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A Research Note

Efficacy of an International Method for Detection of *Salmonella* in Chocolate and Cocoa Products

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ABSTRACT

The methods of the International Office of Cocoa and Chocolate and International Sugar Confectionery Manufacturers' Association (IOCC/ISCMA), and of the Health Protection Branch (HPB) were compared for their ability to detect *Salmonella* in chocolate and cocoa products. Of 152 samples tested, 13 contaminated samples were identified, 10 by the HPB and 8 by the IOCC/ISCMA method. Prolonged (48 h) incubation of enrichment media produced two false-negative results each with the Muller-Kauffman tetrathionate and the selenite cystine broths, exerted no effect on tetrathionate brilliant green, and identified one additional positive sample with the selenite brilliant green broth. More samples were found positive on bismuth sulfite than on brilliant green and brilliant green sulfa agar media. The present study underlines the limited sensitivity of both standard methods and questions the determinant role of casein in the neutralization of toxic agents in cocoa products.

Recent outbreaks of human salmonellosis from milk chocolate (*4,6,10*) underline the importance of sensitive and cost-efficient methods for detection of *Salmonella* in chocolate confectionery. Standard methods generally recommend preenrichment of samples in non-selective broth, enrichment in tetrathionate, selenite or other selective media, and plating on differential agar media (*7,9,11*). Preenrichment of chocolate in reconstituted skim milk powder with added brilliant green is preferred because casein in milk powder neutralizes toxic anthocyanins in cocoa (*13*). In contrast, the method of the International Office of Cocoa and Chocolate and of the International Sugar Confectionery Manufacturers' Association (IOCC/ISCMA), recommends mannitol broth for preenrichment (*8*). The IOCC/ISCMA method also differs from other standard procedures in its use of 10-rather than 1-ml preenrichment transfer volumes and prolonged (48 h) incubation of enrichment cultures.

This paper compares the efficacy of the international IOCC/ISCMA method with a standard cultural procedure for detection of *Salmonella* in naturally contaminated milk chocolate and cocoa products.

MATERIALS AND METHODS

Milk chocolate and cocoa products known or suspected to contain salmonellae were obtained as a result of regulatory activities or in-plant quality control programs. A 100-g sample of product was blended 60 s in 0.05 to 0.2 L of sterile water to obtain a homogeneous fluid preparation. Replicate 25-g portions of the resulting slurry were analyzed in parallel by the methods of the IOCC/ISCMA (*8*) and the Health Protection Branch (HPB;7). In the IOCC/ISCMA procedure, test samples were preenriched overnight in 225 ml of tempered (35°C) mannitol broth. Portions (10 ml) of preenrichment cultures were selectively enriched in 100 ml of Muller-Kauffman tetrathionate (MKT) and selenite brilliant green (SBG) broths at 43°C and 35°C, respectively. Enrichment cultures were streaked on bismuth sulfite (BiS) and brilliant green (BGA) agar media after 24 and 48 h of incubation; plates were examined after 24 and 48 h at 35°C. Suspect colonies were screened biochemically on triple sugar iron and lysine iron agars, and confirmed serologically by somatic and flagellar agglutination reactions. In the Health Protection Branch (HPB) method, 25-g samples were preenriched overnight in 225 ml of reconstituted (10% wt/vol) skim milk powder with added brilliant green (0.002% wt/vol). Portions (1 ml) of preenrichment cultures were selectively enriched overnight in 9 ml of tetrathionate brilliant green (TBG) and selenite cystine (SC) broths at 43°C and 35°C, respectively. Although not part of the standard HPB method, enrichment cultures were incubated for an additional 24 h to permit comparisons with homologous IOCC/ISCMA results. Enrichment cultures were plated on BiS and brilliant green sulfa (BGS) agar media, and suspect colonies were screened biochemically and confirmed serologically as described previously. Incidence of competitive flora on plating media was estimated using the following scale: 1 = absence or scant growth of non-salmonellae; 2 = light growth; 3 = moderate growth; 4 = heavy growth.

