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Rapid Species Identification of Group C Streptococci Isolated from Horses

MARY F. BANNISTER,[1] CHARLES E. BENSON,[1]* AND CORINNE R. SWEENEY[2]

Departments of Pathobiology[1] and Clinical Studies,[2] New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, Pennsylvania 19348

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Two commercial systems, the API 20S (Analytab Products, Plainview, N.Y.) and the Rapid Strep (API System S.A., Montalieu-Vercieu, France), were evaluated for ease of use and accuracy in the rapid identification of group C streptococci isolated from horses. A total of 85 *Streptococcus* isolates were tested, including *S. equi* (67 isolates), *S. zooepidemicus* (13 isolates), and *S. equisimilis* (5 isolates). All *S. equi* and *S. zooepidemicus* isolates were correctly identified within 24 h by the Rapid Strep system. Specific grouping sera was necessary to distinguish between *S. equisimilis* and group G or L strains. The API 20S system did not provide species identification of any of these isolates. An identification of randomly selected isolates to species level was performed by conventional methods and confirmed the identification derived through the Rapid Strep system. Our results indicate that the Rapid Strep system is a valuable aid for species identification of equine isolates of group C streptococci.

The group C streptococci are associated with a variety of equine disease syndromes (1, 6). Identification of these streptococci has clinical and epidemiological significance for the practitioner. Current protocols may require up to 7 days before an identification can be confirmed, particularly in the case of the nonfermentative *Streptococcus equi*, the etiological agent of "strangles" (2). Strangles is characterized by high fever, a serous or mucopurulent nasal discharge, and abscessation of the submandibular or retropharyngeal lymph nodes. These abscesses may drain into the throat area, leading to disseminated abscessation or "bastard strangles" (10). The morbidity associated with an *S. equi* epidemic in a susceptible population can be as high as 100%, whereas mortality is approximately 2% (7, 10). Similar symptoms may be evident in horses with an upper respiratory tract *Streptococcus zooepidemicus* infection which is usually less contagious and more responsive to treatment than an *S. equi* infection (7).

Early laboratory confirmation of an *S. equi* infection can decrease the spread of the disease and the incidence of complications. In a study of farms with histories of endemic strangles the complication rate was 18% (C. R. Sweeney, R. H. Whitlock and C. E. Benson, Proc. 64th Conf. Res. Workers Anim. Dis., 1973, p. 5). Rapid identification of *S. equi* in suspected strangles in horses justifies the isolation of infected horses from uninfected horses. A delay in confirming the presence of *S. equi* increases the opportunity for spread of the etiological agent through the herd. Horses recuperating from clinical signs of strangles often are retained in isolation until *S. equi* can no longer be isolated from nasalpharyngeal swab specimens. A rapid identification of *S. equi* can decrease the associated cost of isolation, as well as loss of revenue from horses that are unable to perform their intended function (e.g., racing) (5, 8). The objective of this study was to compare two rapid identification systems, the Rapid Strep (API System S.A., Montalieu-Vercieu, France) and the API 20S (Analytab Products, Plainview, N.Y.), for their abilities to accurately and rapidly identify the veterinary isolates of group C streptococci to the species level.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. *S. equi* (67 isolates) were obtained from nasal, pharyngeal, or abscessed lymph node swabs of horses exposed to or with clinical signs of strangles. *S. zooepidemicus* (13 isolates) and *Streptococcus equisimilis* (5 isolates) were isolated from clinical specimens submitted to the Microbiology Laboratories, New Bolton Center, University of Pennsylvania, Kennett Square. *S. zooepidemicus* ATCC 6580 was obtained from the American Type Culture Collection, Rockville, Md. All isolates were serogrouped according to the directions of the manufacturer (Phadebact Group C test; Pharmacia Diagnostics, Uppsala, Sweden).

