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Botulism in the Americas

Botulism is a serious intoxication caused by the ingestion of foodstuffs contaminated with neurotoxic poisons produced by *Clostridium botulinum*. As a rule, the contamination is associated with the preparation or inadequate storage of home-canned foods and defects in the industrial processing of canned foods for commercial consumption. Although the disease occurs very sporadically and very infrequently in the Americas, at the present time an outbreak of botulism can be a local clinical and public health emergency. Adequate protection of the population depends on a rapid epidemiological and environmental investigation to identify the source of the toxin and to take immediate control measures. In the last 30 years, early diagnosis and treatment of the disease have substantially changed the botulism case fatality rate even though clinical diagnosis of the disease is sometimes difficult; the shorter the incubation period, the more serious the disease and the higher the case fatality rate, since it means that a larger amount of toxin has been ingested.

Data on cases or suspect cases of botulism are available for Argentina, Brazil, Canada, Chile, and the United States. Most of them are sporadic cases or cases among members of the same family. They are associated with home-prepared or home-canned foods (68 per cent in the United States in 1970-1977) or with industrially-processed commercial foodstuffs (5.5 per cent only in the United States). Botulism occurs in the following forms: food poisoning, infection of wounds, and infant botulism. This classification is important since, in the first form, the toxin produced in the foods is ingested, whereas in wounds and in infant botulism the neurotoxin is produced in vivo by the growth of the bacillus in the infected tissues or in the intestine. Food poisonings predominate in adults and affect women more frequently.

In Argentina (Figure 1) 139 cases of botulism were reported in a 15-year period (1967-1981), nearly always due to food poisoning. The Provinces of Mendoza, Río Negro, La Pampa, and Buenos Aires were the most affected. The nine cases detected in 1979 in Mendoza Province were caused by botulism toxin type A; the outbreak was associated with the consumption of home-canned vegetables.

In Brazil suspected cases of botulism occurred in 1958 when six persons belonging to the same family in the State

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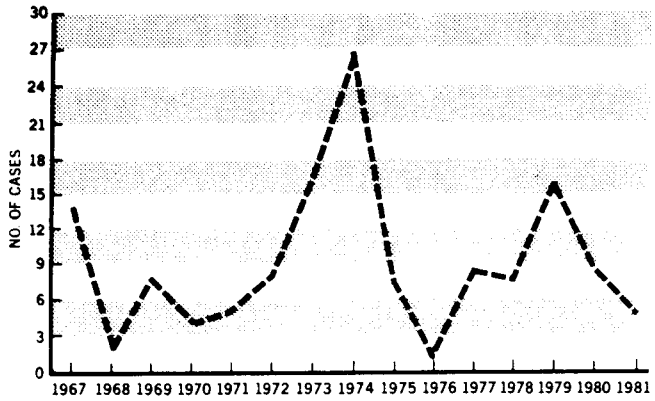
Figure 1. Notified cases of botulism in Argentina, 1967-1981.



of Rio Grande do Sul died and others became ill after eating home-canned boiled fish. In 1981 two suspected cases in Rio de Janeiro were associated with the consumption of industrially prepared chicken pâté.

In Canada one case of botulism was reported in 1979 and was the first case of infant botulism to occur in the country. In 1980 there were three cases of botulism, all in the Province of Quebec. In the first and second case, toxin E was isolated from the patients' serum. Between 1979 and 1980 a total of 15 incidents were investigated as suspected cases of botulism.

In the United States 766 outbreaks of botulism were reported in the period 1899-1977 and caused 1,961 cases. Of these cases, 680 occurred between 1950 and 1977. Figure 2 shows the number of cases and deaths between 1960 and 1980. Of the 45 states that have reported cases since 1899, five are in the western region of the country (California, Washington, Colorado, Oregon, and New Mexico) and reported more than half the cases. Of the total outbreaks between 1899 and 1977, 26.0 per cent were caused by toxin type A, 7.8 per cent by type B, 4.2 per cent by type E, 0.1 per cent by type F, and 0.1 per cent by types A and B combined. Between 1970 and 1977 it was possible to identify the toxin involved in 84 per cent of the cases. Table 1 shows the principal types of foodstuffs that have caused outbreaks of botulism in the United States.

Infant botulism was identified as a separate clinical entity in the United States in 1976. The disease begins with constipation, which is rapidly followed by a neuromuscular paralysis that begins in the cranial nerves and progresses toward the peripheral and respiratory muscles. Cases have been reported in the States of California (37), Pennsylvania (4), Utah (4), and to a lesser extent in Washington, Arizona, Colorado, Montana, Nevada, New Jersey, New York, North Dakota, Oregon, Tennessee, Texas, and Wisconsin.

The diagnosis of botulism is one of the most important aspects in identifying the disease and is established by confirming the presence of botulin toxin in the serum and isolating *C. botulinum* in the suspected food, vomit, gastric contents, or feces of the patient. Other food poisonings produced by toxins such as *Staphylococcus aureus* or *Clostridium perfringens,* salmonellosis or shigellosis, poisonings due to atropine or mushrooms of the genus *Amanita,* and heatstroke can be confused with botulin poisoning.

The administration of antitoxin as a prophylactic measure to asymptomatic persons who have ingested contaminated foods should be carefully weighed since the antitoxin is prepared from horse serum and can cause anaphylactic shocks. For these patients it is recommended that gastric lavages be given, vomiting be induced, or purges with cathartics be administered to eliminate the unabsorbed toxin and that they be kept under strict surveillance.

Before treatment is begun, it is recommended that 20 ml of serum and 50 g of the feces of each person that has ingested contaminated foodstuffs be collected. The specimens should be placed in an unbreakable and sterile container identified with a label that includes the date and the name, age, and sex of the patient. The container should be placed in a water-proof bag packed in ice or in another coolant (never in dry ice) and sent to the laboratory immediately.

Specific treatment consists of the intravenous and intramuscular administration of botulin antitoxin. The efficacy of the treatment is increased by early administration of antitoxin and—as certain studies suggest—its combination with the administration of antibiotics when there are associated infections such as those of the respiratory and urinary systems. Antitoxin is produced in the Mal-

Figure 2. Botulism (foodborne). Reported cases and deaths by year, United States, 1960-1980.

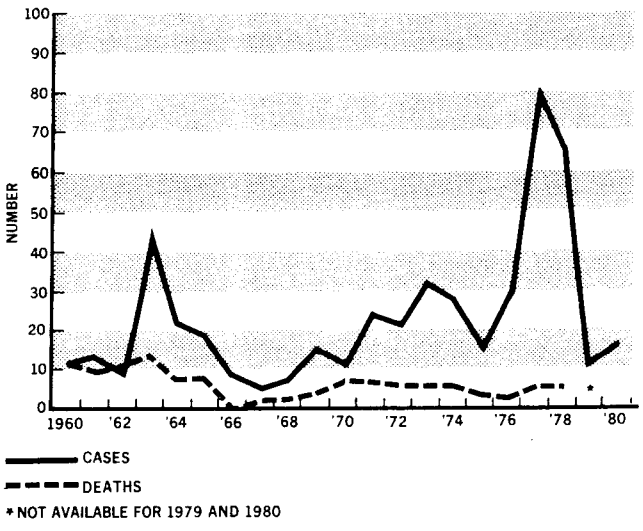


Table 1. Foodstuffs causing botulism outbreaks in the United States of America, 1899-1977.[a,b]

Type of botulin toxin	Vegetables	Fish and fish products	Fruit	Dressings[c]	Beef[d]	Milk and milk products	Pork	Poultry	Other[e]	Unknown[f]	Total
A	115	11	22	17	6	3	2	2	8	9	195
B	31	4	7	5	1	2	1	2	3	3	59
E	1	25							3	1	30
F					1						1
A&B	2										2
Unknown[e]	2	1		1						6	10
Total	151	41	29	23	8	5	3	4	14	19	297

[a]For the period 1899-1973, only outbreaks in which the type of toxin was confirmed are included and, for 1974-1977, all outbreaks.
[b]Prepared by the Centers for Disease Control, Atlanta, Georgia, USA.
[c]Includes outbreaks caused by ketchup, chili, hot sauce, and salad dressings.
[d]Includes one type F outbreak due to venison and one A outbreak due to mutton.
[e]Categories added for the period 1974-1977.

brán Institute (Argentina), Connaught Laboratories (Canada), the Pasteur Institute (France), and the Butantan Institute (Brazil).

