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DETECTION OF CLASSIC AND INVASIVE E. COLI AND SHIGELLA SEROTYPES IN STOOLS BY INDIRECT IMMUNOFLUORESCENCE

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SUMMARY

IIF in the detection of invasive and classic enteropathogenic **E. coli** and **Shigella** serotypes was compared with traditional coproculture methods. IIF results agreed with the coproculture findings in 128 out of 140 cases tested for enteropathogenic **E. coli** (91%) and in 108 out of 112 for **Shigella** (96%). All cases with positive reactions by coproculture were confirmed by IIF. In the control group it were obtained by IIF 12 cases with positive reactions for enteropathogenic **E. coli** and 4 cases for **Shigella**, including two cases of mixed infection by **E. coli** 026/**Sh. dysenteriae** and **E. coli** 0124/**Sh. dysenteriae**. It was discussed the high sensitivity and specificity of the IIF when compared with the traditional methods, being suggested that IIF is a valuable tool in epidemiological studies involving these organisms and an important aid in the stablishment of an early presumptive diagnosis of the acute infantile diarrhea.

INTRODUCTION

The enteropathogenic **E. coli** (EEC) plays an important role in the pathogenesis of infantile diarrhea [1,23]. Specially divised methods and culture media are usually required for the isolation and biochemical-serological identification of these organisms. In addition, the stablishment of a definitive diagnosis takes at least 48 hours.

The use of direct immunofluorescence (DIF) in the detection of EEC was inicially described by WHITAKER in 1958 [24]. This faster and easier method has been used not only as a screening procedure during epidemical situations [1,3,5,6,16], but also during outbreaks due to different serotypes of EEC [7,15,17]. Several authors reported the use of this method in the detection of classic serotypes of EEC from newbornes and children, with special advantages to specificity and sensitivity [3,5,6,8,12,14,22].

Although coproculture and biochemical-serological identification have been routinelly utilized in the detection of **Shigella**, some Authors evaluated the DIF as a method to detect such pathogens [4,19,20,21].

The use of indirect immunofluorescence (IIF) has been restricted to some classic serotypes of EEC from human [11,12] and animal [25] origen. This method would provide the investigation of a variety of microorganisms using only one anti-immunoglobulin conjugate.

The present report was undertaken to compare the use of IIF with the usual laboratory methods in the identification of classic and invasive **E. coli** and **Shigella** serotypes from normal and diarrheic stools. It was investigated by this technique cross reactions between these serotypes of **E. coli** and **Shigella**, which had already been described using agglutination and DIF reactions [4,9].

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MATERIALS AND METHODS

E. coli serotyping — It was done as described by EWING[9]. Rabbit hiperimmune antisera were produced against the following classic and invasive **E. coli** O serogroups: 026, 055, 086, 0111, 0119, 0125, 0126, 0127, 0128 and 028; 029, 042, 0112, 0124, 0136, 0143, 0144, 0152 and Saigon, respectivelly. The sera were titrated by the tube agglutination method, against homologous and heterologous strains, the latter being used to detect cross-reactions.

Indirect Immunofluorescence (IIF) — Fluorescent anti-rabbit immunoglobulins (Hyland Division, Travenol Laboratories Inc., Costa Mesa, California, USA) was suspended in carbonate-bicarbonate buffered glycerol solution (pH 9.0) and kept at −20°C until use. The conjugate was titrated in the reaction of a known antigen (**E. coli** 0111) against its homologous antiserum and also against an heterologous antiserum (anti-**E. coli** 0119) that did not cross react in the tube agglutination test. All the **E. coli** antisera were then titrated with the homologous strains and tested for cross reactions with heterologous organisms.

Polyvalent pools — **E. coli** antisera were grouped into 4 pools, keeping the cross reactive sera within the same pool:

Pool A: 026, 055, 086, 0111, 0119; Pool B: 0125, 0126, 0127, 0128; Pool C: Saigon, 0112, 028, 0124, 0144, 042; Pool D: 0152, 0136, 0143, 029.

Shigella antisera — Rabbit hyperimmune **Shigella** antisera (Lederle Laboratories) were titrated by IIF. Each antiserum reacted with all the serotypes within one species. The sera were also evaluated at the respective titres by IIF against heterologous **Shigella** strains to look for cross reactions.