Identification by the Rapid Strep system. The Rapid Strep strips and required reagents were purchased from DMS Laboratories, Inc., Flemington, N.J. The strips were inoculated according to the instructions of the manufacturer. The inoculum was harvested with a sterile swab from an overnight culture grown at 37°C on Trypticase Soy Agar (BBL Microbiology Systems, Cockeysville, Md.) or Columbia TSA Agar (BBL) containing 5% sheep blood (Interstate Media, Oakdale, Pa.) and was suspended in 2 ml of sterile distilled water to a turbidity greater than a no. 4 McFarland standard. The first half of the strip was inoculated with this suspension, and the remaining volume was diluted into the Rapid Strep medium, for use in the inoculation of the second half of the strip. The Rapid Strep strips were incubated for 4 h at 37°C. The specific reagents were added to eight of the cupules as directed by the manufacturer, and the reactions were recorded. The strips were reincubated overnight, and the reactions of the remaining 12 cupules were recorded. Identification was obtained by comparing the derived seven-digit code number with those in the codebook of the manufacturer.

Identification by the API 20S system. The API 20S strips and required reagents were purchased from Analytab Prod-

TABLE 1. Group level identification by the API 20S

Organisms (n)	No. (%) identified as GLLS[a]		No. (%) with no identification
	Group G	Group C	
S. equi (67)	67 (100)	0 (0)	0 (0)
S. zooepidemicus (13)	10 (77)	2 (15)	1 (8)
S. equisimilis (5)	0 (0)	0 (0)	5 (100)

[a] GLLS, Good likelihood, low selectivity. The first choice provided by the API 20S codebook for group level identification of the isolates tested was group G for 91%, and group C for 2%; no identification possible for 7% of the isolates.

ucts, Plainview, N.Y. The strips were inoculated according to the recommendations of the manufacturer with an inoculum prepared from an overnight culture grown on Trypticase Soy Agar containing 5% sheep blood at 37°C. The bacteria were harvested with a sterile swab and suspended in 2.5 ml of 0.85% saline to at least a no. 1 McFarland standard. Three drops of this suspension were added to each cupule, and the strip was incubated at 37°C for 4 h in the humidor tray. Specific reagents then were added to five cupules, and all reactions were recorded. The resultant seven-digit code number was compared with those in the codebook of the manufacturer for identification.

Identification by conventional methods. Streptococci were characterized by a modified version of the scheme described by Diebel and Seeley (3). Biochemical tests included hemolysis and fermentation of lactose, sorbitol, and trehalose. All carbohydrate fermentation tubes were incubated for 7 days before being recorded as negative (2). All isolates were serogrouped by the Phadebact Group C test.

RESULTS

The rapid identification systems were compared with conventional identification methods in an initial screen of 20 *S. equi* isolates, 1 *S. equisimilis* isolate, and 3 *S. zooepidemicus* isolates, including the ATCC reference strain. The Rapid Strep system identified all of the *S. equi* and *S. zooepidemicus* isolates without the use of additional tests. The identifications were classified by the manufacturer as excellent, indicating a percentage of identification equal to or greater than 99.9. The *S. equisimilis* isolate was identified to the species level as *S. equisimilis* and group G or *S. equisimilis* and group L. This identification indicated low discrimination between taxa. The identification of *S. equisimilis* was confirmed by serological grouping. The API 20S system identified these isolates only to the serogroup level.

After this initial test period, the identification provided by the Rapid Strep strip was used for identification of beta-hemolytic streptococci in our laboratory, with occasional confirmations by conventional methods. A total of 61 additional isolates were identified (47 *S. equi*, 10 *S. zooepidemicus*, and 4 *S. equisimilis*). For all isolates tested, the Rapid Strep strip provided an identification within 24 h. This incubation period was necessary for the acidification of the carbohydrate cupules to be evident. After 24 h, all reactions were clear and easy to interpret. We did not note the discrepancies in the fermentation patterns of the Rapid Strep system described by Poutrel and Ryniewicz (9). The directions of the manufacturer on storage and expiration date should be strictly observed, as outdated or improperly stored reagents will not give reliable results.