The CDC operate as the reference agency for the United States, provide advice on treatment, distribute the antitoxin, and recommend the following procedure:[1]

After an appropriate skin test, administer 1 vial of trivalent antitoxin intramuscularly and 1 vial by the intravenous route. If the patient remains stable or improves, do not administer any more antitoxin. If the disease progresses, after 4 hours administer 2 further vials as indicated above. A larger amount should not be administered to the same patient. The antitoxin administered by the intramuscular route is slowly released over a period of weeks and helps to neutralize any toxin additionally absorbed by the gastrointestinal system. Each trivalent vial contains:

- Type A—7,500 international units, equivalent to 2,698 U.S. units.
- Type B—5,500 international units, equivalent to 2,075 U.S. units.
- Type E—8,500 international units, equivalent to 8,500 U.S. units.

The use of antibiotics is indicated in the treatment of infectious complications such as those of the respiratory or urinary systems. The laboratories that distribute the antitoxins should use the most rapid means available for dispatching the product and inform the health service of the mode of transportation and the date of arrival. All the patients should be kept under strict medical surveillance while their vital capacity is being evaluated and measured. The use of tracheotomy should always be considered for patients with a respiratory disability, which calls for continuing surveillance and the recording of the respiratory function.

The U.S. Public Health Service has succeeded in reducing the botulism case fatality rate (from 60 per cent in 1899-1949 to 15.7 per cent in 1970-1977) through appropriate patient treatment.

In Latin America problems that impede the control of botulism outbreaks may occur. They include delay in diagnosis due to the shortage of specialized laboratories; inadequate collection, storage, and dispatch of specimens, and lack of knowledge of the specific treatment on the part of health services. In Argentina and the United States useful guidelines for the control of botulism have been prepared. It should be emphasized that a reduction in botulism mortality is primarily obtained through early diagnosis and appropriate medical care of the patient.

(*Source:* Epidemiological Surveillance, Division of Disease Prevention and Control, PAHO.)

[1]Centers for Disease Control: *Botulism in the United States, 1899-1977, Handbook for Epidemiologists, Clinicians, and Laboratory Workers,* May, 1979.

Diseases Subject to the International Health Regulations

Cholera, yellow fever, and plague cases and deaths reported in the Region of the Americas as of 31 August 1982.

Country and administrative subdivision	Cholera cases	Yellow fever		Plague cases
		Cases	Deaths	
BOLIVIA	–	*93*	*34*	*1*
Beni	–	1	–	–
Cochabamba	–	2	–	–
La Paz	–	2	2	1
Santa Cruz	–	88	32	–
BRAZIL	–	*20*	*20*	*39*
Bahía	–	–	–	1
Ceará	–	–	–	32
Maranhão	–	4	4	–
Mato Grosso	–	1	1	–
Mato Grosso do Sul	–	13	13	–
Pará	–	2	2	–
Pernambuco	–	–	–	6
COLOMBIA	–	*1*	*1*	–
Cundinamarca	–	1	1	–
PERU	–	*8*	*8*	*11*
Loreto	–	6	6	2
Madre de Dios	–	2	2	–
Piura	–	–	–	4
San Martín	–	–	–	4
Ucayali	–	–	–	1
UNITED STATES	*1*	–	–	*4*
Arizona	–	–	–	2
California	1	–	–	–
New Mexico	–	–	–	1
Texas	–	–	–	1

– None.

Rabies in Valle del Cauca, Colombia

Since August 1980 there has been a marked increase in the number of cases of animal rabies in the Department of Valle del Cauca. The municipalities in the south are the most affected by this outbreak, which up to the end of 1981 continued with high levels of incidence despite the efforts made to control it. Data on the outbreak, considered to be of major importance to health workers, are presented below. A period of five years is examined plus added data for 1981.

During 1976-1980, a total of 279 cases of animal rabies were reported in the Department of Valle del Cauca and confirmed by the Virology Laboratory of the Universidad del Valle, in Cali (Table 1).

An examination of the data for the early years of the period shows an increase in the number of cases every two years and an abnormal situation in 1980 (marked by the average monthly increase in cases beginning in September).

In 1981 the number of cases was twice that of the peak year of the five-year period 1976-1980, and 1.3 times higher than in the five previous years (Figure 1).

Rabies was present in 1976-1980 (60 months) in 12 of

Table 1. Cases of animal rabies in the Department of Valle del Cauca, Colombia, 1976-1980.

Year	1976	1977	1978	1979	1980	Total	%
Dogs	14	44	19	39	139	255	91.4
Cats	0	3	1	5	11	20	7.2
Rats	0	0	1	0	2	3	1.1
Goats	0	0	0	1	0	1	0.3
Total	14	47	21	45	152	279	100

the 42 municipalities in the Department of Valle del Cauca (Figure 2). The municipalities that had the largest number of months with cases of rabies were: Cali, 37 months (61.7 per cent), Palmira, 16 (26.7 per cent), and Yumbo, 12 (20.0 per cent).

Canine rabies showed a tendency to persist with variations every two years in the first four years of the five-year period. The increase in the rate in 1980 was due to an outbreak that began in the second semester.

The Virology Laboratory of the Universidad del Valle reported a total of 387 examinations positive for rabies in animals from the Departments of Valle del Cauca (279)

Figure 2. Percentage of months with cases of rabies, Department of Valle del Cauca, Colombia, by municipalities, 1976-1980 (60 months).