RESULTS AND DISCUSSION

A total of 152 samples of 9 different chocolate and cocoa products was analyzed in parallel for *Salmonella* by the IOCC/ISCMA and the HPB methods. Thirteen contaminated samples containing 6 serovars were identified by the two methods combined (Table 1). Ten samples were detected by the HPB and 8 samples by the IOCC/ISCMA methods (Table 2). Reasons for the limited sensitivity of both methods and lack of agreement between homologous results are uncertain. Non-homogeneous distribution of salmonellae in the original test material cannot account for discrepant results because replicate portions of individual slurries were tested

TABLE 1. Salmonella *in milk chocolate and cocoa products.*

Food	Country of origin	No. of samples tested	No. of positive samples	Serovar (No. of strains)
Milk Chocolate				
Footballs	Canada	65	3	*S. eastbourne* (3)
Beetles	West Germany	3	1	*S. typhimurium* (1)
Balls	Canada	14	0	NA[a]
Nut bars	Greece	25	5	*S. tennessee* (5)
Bars	Italy	2	2	*S. napoli* (2)
Cocoa products				
Chips	U.S.A.	6	1	*S. senftenberg* (1)
Cocoa powder	Netherlands	13	0	NA
Cocoa beans	Ivory Coast	21	1	*S. africana* (1)
Mocha	Canada	3	0	NA
TOTAL		152	13	

[a]Not applicable.

in parallel. The demonstrated equivalence of a 1.0-ml (HPB) and 10.0-ml (IOCC/ISCMA) preenrichment transfer volume (*3*) also fails to support the determinant role of this parameter in the final outcome of analyses with each method. A serovar-dependent response was not apparent. Although the marginal superiority of the HPB method could be attributed to preenrichment in reconstituted milk powder and casein neutralization of toxic agents in chocolate and cocoa products, the protective effect of milk is somewhat limited as seen in the incidence of three false-negative results with the HPB method (Table 2). Earlier work demonstrated that rehydration of milk powder at reduced water activity (a_w) markedly increased isolation of *Salmonella* in this product (*12*); a similar effect was apparent in rendered products and animal feeds (D'Aoust, in press). Possibly, consecutive rehydration of chocolate and cocoa products at low a_w and preenrichment in reconstituted milk powder would increase method sensitivity.

TABLE 2. *Sensitivity of standard cultural procedures.*

		No. of Salmonella-positive samples[a]			
		HPB		IOCC/ISCMA	
Food	Total	24 h	48 h	24 h	48 h
Milk chocolate					
Footballs	3	3	3	1	1
Beetles	1	0	0	1	1
Nut bars	5	3	3	4	4
Bars	2	2	2	2	2
Cocoa products					
Chips	1	1	1	0	0
Cocoa beans	1	1	1	0	0
TOTAL	13	10	10	8	8

[a]Detection of salmonellae in 24- and 48-h enrichment cultures.

Productivities of the TBG and SC enrichment broths were similar, and incubation for an additional 24 h adversely affected the performance of the SC and MKT enrichment broths (Table 3). SBG enrichment plated on BGA was the only combination that yielded an additional positive sample with prolonged (48 h) incubation. These results contrast with earlier reports on increased rates of isolation with extended periods of incubation (*2*). The superiority of BiS in the present study concurs with earlier findings (*1,5*) and reiterates the diagnostic value of this medium because of its ability to repress the growth of competitive flora and identify atypical *Salmonella* biotypes. BiS in combination with SBG provided the most selective conditions against non-salmonellae (Table 3). Examination of IOCC/ISCMA plates after 48 h of incubation failed to identify additional positive samples.

The present study indicates that the HPB and IOCC/ISCMA standard methods are not entirely reliable and suggests that preenrichment of chocolate and cocoa products in reconstituted milk powder marginally increases method sensitivity. Possible synergism between rehydration of test material at low a_w and preenrichment in reconstituted milk powder with added brilliant green would need to be investigated.

TABLE 3. *Productivity of enrichment-plating conditions.*

	No. of *Salmonella* isolations							
	HPB (*10*)[a]				IOCC/ISCMA (*8*)			
Plating media	TBG_{24}	TBG_{48}	SC_{24}	SC_{48}	MKT_{24}	MKT_{48}	SBG_{24}	SBG_{48}
BiS	10 (2.0)[b]	10 (3.0)	10 (3.0)	9 (3.0)	8 (2.0)	8 (2.0)	8 (1.0)	8 (1.0)
BGS	9 (3.0)	9 (3.0)	8 (3.0)	7 (3.0)	-[c]	-	-	-
BGA	-	-	-	-	8 (3.0)	6 (4.0)	7 (3.0)	8 (3.0)

[a]Total number of positive samples identified by the method.
[b]Median value of competitive flora after 24 h of incubation.
[c]Not applicable.

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