A Survey to Assess Potential Disease Hazards Along Proposed Sea Level Canal Routes in Panamá and Colombia VII. Survey for Salmonella Antibodies in Man

Miguel Kourany, Ph.D.
Manuel A. Vásquez, M.D.

A previous report described the results of a bacteriological survey for enterobacterial pathogens among personnel conducting engineering feasibility studies for a proposed sea-level canal in a remote region of eastern Panamá.[1] The proposed canal route through this region is known as Route 17. Indigenous residents, both Indians and non-Indians of this region, employed as support personnel and laborers for the Canal project, were similarly studied. This led to an estimate of the prevalence, variety, and relative frequency of the Enterobacteriaceae associated with diarrheal disease in this section of the country and measured the potential for enteric infections in foreign individuals who entered the region.

The present communication is concerned with the prevalence of agglutinating antibodies to the more common salmonellae in the sera of US citizens and local Panamánian personnel hired for the canal feasibility studies in Darién.

Methods

Field Procedures. This investigation took place in Santa Fé, Darién Province, Route 17 Base Camp for the Atlantic-Pacific Interoceanic Canal Study Commission (A-PICSC), where feasibility studies for a possible sea-level canal in eastern Panamá were conducted, and in six subcamp sites throughout this region where personnel worked and lived while away from the base camp at Santa Fé. Detailed characteristics of the base camp, the living conditions of the personnel, and other pertinent information have been described previously.

The plan of study was to determine the presence of *Salmonella* antibodies and immunoagglutinin change, either in number or titer, in non-indigenous individuals engaged in the canal feasibility studies, before entering the route area, at three to four-month intervals while there, during any illness, and at termination of the work.

In addition, locally hired personnel, Indian and non-Indian residents of the Darién Province, and, where possible, some other persons not employed but living along Route 17, were to be similarly examined. The last two groups were thought of special interest and represented an excellent source of information on the *Salmonella* groups indigenous to the area.

Survey sera were collected from March 1966 through February 1968. The blood specimens were obtained from each individual by venipuncture and held for four hours at room temperature to permit the formation of a clot. The tubes were then refrigerated at 4°C. Once or twice a week the clotted specimens were sent by air to the Gorgas Memorial Laboratory in Panamá City where the sera were separated from the blood upon arrival. All sera were placed in sterile tubes and kept frozen until ready for serological testing.

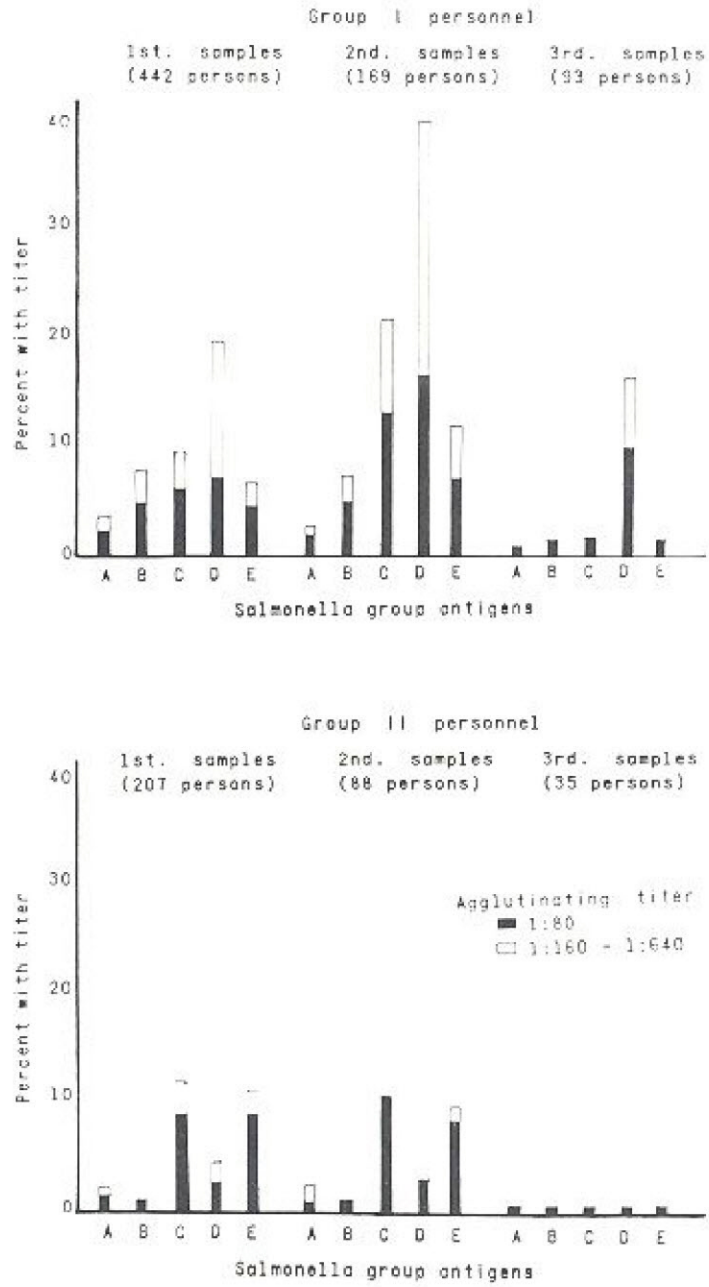

Fig. 1. Distribution of antibodies at titers of ≥ 1:80 to *Salmonella* group antigens in unpaired sera collected from A-PICSC personnel.