The API 20S system did not identify any of the group C streptococci to the species level. *S. equi* and *S. zooepidemicus* isolates were identified as good likelihood, low selectivity to the group level (Table 1). For 91% of the isolates tested, the first choice provided by the API 20S codebook for group level identification was group G, for 2% the first choice was group C, and for 7% there was no identification possible. The *S. equisimilis* isolates could not be identified through the API 20S method. We attempted to identify these strains to the species level based on the fermentation of the carbohydrates in the API 20S strip; however, there were discrepancies between the API 20S system and the conventional methodology (Table 2). Sorbitol was not fermented by the *S. zooepidemicus* isolates in the API 20S system but was fermented in both the Rapid Strep system and in conventional tubed media. Lactose was fermented by 62% of *S. zooepidemicus* strains when tested in the API 20S system but was fermented by all of the isolates when tested in the Rapid Strep system. The utilization of glycerol in the API 20S system was not consistent with conventional identification schemes for *S. zooepidemicus* and *S. equisimilis*, but the result was correct (negative) for 98% of the *S. equi* tested. Species identification of these isolates was impossible when the API 20S system was used.

DISCUSSION

Many commercial diagnostic microbiology systems have been developed without reference to animal pathogens and should not automatically be assumed applicable to veterinary diagnostic problems. Although conventional methods are adequate for identification of many veterinary microbiology isolates, the need for an accurate and rapid identification system is important for prompt control and treatment of some infections. One example is the equine pathogen *S. equi*. This pathogen is unimportant to the human hospital microbiology laboratory, a fact reflected in the reference data base of most commercial rapid identification products. *S. equi* can be presumptively identified by conventional methods at 24 h; however, a longer incubation period (up to 7 days) is required to rule out delayed fermentation reactions (2). Our study established the usefulness of the Rapid Strep strip as a dependable alternative to the conventional method for the rapid (24-h) identification of equine strains of group C streptococci. An evaluation of the Rapid Strep strip for human streptococcal isolates reported by Facklam et al. (4) presents similar conclusions. However, Poutrel and Ryniewicz (9) noted that streptococci isolated from bovine mastitis appear to present a more difficult identification problem for this system. This dichotomy strongly indicates the need for further modification of systems used in veterinary microbiology. As shown in our study, the API 20S

TABLE 2. Comparison of carbohydrate fermentations in *S. equi*, *S. zooepidemicus*, and *S. equisimilis* by three diagnostic systems

Species	% Positive carbohydrate[a] fermentations by								
	API 20S system			Rapid Strep system			Conventional methods[b]		
	L	S	T	L	S	T	L	S	T
S. equi	0	0	0	0	0	0	0	0	0
S. zooepidemicus	62	0	0	100	100	0	100	100	0
S. equisimilis	0	0	100	0	0	100	0	0	100

[a] L, Lactose; S, sorbitol; T, Trehalose.
[b] Initial screen of 24 isolates.

system is inappropriate for identification of streptococcal group C isolates of equine origin. The composition of the API 20S strip and the insufficient data base for the identification of veterinary isolates restrict the usefulness of this system in veterinary diagnostic microbiology.

There is another component to consider in these results besides the usefulness of the test system. The veterinary diagnostic laboratory or office laboratory generally has limited resources and space. An accurate and inexpensive method for correctly identifying streptococcal isolates is an important consideration for the laboratory supervisor or technician. The cost is comparable for both commercial strips, and both are less expensive, less bulky, and have a longer shelf life than the prepared tubed media. Specific grouping sera is necessary for the identification of *S. equisimilis*. However, commercial distributors of streptococcal antisera were not able to accommodate a request for only group C antisera. Indeed, had it not been for the generosity of a sales representative, we would have been forced to purchase a kit containing groups A, B, C, D, and G to acquire the appropriate grouping sera. The unused sera would have been discarded, a poor use of limited laboratory funds.

S. equisimilis is not a significant equine pathogen (6) and is usually of little concern, except in special cases (e.g., uterine abscesses). In this instance, the recognition of beta-hemolytic streptococci is sufficient evidence to commence a treatment regimen. Differentiation of *S. equi* from other group C strains (in particular, *S. zooepidemicus*) is important because *S. equi* infections are highly contagious. Therefore, the rapid identification of *S. equi* from submitted specimens is a high priority for the clinician and farm manager. For this reason, we evaluated the results of two commercial *Streptococcus* identification strips to establish a more reasonable and rapid diagnostic approach. The Rapid Strep strip provides a valuable aid in speciating the beta-hemolytic group C streptococci in an efficient and cost-competitive manner.

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