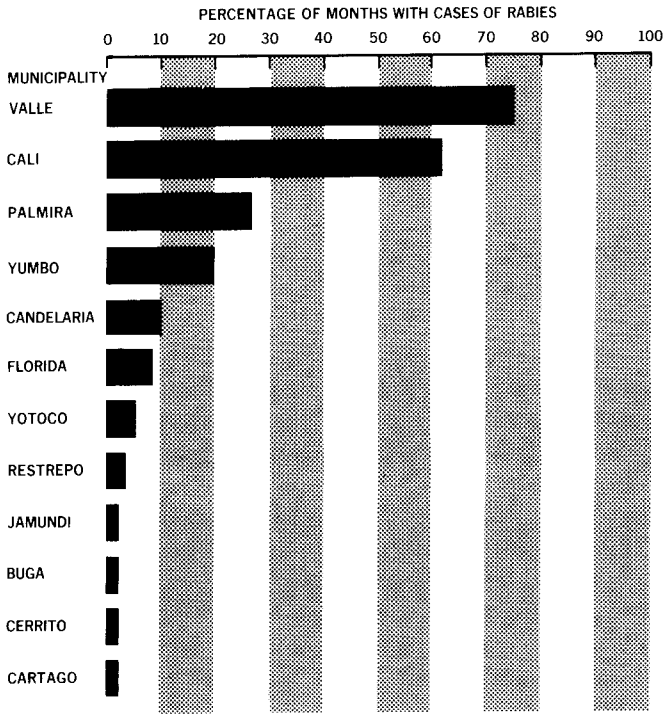
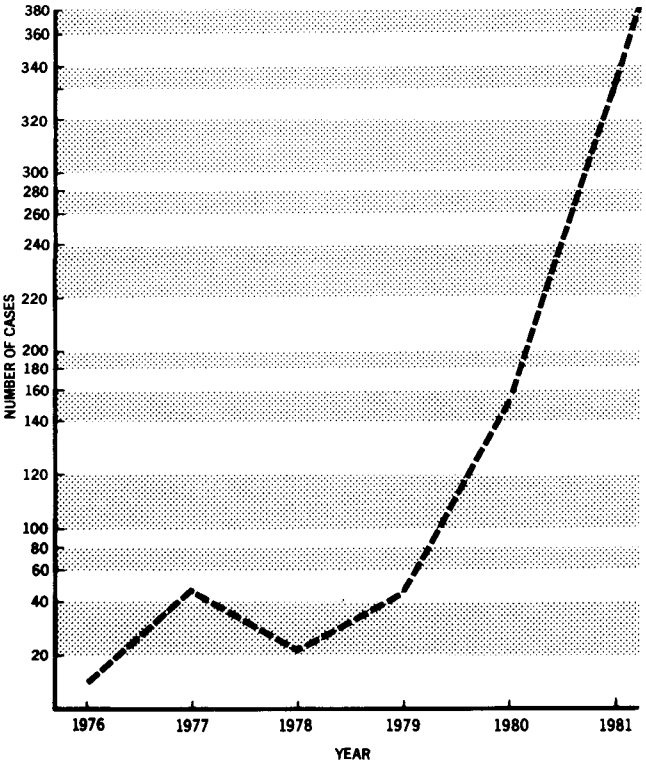


Figure 1. Cases of rabies in animals, Department of Valle del Cauca, Colombia, 1976-1981.



and Cauca (108). The positivity rate of the specimens was determined by the Seller test (78 per cent), by immunofluorescence (11.4 per cent), and by inoculation (10.6 per cent).

The diagnostic data are interesting to note, since the persons responsible for treating patients exposed to rabies must be aware that the direct test (Seller) is not definitive and that, in some cases, immunofluorescence can be negative. The inoculation test confirms the results, between 10 and 15 days after dispatching the specimen to the laboratory.

The human mortality caused by rabies, reported between September 1979 (the last cases had occurred in 1972) and December 1981, was 9 deaths: 6 in men and 3 in women. Seven of the deceased were between 5 and 15 years of age (Table 2).

In 1981 animal rabies occurred in 14 of the 42 municipalities located in the flat region of the Department and from its center toward the south. The most affected municipalities were Cali (with cases reported in each of the 12 months) and Palmira (in 9 out of the 12 months). A total of 382 cases were reported: 362 in dogs, 18 in cats, 1 in a horse, and 1 in a rat.

Table 2. Reported deaths from human rabies in Valle del Cauca, September 1979-December 1981.

Date of death	Age (years)	Sex	Origin
September 22-79	12	M	Yumbo
December 12-80	8	M	Cali
April 9-81	11	M	Cali
April 16-81	13	M	Cali
April 26-81	8	F	Cali
May 4-81	49	F	Cali
June 21-81	13	M	Cali
July 21-81	12	M	Florida
August 10-81	4	F	Cali

The number of cases each month ranged between 12 and 49, with a monthly average of 31.8 cases, which represents about one case of rabies a day. The highest case frequency was observed in March and the lowest in October. The municipalities with the greatest number of cases were: Cali (73.0 per cent), Palmira (6.3 per cent), Yumbo (5.2 per cent), and Florida (3.6 per cent).

It should be emphasized that, of the 239 dogs positive for rabies investigated in Cali in the first eight months of 1981, a total of 116 (48.5 per cent) had been vaccinated; the remaining 123 (51.5 per cent) had not been vaccinated.

(Source: Boletín Epidemiológico,
Health Service of Valle del Cauca, Colombia,
Year 7, number 1, 1981.)

Editorial Comment

The Department of Valle del Cauca is one of the most economically developed geographic areas of Colombia. The occurrence of rabies in the Department took on epidemic proportions in 1980 and 1981, with 152 and 382 cases of animal rabies, respectively. The slow but continuing increase of rabies in cats warrants attention. Almost all the human and animal cases were concentrated in localities situated in the valley itself; for unknown reasons, no cases were reported in large communities located outside the flat region of Valle—such as Buenaventura, Caicedonia, and Sevilla. The Department had not reported any cases of human rabies since 1972.

The comments and observations that appear in the report on the diagnosis and treatment of persons bitten, based on the direct test (Seller), immunofluorescence, and the inoculation test, are significant. According to the Sixth Report of the WHO Expert Committee on Rabies (WHO Technical Report Series 523, 1973), in the hands of a competent technician, the fluorescent antibody test is at present the best method available for the rapid diagnosis of rabies. However, the Committee itself notes that the microscopic examination of the Negri bodies in the brain tissue, the isolation of rabies virus in tissue specimens, and, when necessary, the corroborative test of the neutralization of the virus by serum are becoming very important as laboratory methods for the diagnosis of rabies.

Some of the most important rabies control methods should be recalled:

1. Increase in epidemiological surveillance of the disease, including collection and analysis of data, especially on morbidity in domestic animals and man, as well as information on animal bites, on vaccinations administered to the susceptible population, and postvaccinal reactions. The heads of suspect animals should be appropriately dispatched to the laboratory.
2. Immediate epidemiological investigation both of the suspected persons and of the animals bitten in order to determine the prophylactic measures to be taken. In most human cases of rabies, the inoculation period is 3-8 weeks after exposure. Immediate and adequate treatment of all bites and scratches that may be infected with rabies virus is of vital importance. The health authorities should also be aware of the recommendations on vaccination and treatment of the WHO Expert Committee on Rabies in its Sixth Report.
3. General vaccination of dogs and cats. (The increase in cat rabies noted in Valle del Cauca, Colombia, may pose a serious problem which requires the inclusion of cats in vaccination programs. Since the beginning of the 1970s, vaccinated dogs and cats have been identified by means of colored collars in the Department of Valle.)
4. Elimination of stray dogs, especially in the event of outbreaks of rabies.
5. Continuous training of persons responsible for rabies control programs.
6. Epidemiological surveillance of the wildlife population (in particular mammals, carnivores, and primates) is unfortunately not possible at present in many geographic areas of the Americas. However, this situation is not identical in all countries and may change. The best way of monitoring the problem is to systematically examine the brains of animals, such as foxes, coyotes, raccoons, etc., found dead or behaving in an abnormal way.
7. The principal methods employed to control rabies in bats *(Desmodus rotundus)* by elimination of the vampire, include the use of anticoagulants on cattle or captured vampires so that these vampires can then contaminate their peers.