Individual serum samples were grouped into three cate-

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gories depending on the personnel status of the individual from whom the serum was obtained. Classification was as follows: *Group 1*, non-residents of Darién Province hired for the proposed canal project on Route 17. *Group 2*, residents of the Darién Province, Indians and non-Indians, hired for the project. *Group 3*, residents of the Darién Province not hired for the project, including Indians living within the area of Route 17.

Laboratory procedures. All sera were tested for the presence of *Salmonella* antibodies of groups A, B, C, D, and E (A–E). Multiple serum samples from the same individual were always tested together. Presence of the serum agglutinins was demonstrated by the standard tube agglutination test,[2] using commercially prepared *Salmonella* somatic group A–E antigens purchased from Lederle Laboratories, Pearl River, New York.

The test consisted of adding a constant amount of each of the *Salmonella* antigens to series of tubes containing two-fold dilutions of each serum. The tubes were shaken thoroughly and incubated overnight in a water bath at 50°C. The degree of agglutination in each tube was recorded and the titer of antibodies in the serum expressed as the reciprocal of the highest dilution giving definite agglutination. All titers were recorded but an agglutination titer of 1:160 or greater was considered positive in single specimens. In addition, a fourfold rise in antibody titer was required for significance in tests of serum pairs or in multiple specimens from the same individual.

A control tube containing antigen and saline only was always included with each unknown serum sample and had to show a negative result; a standard serum of known titer for each of the antigens used was assayed with each day's run of unknown sera. All serological findings were tabulated and analyzed according to the three categories employed in this paper.

Results

Antibodies to Salmonella antigen groups A–E were studied in a total of 1,158 serum samples collected from 649 A-PICSC personnel (Groups 1 and 2) hired for the canal project, and from 22 local residents (Group 3) in the area of Route 17. Many of these sample sets are incomplete, since there was a high turnover of the A-PICSC personnel. Table I shows the number of sera collected throughout the study period, extending from March 1966 through November 1968, for each of the three groups.

Antibody titers against *Salmonella* antigens in those samples of unpaired sera drawn three to four months apart from individuals of Groups 1, 2, and 3 ranged from 1:40 to 1:640. Although Group 1 had slightly more individuals with titers of 1:80 or greater to *Salmonella* antigen groups A–E than the two other groups, the data show generally low levels of antibody (1:80 or less) in the initial serum samples (Fig. 1). In the second sample series, antibody titers either remained the same or actually decreased for Group 2, but a relatively greater percentage of individuals in Group 1 exhibited higher antibody titers, particularly to *Salmonella* D (Fig. 1).

A lack of immunological response was characteristic of Group 3 sera. There was a marked absence of agglutinins at 1:80 dilution. When present, the titer was always less than 1:40.

TABLE I

SERA SAMPLES TESTED FOR AGGLUTINATING ANTIBODIES AGAINST SALMONELLA GROUP ANTIGENS, ROUTE 17, DARIEN PROVINCE, PANAMÁ MARCH 1966–NOVEMBER 1968

Bleeding No.	Number of Sera			Total
	Group 1	Group 2	Group 3	
1	442	207	22	671
2	169	88	8	263
3	93	35	1	129
4	55			55
5	27			27
6 & 7	11			11
Total	797	330	31	1158

When agglutinating titers of 1:160 or greater were examined in unmatched serum series, sera from Group 1 personnel revealed the presence of group D antibody at this dilution in all serial samples, while antibodies A, B, C, and E were detected only in the first two samples (Fig. 1). *Salmonella* antibodies were also detected at the 1:160 dilution in the first and second serum series of Group 2 personnel. Antibodies to C and E were most prevalent, but at relatively low frequencies (Fig. 1). Antibodies were not detected in Group 3 sera at this dilution.

Table II summarizes antibody titer changes to *Salmonella* antigens in paired and multiple sera from A-PICSC personnel and local residents. For Group 1, 21 (12.4 per cent) of 169 persons showed a fourfold rise in antibody titer. This response was primarily to *Salmonella*, group D. In Group 2 personnel, nine (10.2 per cent) of 88 individuals whose matched sera were tested showed titer rises for *Salmonella* group C and for *Salmonella* group E (Table II). No antibody rise was noted in Group 3 individuals but here the sample size was very small.

