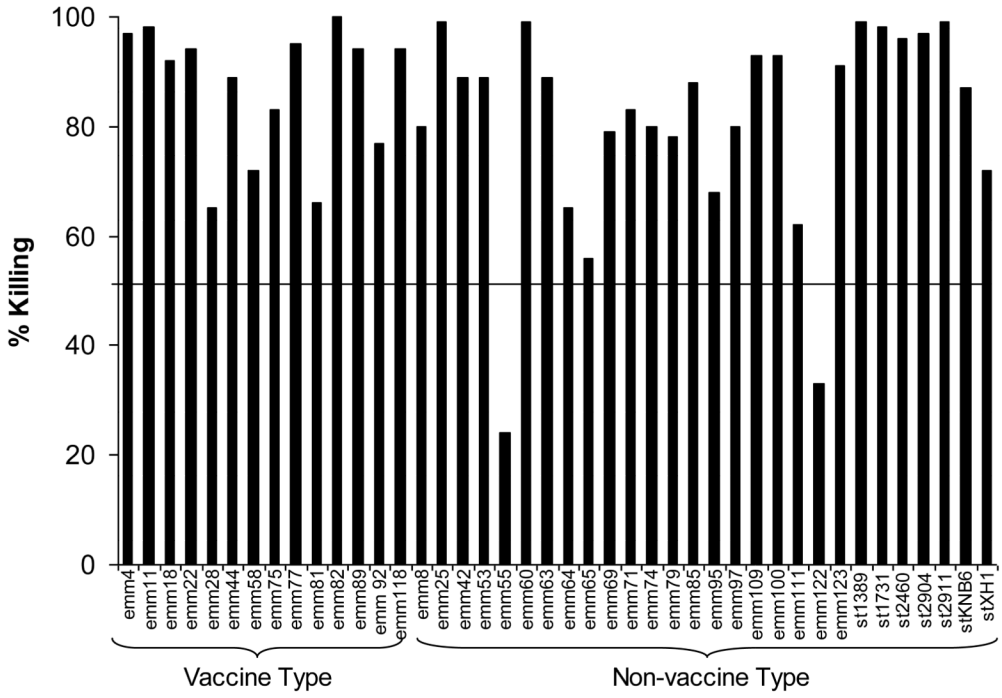

Figure 1.

Indirect bactericidal activity of 30-valent GAS vaccine rabbit antiserum against selected isolates of GAS recovered from symptomatic school children in Bamako, Mali. Indirect bactericidal tests were performed using non-immune human blood mixed with the test isolate and either pre-immune serum or 30-valent vaccine antiserum. Bactericidal killing was calculated as described in the text.

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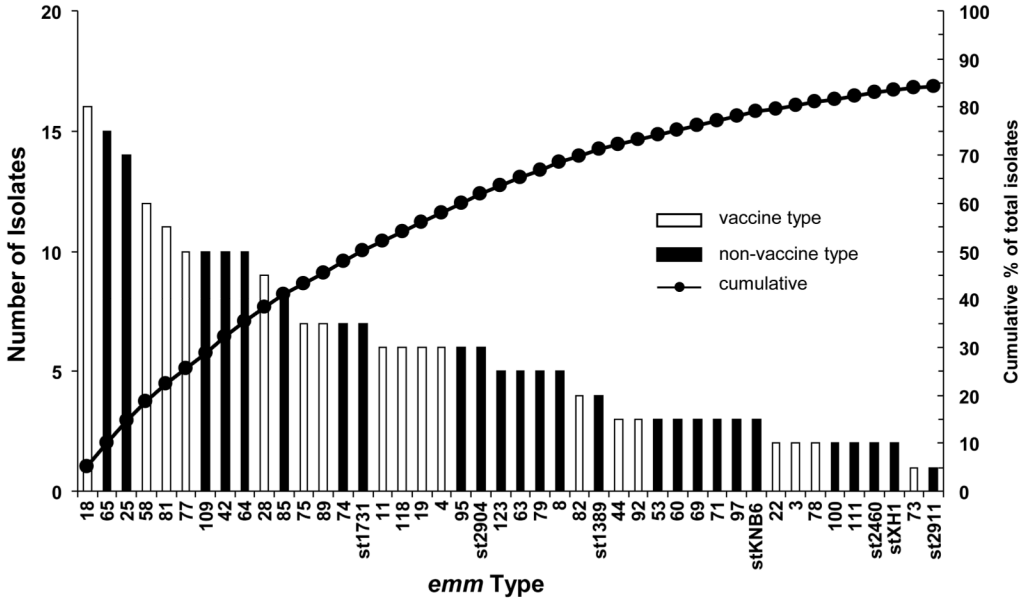

Figure 2.

Summary of GAS *emm*-types that showed >50% bactericidal killing in the presence of 30-valent vaccine antisera. Non-vaccine *emm*-types that were cross-opsonized by the rabbit antisera (solid bars) accounted for 47% of the total GAS in the collection. Together with vaccine *emm*-types in the collection (open bars), potential coverage of the vaccine could reach 84% of the total GAS recovered (solid circles).