Isolation of Pathogenic Enterobacteria in Children with Acute Diarrhea, Argentina, 1971-1978

Between 1971 and 1978 a total of 4,229 stool cultures were made on children with acute diarrhea who were being treated in hospitals in Buenos Aires and its environs. The ages of the children ranged from the first days of life to five years. Specimens were collected by means of a rectal swab before the administration of any treatment with antimicrobial drugs, and the material was processed within three hours of its collection. For the isolation and biochemical and serological identification of the bacteria, methods developed in recent decades for the culture of aerobic and anaerobic bacteria were used. The antigenic structure of *Salmonella, Shigella,* and infantile enteropathogenic (IEP) *Escherichia coli* was determined by means of immune sera prepared by the Carlos G. Malbrán National Institute of Microbiology.

Invasion of the intestinal epithelium is the means by which *Salmonella, Shigella non dysenteriae,* and *E. coli* enteroinvasive serotypes penetrate and damage the epithelial mucosa, thus provoking the secretion of water and electrolytes, which leads to diarrhea. *E. coli* also produce enterotoxins but the modus operandi of IEP *E. coli* is not yet known. The above-mentioned serotypes were the first involved in infant diarrheas. Some acted by an invasive mechanism similar to that mentioned for *Salmonella* and *Shigella* and others by the production of enterotoxins. Some authors discuss their etiological importance since they do not find any relationship with either of those two mechanisms.

With respect to potentially pathogenic germs *(Staphylococcus aureus, Pseudomonas aeruginosa, Providence,* and *Aeromonas)* their incidence is considered since they are not normal constituents of the intestinal flora. During episodes of acute diarrhea, the ecological imbalance produced in the intestinal flora favors adhesion and colonization by these opportunist bacteria. When the physical conditions of the host are compromised, as in the case of malnourished children or children with deficient defense mechanisms, these microorganisms may spread to other sites. Although their pathogenic role in acute diarrhea is not yet accurately known, some of their biological characteristics could implicate them as causes of bacterial infectious diarrheas.

Staphylococcus aureus can induce secretion of fluids and electrolytes by means of two mechanisms: inflammation and production of enterotoxins.

Five enterotoxins of *S. aureus* (A, B, C, D, and E) have been described, and many of them have been incriminated in food poisonings. In the United States it has been reported that between 69 and 75 per cent of the outbreaks are related to A or A and D toxins. Specifically, enterotoxin B induces a secretory mechanism in the small intestine of rats and leaves the absorption mechanism intact.

The approach that regards a strain of staphylococcus that produces coagulase as pathogenic is not always valid since, as Breckinridge and Bergdoll[1] point out, in a case of food poisoning, a coagulase-negative enterotoxin producing *Staphylococcus* was isolated.

Pseudomonas aeruginosa is also capable of producing enterotoxins with a capacity to induce an accumulation of fluid in the small intestine of rabbits; these are apparently different from other *Pseudomonas* toxins (hemolysin, protease, lecithinase, or lethal toxin).

Foremost among the bacteria that play an important role in acute diarrheas are the *Enterobacteriaceae,* some members of the genus *Vibrio* and the potentially pathogenic group. The importance of bacterial toxins is also known since some of them are produced by microorganisms so far considered part of the "normal flora," for example, *E. coli* that does not belong to the IEP group and *Klebsiella pneumoniae.* In recent years the existence of a new group of viruses known as "rotavirus" or "duovirus," which are responsible for many of these diarrheal diseases, has been demonstrated.

This article presents the results of the isolation of enterobacteria traditionally considered enteropathogenic—*Salmonella, Shigella,* and IEP *E. coli*—isolated from children with acute diarrheal diseases. At the same time the potential enteropathogens and the associations between the two groups were studied.

Figure 1 presents the comparative percentages of isolations of *Salmonella, Shigella,* and IEP *E. coli* in the period 1968-1978. The genus *Salmonella* predominates, with values ranging between 20 and 30 per cent in the period 1971-1976 although a downward trend is to be noted in the following two years in which the figures fell to 13.6 and 11.2 per cent. Next in importance is the IEP *E. coli* group, with marked annual fluctuations, which range between 6.1 and 18.4 per cent. In the main, the genus *Shigella* shows a low incidence and a steady range of between 4.8 and 6 per cent in 1972-1976 although subsequently there was an increase of 10.7 and 8.7 per cent, respectively.

Taking the three genera together, an etiological agent was detected in 40 per cent of the stool cultures analyzed

[1]Breckinridge, J. C., and M. S. Bergdoll. Outbreak of foodborne gastroenteritis due to a coagulase-negative enterotoxin producing *Staphylococcus. N Eng J Med* 284:541, 1971.

Figure 1. Percentages of pathogenic enterobacteria isolated from children with acute diarrhea, Argentina, 1968-1978.

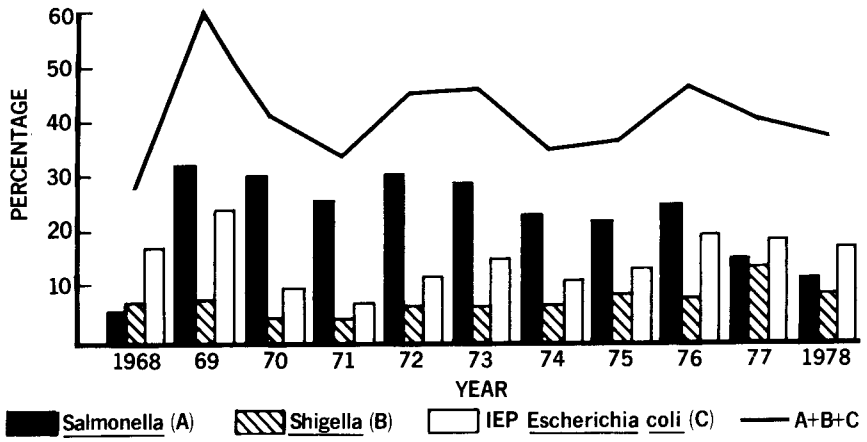
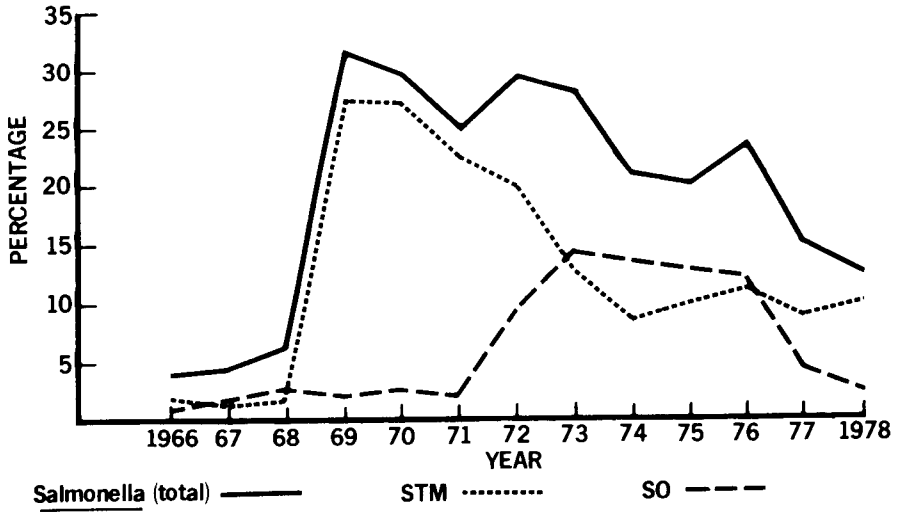


Figure 2. Annual evolution, in percentages, of *Salmonella typhimurium* (STM) and *Salmonella oranienburg* (SO) isolated from stool cultures, Argentina, 1966-1978.



since 1970. Up to 1968, the group most frequently involved was IEP *E. coli,* which peaked in 1969 with 24.3 per cent and in subsequent years did not exceed 18.4 per cent. In 1969, the peak level (60 per cent) of isolations of enteropathogens was reached, due to an increase in the incidence of the IEP *E. coli* group, and *Salmonella* (32 per cent), which up to 1968 had remained below 6 per cent.

