Taxonomic Note: a Proposal for Reviewing the Interpretation of the CAMP Reaction between *Listeria monocytogenes* and *Rhodococcus equi*

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The discrepancies between the current description of the CAMP test between *Listeria monocytogenes* and *Rhodococcus equi* in the latest edition of Bergey's Manual of Determinative Bacteriology (*L. monocytogenes* is described as CAMP test negative with *R. equi*) and routine findings (positive reactions are usually described in many laboratories) make it advisable to review the current interpretation of the CAMP test to avoid confusion among people working in microbiological laboratories. Overall, 98.4% of the *L. monocytogenes* strains examined in this study, regardless of their source or the intensity of their hemolytic activity, displayed a synergic hemolytic reaction (CAMP phenomenon) with *R. equi*, indicating that *L. monocytogenes* can generally be considered CAMP positive with *R. equi*. We propose that *L. monocytogenes*, together with *Listeria ivanovii*, should be considered CAMP test positive with *R. equi* (circular or racket and semicircular or shovel shapes, respectively).

Despite the numerous tests of phenotypic and genotypic character that have appeared in the scientific literature in the last years, hemolytic activity, together with the acidification pattern of a few sugars, is considered an essential criterion for the characterization and identification of the different species of *Listeria* (13, 16). As the interpretation of the hemolytic activity of *Listeria* strains on blood agar plates is sometimes problematic (2), a synergistic hemolytic reaction (CAMP test) with *Staphylococcus aureus* and *Rhodococcus equi* was proposed (11). The CAMP test is considered to be a fundamental taxonomic criterion (13), being especially useful for differentiating between very weakly hemolytic strains of *Listeria monocytogenes* and nonhemolytic strains of *Listeria innocua*, as both species are very similar biochemically and are frequently isolated simultaneously. In the latest edition of Bergey's Manual of Determinative Bacteriology, *L. monocytogenes* and *Listeria seeligeri* are described as CAMP positive with *S. aureus* and CAMP negative with *R. equi* (SA+/RE–). The typical CAMP reaction of *Listeria ivanovii* is SA–/RE+, while the CAMP reaction for both *L. innocua* and *Listeria welshimeri* is SA–/RE– (13).

While the interpretation of the CAMP test between *S. aureus* and *L. monocytogenes* has been widely accepted, the interpretation of the CAMP test between *L. monocytogenes* and *R. equi* is more controversial. In spite of the fact that *L. ivanovii* is the single species of *Listeria* considered CAMP positive with *R. equi*, we have reported clear synergistic hemolytic reactions (CAMP test positive) with many *L. monocytogenes* strains of different origin (collection, clinical, environment, food, and feedstuffs), indicating the reproducibility of the CAMP phenomenon between both species (4, 16–19). Similar cooperative hemolytic reactions are observed when diffusion CAMP assays are performed with supernatant cultures of these species (3). Table 1 shows the *R. equi* CAMP results with different collec-

tion strains and field isolates of *L. monocytogenes*. Overall, 98.4% of the *L. monocytogenes* strains gave a positive CAMP reaction with *R. equi*. Only 6 of 373 strains, identified biochemically as *L. monocytogenes*, were nonhemolytic and were also CAMP test negative with *R. equi*, while we have not found any hemolytic strain of *L. monocytogenes* that gives a negative CAMP reaction with *R. equi*. Positive CAMP reactions between *L. monocytogenes* and *R. equi* have frequently been reported by other authors as well (7, 8, 10, 12, 14, 20). The CAMP reactions of *L. monocytogenes* and *L. ivanovii* with *R. equi* differ in the shape of the zone of synergic hemolysis. The CAMP test between *L. ivanovii* and *R. equi* produces a semicircular or shovel shape, while the CAMP test between *L. monocytogenes* and *R. equi* displays a circular or racket shape (16, 17) (Fig. 1). We have found total reproducibility in performing the CAMP test on Columbia sheep blood agar base (Biomérieux) with *R. equi* STCC (Spanish Type Culture Collection) 4568 after 24 to 48 h of incubation at 37°C (16).