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Table 1

Summary of GAS emm-types and functional bactericidal assays

Variable	No. of *emm*-types (%)	No. of Isolates (%)[a]
Total *emm*-types and isolates in the collection	67 (100)	305(100)
Vaccine *emm*-types in the collection	18 (27)	113 (37)
Non-vaccine *emm*-types in the collection	49 (73)	192 (63)
Vaccine *emm*-types tested	14 (21)	102 (33)
Vaccine *emm*-types killed >50%	14 (21)	102(33)
Non-vaccine *emm*-types tested	28 (42)	159(52)
Non-vaccine *emm*-types killed >50%	26 (39)	144 (47)
Total *emm*-types killed >50%	40 (60)	246 (81)
Total *emm*-types killed + vaccine types not tested[b]	44 (66)	257(84)
Non-vaccine *emm*-types killed among non-vaccine types tested	26/28 (93)	
Total *emm*-types killed among total *emm* types tested	40/42 (95)	

[a]The number isolates in the collection represented by the various *emm*-types. The number and percent killed have been extrapolated based on results from the *emm*-types tested.

[b]The four vaccine *emm*-types present in the collection that were not tested were previously shown to be opsonized by the 30-valent vaccine antisera [10].

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Table 2

Protein sequence identities between the 26 non-vaccine *emm*-types that were opsonized and 30-valent vaccine proteins.[a]

Non-vaccine *emm*-type	30-Valent Vaccine Sequence Identity	% Identity	30-Valent Vaccine Sequence Identity	% Identity	30-Valent Vaccine Sequence Identity	% Identity
25.1	Emm58.0 (2-37)[b]	50	Emm87.0 (1-33)	52.9	Emm82.0 (1-38)	41
65.0	Emm44.0 (4-40)	75.7	Emm11.0 (16-50)	54.1	Emm118.0 (17-48)	43.8
109.1	Emm28.0 (10-50)	72.7	Emm22.0 (19-50)	90.6	Emm4.0 (13-50)	50
42.0	Emm11.0 (20-50)	71.9	Emm44.0 (11-46)	56.8	Emm81.0 (12-35)	58.3
64.4	Emm83.1 (2-48)	55.3	Emm24.0 (23-33)	54.5		
74.0	Emm14.3 (3-49)	55.3	Emm29.2 (3-43)	39	Emm19.0 (38-50)	46.2
85.1	Emm11.0 (6-50)	87.5	Emm44.0 (10-46)	59.5	Emm81.0 (12-44)	48.5
95.0	Emm82.0 (22-29)	75				
123.0	Emm83.1 (30-48)	73.7	Emm4.0 (10-32)	56.5	Emm29.2 (23-40)	55.6
st1731.1	Emm78.0 (5-50)	68.8	Emm22.0 (1-50)	51.1	Emm4.0 (19-50)	54.5
st2904.2	Emm118.0 (24-50)	76.9	Emm49.0 (29-50)	73.9	Emm44.0 (14-41)	46.4
8.0	Emm78.0 (1-50)	66	Emm4.0 (42-49)	87.5	Emm78.0 (15-27)	53.8
63.0	Emm4.0 (18-37)	57.9	Emm44.0 (16-47)	45.2	Emm11.0 (21-50)	45.2
79.0	Emm87.0 (4-50)	70.2	Emm58.0 (3-40)	57.9	Emm82.0 (4-41)	42.1
97.1	Emm3. 1 (41-50)	70	Emm114.0 (28-34)	71.4		
stKNB.6	Emm49.0 (8-50)	97.7	Emm118.0 (8-50)	78.7	Emm89.0 (15-41)	55.6
st1389.0	Emm22.0 (31-37)	62.5	Emm58.0 (10-17)	62.5	Emm3. 1 (45-48)	100
69.1	Emm44.0 (3-39)	75.7	Emm11.0 (16-50)	54.1	Emm118.0 (17-48)	43.8
71.1	Emm5.14 (1-25)	53.8	Emm18.0 (28-50)	59.1		
53.4	Emm83.1 (30-50)	76.2	Emm18.0 (31-42)	66.7	Emm29.2 (23-39)	47.1
60.0	Emm78.0 (5-38)	58.8	Emm22.0 (1-31)	68.8	Emm89.0 (25-37)	69.2
100.2	Emm18.0 (4-46)	44.2	Emm24.0 (1-50)	40.8	Emm18.0 (30-50)	56.5
111.0	Emm83.1 (32-41)	50	Emm22.0 (24-34)	54.5	Emm19.0 (36-45)	60
st2460.0	Emm58.0 (27-50)	75	Emm83.1 (31-40)	80	Emm58.0 (29-50)	40.9
stXH1.0	Emm11.0 (24-37)	92.9	Emm92.0 (10-46)	51.4	Emm44.0 (9-26)	66.7
st2911.0	Emm18.0 (40-49)	60	Emm83.1 (18-40)	43.5	Emm4.0 (21-28)	75

[a]Sequence identities for regions of vaccine peptides of fewer than seven consecutive amino acids were not tabulated, thus some fields are blank.

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[b] Emm designates the peptide fragment within the 30-valent protein that shares identity with the N-terminal 50 amino acid sequence of the M protein from the non-vaccine *emm*-type. The numbers in parentheses indicate the amino acid sequence locations within the peptides that exhibit the sequence identities.