Stool smears and bacteria — The smear slides and bacteria were obtained from clinical laboratories which performed DIF and/or culture and serology for detection of invasive and classic enteropathogenic **E. coli** and culture and serology for **Shigella.** 169 Stool smears were received and divided into three groups: 57 were from positive cases for classic or invasive **E. coli,** 29 from positive cases for **Shigella** and 83 from negative cases for pathogenic enterobacteria which constituted the control groups. The fixed stool smears were kept at 4°C and submitted to IIF up to 48 hours after receiving. Stool smears from positive cases runned together with smears of the one respective isolated and serologically confirmed bacteria. All cases including of the **Shigella** and control groups were confronted at first with polyvalent **E. coli** antisera and when a positive result was observed (characteristic bacterial morphology and fluorescence intensity from 2 to 4+) another slide was tested against the respective monovalent antisera. In all cases the identification of the isolated bacteria was confirmed through the biochemical tests described by FONTES[10] and by the IIF done with the pure culture of the isolated bacteria.

RESULTS

The intensity of the reaction obtained between each bacteria and with the homologous monovalent **E. coli** antiserum was as strong as with the pooled sera, in contrast with the very weak or negative reaction obtained when the heterologous monovalent sera belonging to the same pool were used. Similar results were obtained with the **Shigella** antisera against heterologous **Shigella** strains.

We detected 14 distinct enteropathogenic **E. coli** serogroups: 8 belonging to classic serogroups (026, 055, 086, 0111, 0119, 0125, 0126, 0128) and 6 belonging to invasive ones (0112, 028, 0144, 042, 0143, 029) (Table I). The results with IIF showed a high correlation with findings obtained by traditional culture and serological methods (Tables I and II). In 140 cases tested by IIF for enteropathogenic **E. coli** (57 positive for invasive and classic **E. coli** serotypes and 83 controls) was obtained 91% (128 cases) of correlation between this method and coproculture. All cases with positive coproculture were confirmed by IIF. The remaining 12 cases belonging to the control group showed positive IIF results although being negative by coproculture.

In 112 cases tested for **Shigella** (29 positive and 83 controls) 96% (108 cases) of correlation between the two technics was obtained (Tables III and IV). The other 4 cases were positive only by IIF as it was also observed in the groups tested for enteropathogenic **E. coli.** One case presented mixed infection (**Shigella sonnei** and **E. coli** 0128) and both organisms

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T A B L E I

Indirect immunofluorescence in 57 strains of enteropathogenic **E. coli** positive feces (*) and 83 negative controls by culture-agglutination

Culture/agglutination			Indirect immunofluorescence			
			Number of positive reactions			
	Isolated enteropathogen	Number of cases	Against monovalent homologous antiserum	crossed reactions (against heterologous antiserum of homologous pool)		Negative reactions
				+ and ++	+++ and ++++	
Positive	026	5	5	—	—	—
	055	2	2	—	—	—
	086	4	4	—	—	—
	0111	19	19	2(026)	—	—
	0119	9	9	—	—	—
	0125	1	1	—	—	—
	0126	3	3	—	—	—
	0128	8	8	—	1(0125)	—
	0112	1	1	—	—	—
	028	2	2	—	—	—
	0144	1	1	—	—	—
	042	1	1	—	—	—
	0143	2	2	—	—	—
	029	1	1	—	—	—
Negative				12		71

(*) It were included the mixed infection cases: 0111/028 (1)
0111/0119 (1)
0128/**Sh. sonnei** (1)

T A B L E II

Positive and negative cases detected by immunofluorescence and coproculture, in 140 stool specimens studied for enteropathogenic **E. coli**

		Coproculture		Total
		Positive	Negative	
Immunofluorescence	Positive	57	12	69
	Negative	0	71	71
Total		57	83	140

were detected either by coproculture or by IIF (Table III).

Positive reactions by IIF for two different organisms were observed in two control cases where **E. coli** 026 and **S. dysenteriae** and **E. coli** 0124 and **S. dysenteriae** were respectivelly detected (Table V).

DISCUSSION

IIF in the detection of invasive and classic enteropathogenic **E. coli** and **Shigella** serotypes was compared with traditional coproculture methods.

There was a good correlation between both methods. IIF results agreed with the coproculture findings in 128 out of 140 cases tested for enteropathogenic **E. coli** and 108 out of 112 tested for **Shigella** corresponding respectivelly to 91 and 96% of agreement. There are some reports comparing the results obtained by DIF and coproculture in the detection of enteropathogenic **E. coli** [1,3,5,14,22] and **Shigella** [18,20] with correlations of 84.3 to 92% and 70.8 to 95.6% respectivelly. Although we compared coproculture with IIF, our data are similar to that showed on the literature using DIF.