The exosubstances involved in the synergistic hemolytic reaction of *L. monocytogenes* and *L. ivanovii* with *R. equi* have been identified. The CAMP phenomenon between *L. ivanovii* and *R. equi* involves a sphingomyelinase C and a cholesterol oxidase, respectively (10). Listeriolysin O (LLO) is the cytolytic factor of *L. monocytogenes* involved in the CAMP reaction with *S. aureus* and *R. equi* (10), while the enzymes of *S. aureus* and *R. equi* implicated in the cooperative hemolytic reaction with *L. monocytogenes* are, respectively, a sphingomyelinase and a cholesterol oxidase (3, 7).

Two reasons have usually been argued to reject a positive CAMP reaction between *L. monocytogenes* and *R. equi*. First, this CAMP reaction does not display the typical shovel-like shape observed with *L. ivanovii*, and second, many of the investigators reporting positive CAMP reactions for *L. monocytogenes* used strains of *R. equi* different from the collection strain CIP 5869 indicated by Rocourt et al. (11). Regarding the shape of the CAMP reaction, now that the exosubstances involved in the CAMP reaction between *L. monocytogenes* and *R. equi* have been determined, the fact that the CAMP reaction between both species does not display a typical shovel-like

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TABLE 1. Hemolytic activity and CAMP reaction with *R. equi* of different collection strains and field isolates of *L. monocytogenes*^a

Source	Serovar	Hemolytic activity ^b	CAMP reaction with <i>R. equi</i> ^b
Collection strains ^c			
NCTC 8817	ND ^d	+	+
NCTC 4885	ND	++	+
NCTC 5105	3a	+++	+
NCTC 7973	1/2a	++	+
NCTC 5214	4a	+	+
NCTC 5348	1/2c	+	+
SLCC 3551	4b	+/-	+
SLCC 2482	7	+	+
SLCC 2378	4e	+	+
NCTC 10357 ^e	1/2a	-	-
SLCC 4324	4b	+	+
SLCC 5132	1/2a	+	+
Scott A	4b	++	+
Clinical isolates			
Encephalitis in sheep (27) ^f	4b	+/++ (26) ^g	+(26) ^g
Feed/Foods isolates			
Silage (30)	4b	+/++ (29)	+(29) ^g
Chicken (12)	ND	+/++ (12)	+(12)
Soft cheese (40)	ND	+/++ (40)	+(40)
Frozen foods (51)	ND	+/++ (48)	+(48) ^g
Environmental isolates			
Slaughterhouse (179)	ND	+/++ (178)	+(178) ^g
Fish industry (28)	ND	+/++ (22)	+(22)

^a Data obtained from *L. monocytogenes* strains isolated in our laboratory and references 4, 17, and 19.

^b Hemolytic activity and *R. equi* CAMP reaction were determined in Columbia sheep blood agar. The hemolytic activity in the clinical, feed/food, and environmental isolates is an estimation of the hemolysis average displayed by all the strains included in each group. +/-, very weak hemolysis (sometimes it is necessary to remove the colonies to see the zone of hemolysis); +, weak hemolysis; ++, moderate hemolysis; +++, strong hemolysis.

^c NCTC, National Collection of Type Cultures; SLCC, Special *Listeria* Culture Collection.

^d ND, not determined.

^e Nonhemolytic type strain of *L. monocytogenes* (5). As LLO is involved in the CAMP phenomenon, the nonhemolytic strains of *L. monocytogenes* do not display RE+ CAMP reactions.

^f The number of strains that were analyzed, that were hemolytic, and that displayed RE+ CAMP reactions are shown in parentheses (as appropriate).

^g Six strains of *L. monocytogenes* (identified biochemically) isolated from a clinical case of encephalitis in sheep (1), a slaughterhouse environmental sample (1), silage (1), and frozen foods (3) were nonhemolytic and were also CAMP test negative with *R. equi*.

shape should not be the reason to consider this test negative. Weakly, moderately, and strongly hemolytic strains of *L. monocytogenes* give positive CAMP reactions with *R. equi* (Table 1). Keeping in mind that LLO is involved in this synergistic hemolytic reaction, the variations in the size of the zone of synergic hemolysis that are observed with different *L. monocytogenes* strains are probably associated with variations in the ability of these strains to secrete LLO (Fig. 1 and 2). Weakly hemolytic strains of *L. monocytogenes* display positive CAMP reactions that are smaller than those produced by stronger hemolytic strains. This fact can be clearly observed in Fig. 1, in which *L. monocytogenes* NCTC 8817 (weakly hemolytic) displays a CAMP reaction that is smaller than that of *L. monocytogenes* NCTC 7973, while the reaction of the latter strain is smaller than that produced by *L. monocytogenes* NCTC 5105 (strongly hemolytic) (Fig. 2). Only nonhemolytic strains of *L. monocytogenes* (e.g., strain NCTC 10357 in Fig. 2) were CAMP negative (as expected considering that LLO is implicated in