An analysis of the results of *Salmonella* isolations in 1969 showed that 84.7 per cent of the strains represented solely the serotype *S. typhimurium* (STM). This predominance, which persisted up to 1971, when STM represented 93.3 per cent of all *Salmonella,* was due to an epidemic outbreak that spread to various areas of Argentina. All the health centers in which acute diarrheal diseases were investigated—for example, in Tucumán, Rosario, and the Province of Buenos Aires—reported similar results that agree with the observations published by WHO[2] concerning the prevalence of that serotype in the world in both humans and animals.

Beginning in 1972 isolations of the STM serotype began to decline while the findings of another serotype *S. oranienburg* (SO) gradually increased and in the years subsequent to 1976 exceeded those of STM. Figure 2 shows the annual changes in STM and SO in percentages of isolations from the stool cultures analyzed. In 1976 there was

[2]Surveillance of *Salmonella* other than *S. typhi* and *S. paratyphi,* 1971. *Weekly Epidem Rec* 48:385-388, 1973.

Table 1. Annual distribution of the principal serotypes of *Salmonella, Shigella,* and infantile enteropathogenic *Escherichia coli.*

Year	1971	1972	1973	1974	1975	1976	1977	1978	Total	%
No. of stool cultures	884	452	583	567	469	461	411	402	4,229	
Serotypes:										
Salmonella									942	22.27
S. typhimurium	211*	91	72	44	37	42	26	32		
S. oranienburg	7	36	82	73	50	54	20	8		
Other	4	6	9	5	7	11	10	5		
Shigella									232	5.48
Sh. flexneri	22	17	24	22	19	23	36	29		
Sh. sonnei	2	4	2	7	3	5	8	6		
Other	0	1	0	0	2	0	0	0		
IEP *Escherichia coli*									517	12.22
0111:B_4	24	34	70	33	40	47	36	25		
0119:B_{14}	15	10	4	2	1	10	12	15		
055:B_5	4	0	2	10	7	11	13	11		
Other	11	5	6	9	5	17	8	20		

*The figures correspond to the number of strains isolated.

a reversal of the situation with SO decreasing while STM again predominated, although the total figure for *Salmonella* tended to decrease.

Table 1 shows the annual distribution of the most frequent serotypes of *Salmonella, Shigella,* and IEP *E. coli.* Of the four subgroups of *Shigella* investigated, that of *Sh. flexneri* was most frequently found, serotype 2 and *Sh. sonnei* predominating. The remainder were *Sh. boydii,* since no *Sh. dysenteriae* were isolated.

With respect to IEP *E. coli,* the 0111:B_4 was the most frequent serotype followed by 0119:B_{14} and 055:B_5. Smaller proportions of the remaining serotypes were found.

With respect to *Salmonella* the serotypes most frequently obtained were STM and SO while others, like *S. typhi, S. panama, S. anatum,* and *S. newport,* were less frequent.

Table 2 presents the findings of potentially pathogenic bacteria. As may be seen, the values of *Pseudomonas* are significant, high in the first three years and with a tendency to decrease up to 1976. From 1977 their proportion increased to 12.6 per cent. It is noteworthy that in the years in which there was an increase in the isolations of *Pseudomonas,* the increase was correlated with a decrease in the findings of *S. aureus* and that over a period of eight years the average values were similar for the two genera.

Table 2. Annual prevalence of potentially pathogenic bacteria in children with diarrheal diseases.

Year	No. of stool cultures	*Staphylococcus aureus* %	*Pseudomonas* %	*Providence, Aeromonas,* and others %	Total
1971	884	3.61	14.59	1.35	19.55
1972	452	7.07	28.98	1.54	37.59
1973	583	5.66	12	1.88	19.54
1974	567	10.75	7.05	1.94	19.74
1975	469	17.05	8.31	4.67	30.03
1976	461	16.26	9.54	2.38	28.18
1977	411	17.27	10.94	4.86	33.09
1978	402	13.68	12.68	3.48	29.85
Total	4,229	10.38	12.39	3.14	25.91

The genus *Aeromonas* has recently been incriminated as the causal agent of acute diarrheas in Asian countries.[3] It has been demonstrated that 11 strains of *Aeromonas* isolated from children with diarrhea in Ethiopia produced enterotoxins. Our data show a low incidence of this group and of the *Providence* group.

S. aureus fluctuated between 3.6 and 17.2 per cent of the total stool cultures analyzed. If the figures for *Providence* and *Aeromonas,* which are potentially pathogenic, are added, the total figure is 25.9 per cent for this group of microorganisms.

The frequency of the simultaneous isolations of the enterobacteria traditionally considered pathogenic and those considered potentially pathogenic is of interest. Table 3 shows that the general average of combinations which include two or three strains is 12.2 per cent. Of this proportion, more than half (6.2 per cent) is due to the concurrence of one enteropathogenic and one potentially enteropathogenic bacterium, followed by the association of two enteropathogens (3.3 per cent). The data so far presented are for the total strains isolated in the stool cultures made.

Table 4 presents an analysis of the prevalence of the two groups of pathogenic and potentially pathogenic bacteria in the children studied. The differences between these and the foregoing figures are due to the associations

[3]Ljunch, A., M. Popoff, and T. Wadstrom. *Aeromonas hydrophila* in acute diarrheal disease. *J Clin Microbiol* 6:96-100, 1977.

Table 3. Annual distribution of associations of pathogenic enterobacteria and potentially pathogenic bacteria in children with diarrheal diseases.

Year	No. of stool cultures	Percentage of associations of two bacteria a	b	c	of three bacteria d	Total a + b + c + d
1971	884	1.9	6.56	0.57	0.75	9.84
1972	452	3.54	7.96	1.55	2.65	15.70
1973	583	5.32	6.35	1.37	1.37	14.41
1974	567	1.94	3.88	0.35	1.23	7.41
1975	469	2.98	5.97	1.91	1.70	12.57
1976	461	6.07	6.72	1.30	0.65	14.74
1977	411	3.64	5.11	1.46	4.86	15.08
1978	402	2.48	6.96	0.74	1.24	11.44
Total	4,229	3.35	6.17	1.09	1.65	12.27

a) Between two recognized pathogens.
b) Between one recognized and one potential pathogen.
c) Between two potential pathogens.
d) Between three bacteria with possible combinations of pathogens and potential pathogens.

Table 4. Prevalence of pathogenic and/or potentially pathogenic agents in children with diarrheal diseases.