Cases with positive reactions and negative coproculture results were observed in 12 of the cases tested for enteropathogenic **E. coli** and in 4 of the cases tested for **Shigella.** These cases could possible be explained by the greater sensitivity of immunofluorescent methods, unespecific reactions and/or specific reactions with non viable organisms [6,14]. The last hypothesis if confirmed, would provide a method for gastroenteritis diagnosis even if under antibiotic therapy when drug levels in stools may inhibit bacterial growth. The lack of information on the therapy of these cases prevented us to

T A B L E III

Indirect immunofluorescence in 29 strains of **Shigella** positive feces and 83 negative controls. Search for crossed reactions against enteropathogenic **E. coli** with polyvalent antisera (A,B,C,D)

Culture/agglutination			Indirect immunofluorescence					
			Number of positive reactions					
	Isolated enteropathogen	Number of cases	Against homologous antiserum	Against anti-enteropathogenic **E. coli** pools				Negative reactions
				A	B	C	D	
Positive	**Sh. dysenteriae**	1	1	—	—	—	—	—
	Sh. flexneri	14	14	1(*)	1(*)	—	—	—
	Sh. boydii	1	1	—	—	—	—	—
	Sh. sonnei	13	13	—	1(**)	1(**)	—	—
Negative					4			79

(*) Fluorescence intensity reaction +++ against the polyvalent antiserum anti **E. coli** but not confirmed for monovalent antisera from this pool

(**) Mixed infection case: **Sh. sonnei/E. coli** 0128

T A B L E IV

Positive and negative cases detected by immunofluorescence and coproculture, in 112 stool specimens studied for **Shigella**

		Coproculture		Total
		Positive	Negative	
Immunofluorescence	Positive	29	4	33
	Negative	0	79	79
Total		29	83	112

T A B L E V

Positive indirect immunofluorescence (*) in stool specimens from the control group

Antisera (number of cases with positive IIF**)	
Polyvalent	Monovalent
A(6)	026(5)
	0111(1)
B(0)	—
	0124(3)
C(5)	0112(2)
D(1)	0152(1)

Sh. dysenteriae (3)
Sh. flexneri (0)
Sh. boydii (0)
Sh. sonnei (1)

(*) Immunofluorescence intensity: +++ and ++++

(**) It were included the mixed infection cases:
026/**Sh. dysenteriae** — (1)
0124/**Sh. dysenteriae** — (1)

take any conclusion about it. In respect to the hypothesis of unespecific reactions, it must be emphasized that all **E. coli** IIF positive control cases reacted with only one pool and with only one monovalent serum within this pool, and that we also had strong and apparently specific reactions with **Shigella** antisera by this technique. In addition, in the diarrheic group unespecific reactions were never observed. We suggest that IIF is as specific as coproculture and constitutes a more sensitive method, as it was observed by other Authors, regarding to DIF [14,15,22,24]. In contrast to some reports [14,15,20,22] we could confirm by IIF all diagnosis made by traditional methods.

Cross reactions between EEC serotypes and **Shigella** did not constitute a major difficult for the diagnosis by IIF. The few cases of **Shigella** infection that were positive with one anti **E. coli** pool did not react with any of the monovalent sera within that pool.

The detection of mixed infections was already reported [13,16]. The identification of two organisms in the control group (Table V) could be explained by an asymptomatic carrier state where the organisms were either non viable or in numbers too reduced to be detected by coproculture. Our findings do not diminished the importance of isolation of the causative agent, but we suggested that IIF is an valuable tool in epidemiological studies involving classic and invasive EEC and **Shigella** and an important aid in the stablishment of an early presumptive diagnosis in the acute infantile diarrhea.

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RESUMO

Detecção de E. coli enteropatogênica clássica e invasora e Shigella em fezes por imunofluorescência indireta

A imunofluorescência indireta (IFI) de sorotipos enteropatogênicos clássicos e invasores de **E. coli** e de **Shigella** foi comparada com os métodos tradicionais de coprocultura e soroaglutinação. Os resultados da IFI concordaram com os da coprocultura em 128 dos 140 casos testados para **E. coli** enteropatogênica (91%) e em 108 dos 112 testados para **Shigella** (96%). Todos os casos com reações positivas por coprocultura foram confirmados por IFI. No grupo controle, onde não haviam sido isolados tais patógenos por coprocultura, foram evidenciados por IFI, 12 casos com reações positivas para **E. coli** enteropatogênica e 4 para **Shigella,** incluindo-se 2 com infecção mista: **E. coli** 026/**Sh. dysenteriae** e **E. coli** 0124/**Sh. dysenteriae.** Foi discutida a alta sensibilidade e especificidade da IFI quando comparada aos métodos tradicionais, sendo sugerido o valor desta técnica em estudos epidemiológicos envolvendo os microrganismos em questão e sua importância no estabelecimento de diagnóstico precoce na diarréia infantil aguda.

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