the synergistic hemolytic reaction). The great majority of the *L. monocytogenes* isolates, regardless of their source, show weak or moderate hemolysis (Table 1) yet display clear RE+ CAMP reactions (Fig. 2). Despite these differences in the size of the CAMP reaction, it seems clear that there is a synergistic hemolytic reaction (CAMP phenomenon) with most of the *L. monocytogenes* strains in their intersection with *R. equi*. From these results it can be stated that *L. monocytogenes* can generally be considered CAMP positive with *R. equi*. In fact, from our experience, the high reproducibility of the CAMP reaction with *R. equi* makes it especially useful as one of the confirmatory tests to differentiate strains of *L. monocytogenes* from *L. innocua*, particularly in cases of weakly hemolytic strains of *L. monocytogenes* (Fig. 2), which display unambiguously positive CAMP reactions with *R. equi*, while *L. innocua* strains are, as expected, RE-.

The differences in intensity reported in the CAMP reaction with *L. monocytogenes* depending on the *R. equi* strain used to perform the test (8, 12) may be explained by variations in the capability of different strains of *R. equi* to secrete cholesterol oxidase (15). Weak CAMP reactions observed with some strains of *R. equi*, specially with very weakly hemolytic strains of *L. monocytogenes*, can be clearly detected by extending the incubation period (18). In practice, the fact that there are not specific recommendations for using a particular strain of *R. equi* in some of the most common manuals of clinical or food microbiology (1, 6, 9, 20) means that many laboratories, using different strains of *R. equi*, usually find positive CAMP reactions for *L. monocytogenes* (7, 8, 10, 14, 16, 17-20). It could be stated, therefore, that among *Listeria* spp., a positive CAMP test with *R. equi* is not exclusively limited to *L. ivanovii*, as it is currently considered to be.

As the CAMP reaction is still a very useful and widely used test for the routine identification of *Listeria* spp. (13, 16), and in order to avoid confusion among people working in clinical and food microbiology laboratories, the current interpretation of the CAMP test between *L. monocytogenes* and *R. equi* should be reviewed. We propose that both *L. monocytogenes* and *L. ivanovii* should be considered CAMP test positive with

FIG. 1. CAMP reactions with *R. equi* STCC 4568 (vertical streak of growth) and *L. monocytogenes* NCTC 8817 (A), *L. ivanovii* ATCC 19119 (B), and *L. monocytogenes* NCTC 7973 (C) on blood agar plates after 48 h of incubation at 37°C. Both *L. monocytogenes* and *L. ivanovii* display synergistic hemolytic reactions (positive CAMP test) with *R. equi*, although with different shapes. *L. ivanovii* displays the typical semicircular or shovel shape, while *L. monocytogenes* produces a circular or racket-shaped CAMP reaction of different size depending of the strain of *L. monocytogenes* tested.

FIG. 2. CAMP reactions with *R. equi* displayed by different collection strains and field isolates of *L. monocytogenes*. The isolates used are as follows: *L. monocytogenes* NCTC 10357 (1), *L. monocytogenes* SLCC 3551 (2), *L. monocytogenes* SLCC 2482 (3), *L. monocytogenes* SLCC 4324 (4), *L. monocytogenes* NCTC 5105 (5), *L. monocytogenes* isolated from a case of encephalitis (6), *L. monocytogenes* isolated from silage (7), *L. monocytogenes* isolated from frozen minced meat (8), and *L. monocytogenes* isolated from a slaughterhouse environment (9). Most of the field isolates, as well as the collection strains, of *L. monocytogenes* are weakly or moderately hemolytic but nevertheless display clearly positive CAMP reactions with *R. equi*.

R. equi (circular or racket and semicircular or shovel shapes, respectively), while *L. monocytogenes* (as well as *L. seeligeri*) is CAMP positive with *S. aureus*. Thus, *L. monocytogenes* and *L. ivanovii* should be described as being SA+/RE+ and SA-/RE+, respectively, for the CAMP test.

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