Year	No. of stool cultures	With pathogens	With potential pathogens	Total
1971	884	32.23	10.86	43.1
1972	452	39.82	19.69	59.51
1973	583	39.96	10.81	50.77
1974	567	32.26	13.77	46.03
1975	469	32.62	20.25	52.87
1976	461	46.63	19.95	63.58
1977	411	35.03	25.30	60.34
1978	402	34.57	20.39	54.97
Total	4,229	35.72	16.31	52.04

of bacteria in one and the same child. More than 35 per cent of the patients with acute diarrheal diseases presented enteropathogenic germs. If the children in which potentially pathogenic bacteria were found are also taken into consideration and if these bacteria actually cause diarrhea, then in half the children (52 per cent) the diarrheal symptoms could be attributed to a bacterial agent.

It should be emphasized that there is a need to complete this study with research into the true function of potentially pathogenic bacteria in the pathology of diarrheal diseases, since their mere presence in a stool culture does not prove a cause and effect relationship.

(*Source:* Eiguer, T., N. Binsztein, and G. Spizzamiglio, Enterobacteria. *Archivos Argentinos de Pediatría* 78 (3): 354-362, 1980; *Boletín Epidemiológico Nacional,* Publication of the Office of National Disease Prevention and Control, and *Vigilancia Epidemiológica,* Ministry of Public Health and the Environment, Argentina, No. 9, 1981.)

Editorial Comment

Laboratory work is one of the fundamental aspects of epidemiological surveillance. The organized collection and prompt dispatch of specimens to laboratories must be the objective of day-to-day practice. The isolations made should be regularly analyzed so the services that treat children with acute diarrhea can do so on the basis of a better knowledge of the agent, which makes for better decisions in the diagnosis, treatment, and prevention of this group of diseases.

The epidemiological use of the laboratory, as is being done in Argentina at the Malbrán Laboratory, is a rational approach to the utilization of this valuable resource. It is also important to increase the exchange of information among countries of the Region.

Social Factors in Malaria Transmission and Control

The Working Group on Social Sciences Applied to Health of the PAHO Advisory Committee on Medical Research (ACMR) has prepared, in accordance with the mandate assigned to ACMR, a *Latin American Bibliography of Social Sciences Applied to Health.* It has also made a study of the social and economic factors that influence malaria transmission and control, in which it pinpoints those that are conducive to more efficient organization and planning of malaria control programs.

The recommendations of the Working Group, which were included in the ACMR report (Caracas, April 1982), cover the following:

- Review of the plan of work for future activities.
- Establishment in PAHO of a program of research on social sciences applied to malaria.
- Establishment of a technical advisory group in this field.
- Convocation of a meeting to discuss economic and social factors relating to malaria.
- Publication of a summary of the social and economic factors relating to malaria transmission and control.
- Appointment of a specialist in social sciences for the WHO Expert Committee on Malaria.

The report of the Working Group is summarized below.

Present Status of Malaria Programs

In the past two decades, domiciliary spraying with residual insecticides has eliminated endemic malaria in 40 per cent of the originally malarious area and in 75 per cent of the human population at risk. The price paid for the spectacular success obtained through comprehensive coverage with insecticides and antimalaria drug administration in some areas has been insecticide resistance of the vector and drug resistance of the plasmodia.

One of the main causes of the failure of the malaria control program in some rural areas is the lack of appropriate human and economic resources, especially at the local level, for ensuring the continuity of activities, in addition to the absence of a comprehensive approach to the problem. The planning and evaluation of malaria control measures have not taken into account the relationships between environmental conditions, induced changes, the sociocultural characteristics of the population, the level of socioeconomic development, and the biological factors involved in the transmission of the disease, all of which are fundamental elements of the epidemiological method.

In 1969 the World Health Assembly recommended a strategy that called for better adaptation to local conditions and needs, and for broader skills in malariologists in order to enable them to better identify epidemiological problems and their magnitude and thus apply technology most appropriate to local conditions utilizing available resources. However, many factors are still hindering the development of malaria control programs which are based on new approaches and structures, and the gaps in scientific knowledge have precluded the application of different strategies.

Many countries are facing a serious malaria problem; some are endeavoring to make good use of the scanty means available, but none can afford to wait for more efficient technologies to emerge.

The social sciences have made increasing and substantial contributions to our knowledge of health problems, and WHO has incorporated them into its training programs, including those in applied research, since 1948. Since the 1950s social sciences have also been included in PAHO programs. Nevertheless, few attempts have so far been made to comprehensively and systematically analyze the social and economic factors that can influence malaria transmission and control.

Malaria eradication programs have been assigning special attention to the evaluation of malaria control measures. In some cases, these evaluations have shown a marked deterioration in the malaria situation due to the difficulty of ensuring the continuity of the programs. Accordingly, the present approach of control programs attempts not only to reach the goals but also to maintain the achievements made, even if this means that the pace is slower.

The purpose of stratification in malaria control is to localize the pathological phenomenon and identify its epidemiological interactions. Stratification studies may begin at the local level, go on to encompass an entire area, and subsequently reach the national level. This process may proceed in the reverse order or simultaneously. The complexity of epidemiological stratification will vary according to the geographic variability of malaria in a country, the information and resources available, and the flexibility of malaria programs.[1]

The information obtained through stratification can help to determine, at the level of a homogeneous epidemiological area, which will vary from country to country, which combination of control measures or which individual actions are more effective in one place and at a given time. Thus the biological indicators for measuring the scope of malaria are analyzed jointly and harmoniously with the social indicators for preparing planning activities and for selecting the most appropriate objectives and strategies. By selecting these objectives and strategies, each "area" can be subdivided on the map into operating strata in which malaria measures will be applied either separately or in combination. The entire approach must be underpinned by a responsive and dynamic system of epidemiological information that makes it possible to promptly correct and gear the program to the new technology and makes it easier to identify problems and formulate working hypotheses to serve as a basis for further research.

Model of Socioepidemiological Study

Recognizing the social variables that interact with the biomedical variables, the Working Group constructed a conceptual model which provides a new analytical framework for the factors that determine the dynamics of malaria transmission and control. Certain principles were identified as essential to strengthening research:

- To establish a more complete and systematic conceptual framework through the joint efforts of malariologists and social scientists.

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[1]Geneva, WHO, Malaria Action Program. *Guidelines for Planning Malaria Control,* 1981.

- To strengthen the training and research capacity.
- To apply the results of the research to malaria programs.

The ecological factors peculiar to the human host and the vector arthropod define the bounds within which social and economic factors can influence the prevention, prevalence, and distribution of malaria, the increase in risk for certain human groups, and the effectiveness and acceptability of control measures. In addition, certain ecological changes that are social in origin may have a major impact on the increase or decrease in the risk of malaria incidence (degree of exposure).

The Working Group recognized that the overall plan of the framework presented is not definitive and that its purpose is to stimulate discussion and to serve as a guide for research programs as well as a basis for the planning of malaria control programs.

Country Proposals

In reviewing proposals for conducting social studies on malaria, the Working Group mentioned activities in the following three countries:

Dominican Republic

After several years in which malaria transmission was virtually interrupted (28 cases in 1968), an increase in incidence occurred in 1977 primarily due to the immigration of agricultural workers from Haiti into sugarcane and other plantations. In view of the worsening problem, the Ministry of Public Health and Social Welfare of the Dominican Republic decided that any long-term strategy should be based on a better knowledge of the socioeconomic determinants of malaria. To that end, it established a research group composed of a number of malariologists, a health educator, an agricultural engineer, social research technicians, and several consultants in epidemiology and sociology.