Discussion

Serological diagnosis by agglutination has been widely used in detecting the presence of brucellosis, leptospirosis, and enteric infections. As a diagnostic tool, it has limited value since it does not readily distinguish between past or present infections. Its main diagnostic value lies in detecting disease where isolation of the agent is difficult or has been missed. In this respect, it assists in the formation of a retrospective diagnosis of a recent illness.[3] The presence of agglutinins in a person's serum may be indicative of active or past infection, depending on the titer against a particular antigen. In some instances, a titer may indicate active infection, while in other cases the same titer may be meaningless because of previous vaccination, or because the individual comes from an area where the disease is endemic.[4]

Since agglutinins of variable titer may occur in normal sera, a positive agglutination test in our unmatched sera was considered to be of little significance, unless the titer was 1:160 or greater. Titer changes also were considered

TABLE II

ANTIBODY TITER CHANGES TO *SALMONELLA* GROUP ANTIGENS AMONG A-PICSC PERSONNEL, ROUTE 17, DARIEN PROVINCE, PANAMÁ MARCH 1966–NOVEMBER 1968

Personnel Group	Individuals Tested[a]	Individuals with Titer Rise[b]		No. Individuals with Titer Rise to *Salmonella* Group				
		No.	%	A	B	C	D	E
1	169	21	12.4		3	1	16	1
2	88	9	10.2			7		2
3	8	0						

[a]Paired or multiple sera from each individual was tested against five *Salmonella* group antigens.

[b]Fourfold or greater rise in agglutinating antibody

of assistance in interpreting the results of the agglutination tests. In sequential serum specimens from the same individual, fourfold changes in the titer were considered to be significant.

The present study revealed low levels of antibodies against *Salmonella* organisms most frequently encountered in enteric disease. Not only were serum titers of a low order, but the number of individuals with positive titers also was small, particularly in residents (Groups 2 and 3) of the Darién region (Fig. 1). On the other hand, in non-resident personnel (Group 1), the frequency of detectable antibody was higher and the proportion of titers greater than 1:160 was greater than in the two former groups (Fig. 1).

Thus, a low order of infection or of previous exposure to the more common salmonellae endemic in this area was suggested by our serological evidence. This is in agreement with recent bacteriological surveys, which demonstrated the presence of Salmonellae belonging to serogroups A, B, C, and D in A-PICSC personnel, and in small wild rodents captured around the vicinity of the canal route who were found to harbor *Salmonella* of serogroups B, D, and E.[5]

Medical histories of Group 1 personnel showed that most of them had been vaccinated with the typhoid-paratyphoid (TAB) vaccine. Titer rise in this group (Table II) may be interpreted as the result of previous vaccination with TAB vaccine before entering Darién Province. The D antigen is the main component of the typhoid bacillus used in the preparation of the vaccine. On the other hand, there is little to suggest that Darién residents (Group 2) have received the TAB vaccine (Ministry of Health records). That group D antibodies were not detected in sequential sera from local personnel (Table II) lends support to the previous statement. Also, Salmonellae belonging to some other serogroups could be prevalent or more common in this region. The serological response in Group 2 personnel was primarily to *Salmonella* group C, a bacterial group made up of strains unrelated to those utilized in TAB vaccines or present in the D group of the Salmonellae. Antibody titer rise to *Salmonella* group C and E antigens (Table II) and the presence of these antibodies at 1:160 or greater dilution in the unmatched sera of Darién residents, is of a diagnostic significance and may well be related to clinical or subclinical infection with Salmonellae of serogroups C and E in Group 2 personnel.

Finally, there may have been infections or contact with salmonellae other than those represented in the A–E antigen series utilized in the present study. These, if present, remained undetected serologically. A few rare *Salmonella* serotypes (*S. enteritidis* Ser Flint, Ser Bonaire, and Ser Wassenar) belonging to serogroup Z, (subgenus IV),[6] have been isolated from sporadic cases of salmonellosis in humans and from animals in the same area of the present study.[1,5]

Summary

This study determined the presence of *Salmonella* antibodies and immunoagglutinin change in non-residents and locally hired Indian and Non-Indian residents, employed as support personnel and laborers for the feasibility studies on a proposed sea-level canal in eastern Panamá.

Survey sera were collected from individuals before entering the route area, at intervals while there, and at termination of the work. Presence of the serum agglutinins against *Salmonella* group antigens A, B, C, D, and E was demonstrated by the standard tube agglutination test.

The study demonstrated an endemic area of salmonellosis of a low order of magnitude in eastern Panamá and an exposure to some of the more common *Salmonella* organisms among the foreign and local personnel engaged in the Canal study.

In unmatched serum series, sera from non-residents revealed the presence of *Salmonella* group D antibody in all serial samples, while antibodies of groups A, B, C, and E were detected only in the first two samples. In serum series of locally hired personnel, antibodies to group C and E were most prevalent but at low frequencies.

In paired and multiple sera from residents and non-residents, 21 (12.4 per cent) of 169 non-residents showed a fourfold rise in antibody titer; this response was primarily to *Salmonella* group D antigen. In locally hired personnel, nine (10.2 per cent) of 88 individuals showed titer rises for Salmonella group C and group E antigens.

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