The research plan included studies of two types:

- One, *retrospective,* based on data from the agricultural census and covering the relationship between the type of land ownership, the area farmed, and the number of paid agricultural workers in most of the municipalities of the country, and the incidence of malaria, the purpose being to make a statistical analysis by means of cross-tabulation and multiple correlation.
- The other, *prospective,* based on the observation of the relationship between the socioeconomic structure, the characteristics of migration, the macro-and micro-socioecological conditions, the activities of the malaria eradication program and the incidence of malaria in eight areas of operation of the Malaria Eradication Service.

Incidence will be analyzed according to five broad sets of factors: modes of production, characteristics of migration, macro-environmental variables, micro-environmental variables, and working conditions.

The importance of this research lies in the fact that it will provide new knowledge on the socioeconomic background of malaria that can be used for coordinating health programs and health policies, on the one hand, and planning and economic development, on the other. The specific results of the study will provide malaria control and eradication programs with the necessary information to establish new strategies for reformulating and reorganizing their activities. In addition, it will furnish a model for the planning and conduct of similar research on other vector-borne diseases in other countries of the Region of the Americas.

Nicaragua

Of special interest is the evaluation of the degree of acceptability of the health programs planned and executed by the "grassroots health seminars" in 1981. The purpose of one of these programs was to reduce the reservoir of plasmodia in the human population through the antimalaria treatment of the entire population affected.

Advantage was taken of the opportunity afforded by the country's current historical situation to mobilize the support of the population for a national campaign for the control of the spread of malaria: a positive factor was the spirit of community participation that could be expected following the revolution. Despite the fact that at the time of the report no statistical data were available on the control of the disease following the health seminars, the fact is that persons with malaria received treatment. Greater coverage (as much as 94 and 96 per cent) was obtained in the two areas with the highest malaria incidence. Subsequent studies will be made to evaluate the effect of the campaign on the incidence of the disease in the various areas of the country.

Guatemala

An investigation is planned to determine the effect of work on the voluntary collaborators, the community in general, and the malaria control program in particular. The study will endeavor to measure: the effectiveness of the network of voluntary collaborators as a drug distribution and epidemiological surveillance system; the cost of the system; and the degree of the population's awareness of the disease.

The importance of this research lies in the fact that Guatemala's experience is an example of the combination of two simultaneous approaches: the collection of blood slides and the conduct of sociocultural surveys. The results will pinpoint the role of community participation and the extent to which the population accepts and uses the services of the malaria program and of the community.

Conclusions

In the past 25 years, malaria eradication strategy has been based almost exclusively on the use of insecticides. The failure of that methodology in some areas has made it necessary to emphasize the need to deepen epidemiological studies and maintain a reasonable balance between social and biological factors that favor the transmission of the disease. This would provide a better understanding of the socioeconomic and historical processes that underlie the causal complex of the problem and make efficient antimalaria activities possible. In addition, it is important to continue to improve the systems for the epidemiological surveillance of the disease and to endeavor to incorporate in a simple way the new social and biological variables which come to light from the research process.

(*Source:* Research Promotion and Coordination, Division of Human Resources and Research, PAHO.)

Use of Locally Available Drinking Water for Preparation of Oral Rehydration Salt (ORS) Solution[1]

Mothers are encouraged to prepare oral rehydration fluid using only clean water. However, most people in rural areas of developing countries have no access to potable water and in some communities the only available water is heavily contaminated with fecal material.[2] Since only some 20 per cent of the population in developing countries has access to clean water, the risks involved in using untreated water to prepare the Oral Rehydration Salt (ORS)[3] solution, and the need to decontaminate water before adding the ORS ingredients, have prompted researchers to initiate various investigations to explore and further expand available knowledge on this question.

The following presents current research findings relating specifically to the growth of enteric bacteria in oral rehydration solutions prepared from ORS, the risks associated with the use of ORS solution which is not bacteria-free, and possible methods of decontaminating either the water used for preparing the ORS solution or the solution itself.

In the Region of the Americas, numerous studies have been conducted in which the growth of enteric bacteria in ORS solutions prepared with various types of water were compared. A study at the University of Maryland[4] used river water from Suriname and Honduras (containing about 10^3-10^5 bacteria/ml) and distilled water after boiling both sources for 10 minutes to prepare the ORS solution. Growths of enteropathogenic bacteria (*Vibrio cholerae, Escherichia coli,* and *Shigella flexneri*) on blood agar were harvested, then diluted with phosphate buffered saline, and added to different aliquots of the solution to achieve final concentration of about 10^2 bacteria/ml. Viable bacteria counts at 0, 6, 12, 18, 24, and 48 hours in solution while standing at room temperature (24-26°C) revealed an increase in the number of *V. cholerae* and *E. coli* at 12 hours, and a 2-3 log increase at 24 and 48 hours. *S. flexneri* did not increase in number and could not be recov-

[1] Excerpts taken from: Oral rehydration with dirty water. *Diarrhoea Dialogue,* Issue No. 4, 1981.

[2] *Lancet* 2:255-256, 1981.

[3] Composition for oral rehydration as recommended by WHO.

[4] Black, R. *et. al.* Proliferation of enteropathogens in oral rehydration solutions prepared with river water from Honduras and Suriname. Unpublished, 1981.

ered at 24 hours in the Suriname and distilled water samples, but was present at a concentration of 10^3/ml in the Honduras river water sample.

In a village-based study in Brazil,[5] water from households with and without a piped supply was boiled, cooled until warm, and then used to prepare the ORS solution. The solution was then kept at a temperature of 29-32°C for 12-18 hours, after which samples were brought to the laboratory in sterile containers on ice and quantified by the Most Probable Number (MPN) technique for fecal coliforms. About 50 per cent of the samples from homes without running water had concentrations greater than 10^3 coliforms/ml as compared with about 19 per cent of samples from homes with running water. The 10 villagers who prepared heavily contaminated fluid were then provided with autoclaved water; again, 90 per cent of the samples were found to contain 10^3-10^5 coliforms/ml.

In a similar experiment, the same group of workers obtained water from a one meter deep well, prepared the ORS solution, and examined it for coliform growth at 37°C after 4, 8, 16, and 24 hours by the same technique. The solution made with river water initially contained about 4 logs more coliforms than that made with well water, but after 16 hours at 37°C both solutions contained about 10^5 coliforms/ml irrespective of the source of water.

Although a strict comparison of study results is difficult due to the lack of uniformity in study design and techniques employed, it seems clear that an ORS solution prepared with untreated water containing organic matter can support the growth of enteric bacteria at the ambient temperature that prevails in the countries where the solution is most likely to be used extensively. Solutions made from distilled, boiled, or autoclaved water may also support the growth of enteric bacteria as these processes do not remove the nitrogen derived from any killed bacteria. The use of boiled water for preparing the ORS solution does not guarantee its sterility since contamination of such water can occur after boiling.

Although unrelated to the Region of the Americas, a more relevant study on the behavior of *E. coli* in oral rehydration fluid made with well water was recently reported from Gambia.[6] The concentration of *E. coli* in well water alone fell slightly during 24 hours storage at 23-30°C. The same study compared the response of children (three months to four years) receiving oral rehydration fluid composed of well water with those whose fluid was made with sterile water.

In the Gambia study, no difference was observed in the isolation rates of pathogenic organisms in the 97 stool specimens obtained from children who received ORS made with "clean" water and in the 87 stool specimens from children who received ORS made with well water, although *Salmonella* and enteropathogenic *E. coli* (EPEC) were isolated from the well water itself. The incidence and duration of diarrhea and the growth rate in the two groups were also found to be similar. It was estimated that ORS solutions prepared from untreated well water and taken by a child one day out of five were unlikely to contribute more than 5 per cent of the total *E. coli* he would ingest with food prepared with water from the same source.

Theoretically, the ORS solution can be chemically decontaminated by adding a suitable bactericidal or bacteriostatic agent to ingredients at the time of packing. Such an agent would need to be:

- effective against the organisms concerned
- non-toxic to man
- effective in the pH of ORS solution
- non-reactive with the ORS ingredients
- non-corrosive for ORS packaging material
- acceptable from the viewpoint of taste, smell, and color of the solution
- non-disruptive of the absorption process in oral rehydration
- inexpensive.

At present, no known compound meets these criteria.

Boiling water is an effective method of decontamination; although it presents disadvantages in the:

- cost of the fuel and difficulty in obtaining it
- time required for boiling and cooling
- risk that, after boiling, the water or the ORS solution prepared with it may become contaminated during measuring, mixing, handling, or storage
- risk that the water may be used for preparing the ORS solution before it has been sufficiently cooled
- risk that users will mistakenly boil the solution after preparation.

A second strategy, based on available information and pending more field research, is to advise mothers to use the cleanest water available when preparing the ORS solution, to boil it whenever possible, and to use the solution within 12 and never after 24 hours. The solution should also be protected from subsequent contamination and stored in a cool, dark place. To those who express concern regarding these recommendations, it should be stressed that the proven benefits of water and electrolyte replacement early in acute diarrea far outweigh the possible risk of using contaminated water.

(*Source:* Enteric Disease Control Program, Communicable Diseases Control, Division of Disease Prevention and Control, PAHO.)

[5]Shields *et al.* Electrolyte/glucose concentration and bacterial contamination in home-prepared oral rehydration solution: A field experience in North Eastern Brazil.

[6]Watkinson, M., N. Lloyd Evans, and A. Watkinson. The use of oral glucose electrolyte solution prepared with untreated well water in acute non-specific childhood diarrhoea. *Trans R Soc Trop Med Hyg* 74: 657, 1980.

Pan American Conference on Health Research Policies

The Conference was held in Caracas, Venezuela, from 25-28 April 1982, and was attended by 110 participants and observers, among them ministers of health from the countries of the Region, representatives of international agencies, medical research councils, universities, and members of the PAHO Advisory Committee on Medical Research.

The Conference was the culmination of a series of subregional and national meetings in progress since 1977; its major objective was to promote the acceptance and implementation of national research policies as an indispensable part of research development, which is itself closely linked with planning and development in the health sector as a whole.

The agenda included presentations on health and health research in general, summaries of the proceedings of national and subregional meetings and working groups which dealt with specific problems in implementing a health research policy, as well as a panel discussion where representatives of international agencies described their perspective on health research promotion.

Recognizing the importance of national research policies in the context of the Plan of Action to implement the Regional Strategies for achieving health for all by the year 2000, the participants declared their firm commitment to the promotion of national health research policies. The final declaration of the Conference affirmed, in essence, that:

- A national health research policy should be established where it does not exist, or strengthened where it does exist.
- Health research is absolutely essential and has as its fundamental objective the development of knowledge and technologies which will lead to the solution of current and future health problems.
- The development of a health research policy requires planning at all levels to secure financial allocations, support for the priority areas of research, training of personnel, and institutional cooperation, as well as the close collaboration between the responsible authorities and the scientific community.
- Programs in health research should be inter- and multidisciplinary.
- Basic and applied research in the epidemiological, behavioral, environmental, and health services fields should be strengthened.
- Information exchange and collaborative research, clearly and practically defined, among countries at all levels of development is essential.
- Universities and institutions of higher education should continue to develop activities which will strengthen the teaching capacity and ensure the future of research.
- The public should be kept informed about research principles and practices.
- The development of procedures for considering the ethical aspects of research involving human subjects and animals should be supported.
- Financing is critical to the whole process and requires a national mechanism to ensure the continuity of financial support for research and research training.

Meeting of the PAHO/WHO Scientific Working Group on the Control of Sexually Transmitted Diseases

In 1976 WHO convened a Working Group on *Neisseria gonorrhoeae.* The Group prepared a report (WHO Technical Report Series No. 616, 1978), on the epidemiology, clinical features, laboratory tests, treatment, and control of gonococcal infections. Similar reports were published after meetings in 1978 and 1980 on nongonococcal urethritis and other sexually transmitted diseases (STDS), and treponemal infections, respectively (WHO Technical Report Series Nos. 660 and 674, 1981 and 1982.)

PAHO/WHO convened a fourth Scientific Working Group on the control of STDs in April 1982. Twenty-four participants representing a broad spectrum of both developing and developed countries met in Washington, D.C., to discuss the epidemiology of STDs and the planning, organization, implementation, administration, and evaluation of STD control programs within the context of primary health care. The recommendations of the group will be published in Spanish and English as part of WHO's Technical Report Series; the publication will serve as a guide to STD program planners, administrators, and trainers for the preparation or improvement of STD control efforts.

Publications

Hospitals in the Americas. Washington, D.C., Pan American Health Organization, PAHO Scientific Publication 416, 1981. (ISBN 92 75 11416 1). 26 pages + 21 tables. Price: US$6.00.

This publication attempts to meet the constant and increasing demand for information on hospital resources, distribution, administrative affiliation, and utilization. It emphasizes the best possible use that can be made of this information in the future in planning and administration of health services; it also recognizes the need to develop better and clearer definitions of their components and to perfect data collecting and processing procedures.

Hospitals, as referral centers for primary health care, will have new and more important functions as efforts toward achieving health for all by the year 2000 get under way. The analysis of data on the quality and type of care provided by these establishments will be of great value in the monitoring and evaluation of country health plans.

This publication, in addition to containing the currently available information on most countries, endeavors to draw attention to the lack of data in this field, and requests health experts in the Americas to contribute new information and procedures for the collection and analysis of data and the efficient management of health establishments and primary health care services in the Region.

Immunization and Primary Health Care: Problems and Solutions. Washington, D.C., Pan American Health Organization, PAHO Scientific Publication 417, 1981. (ISBN 92 75 11417 X). 44 pages. Price: US$6.00.

This report on the regional meetings of the Expanded Program on Immunization (EPI) held in Quito, Ecuador (18-22 May) and Kingston, Jamaica (14-18 September 1981), provides an analysis of the immunization problems facing countries in the Region and their possible solutions formulated in line with the plan of action for implementing the regional strategies to achieve health for all by the year 2000.

By 1990, it is expected that immunization services will be available for all infants against diphtheria, whooping cough, tetanus, tuberculosis, measles, and poliomyelitis, and that coverage will be maintained despite population increases during the final decade of the century. In order to achieve this goal, the national program managers at the meetings on EPI discussed the following topics: the role of primary health care, programming and evaluation, strategies for increasing coverage, supervision and continuing education, the cold chain, community promotion and participation, coordination, staff training, epidemiological surveillance and information systems, administration of resources and financing, and activity timetables. The participants also generated preliminary biennial plans of work for all member countries.

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