# Further Studies of Food Poisoning in Puerto Rico\*

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**GR MANY** years we have been interested in the epidemiology of food infections in Puerto Rico with special reference to the various types of bacteria producing them, and as to whether in this Island they show special modalities or characteristics different from those found in the temperate zone.<sup>1-6</sup>

We have not been able as yet to study any case of botulism. That the condition exists in Puerto Rico we do not doubt, since local conditions are suitable for the contamination of food and the climate is a favorable factor for the development and growth of the organism and the elaboration of its exotoxin.

The present communication we are limiting to epidemiological studies of a number of outbreaks of food infections produced by bacilli of the Salmonella Group, the toxic products of the staphylococcus or cases which epidemiologically suggest food infections, yet in which no definite etiological agent could be found.

#### OUTBREAKS PRODUCED BY ORGANISMS OF THE SALMONELLA GROUP

Since 1933, when we published our studies on food poisoning in Puerto Rico,<sup>1</sup> we have been able to study four large outbreaks of food infections, produced by organisms of the Salmonella Group.

### THE GOYCO SCHOOL OUTBREAK

The Goyco School is a public school located in a suburb of San Juan, in which there are approximately one thousand one hundred pupils.

The school normally runs a daily lunch room for indigent children. On October 27, 1933, eighty-four persons partook of lunch, and within several hours sixty-four suffered with symptoms of food poisoning. The lunch consisted of boiled, salted codfish which had been placed in water the night before and allowed to stand until the next morning when it was cooked. Besides the codfish there were also served boiled potatoes, sweet potatoes, yautías, plantain and lettuce salad.

Within two to twelve hours after eating the lunch, sixty-four persons

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were taken severely ill with acute gastro-intestinal symptoms which lasted anywhere from three to twelve hours; in the more severe cases, twenty-four. Nausea was the most constant symptom. The percentage occurrence of the symptoms in those affected was as follows:

Nausea	Headache
Vomiting	Fever
Abdominal pain	Pain in joints
Dizziness	Muscle cramps 12.5%
Diarrhea	Visual symptoms 3.1%

A complete epidemiological survey was carried out.\* In Table No. 1 is shown the relation of the appearance of the symptoms to the various items of the diet. The epidemiological survey pointed to the codfish as the probable causative agent of the poisoning.

A group of children in the school room about three hours after the meal suddenly complained of pain, this being the first severe warning as a possible poisoning from food. The Principal of the school ate some codfish and potatoes about ten o'clock in the morning and two hours later noticed slight abdominal cramps and nausea.

#### TABLE I

Relation of Persons Eating Food and the Appearance of Symptoms, Goyco School Outbreak

	Percent out of total of 86								
	Cod- fish	Pota- toes	Let- tuce	Sweet pota- toes	Yau- tía	Plan- tain	Mix- ture		
Persons eating food and developing									
symptoms	73.8	66.7	59.5	69	67.8	54.8	76.2		
without eating food	2.4	9.5	16.7	8.1	8.3	21.4			
Ate food without de- veloping symptoms	14.3	9.5	15.5	22.6	17.8	17.8	23.8		
Did not eat food or develop symptoms	9.5	14.3	8.3	1.2	6	6			

In the epidemiological survey it was revealed that a nearby school, the Castelar, with a lunch room for indigent children, prepared its lunch that day in the kitchen of the Goyco School, although the menu was entirely different. None of the children of the Castelar School was

\* This survey was in charge of Dr. R. Román Benítez, Medical Health Officer of the Department of Health of Puerto Rico.

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taken sick, yet one of its employees who ate of the menu of the Goyco School became ill.

Sanitary conditions of the kitchen of the Goyco School were good.

The remnants of the food served during the lunch were collected and brought to the Laboratory for bacteriological examination.

The presence of an organism of the Salmonella Group was revealed, whose morphological and biochemical characteristics can be seen in Table 4, culture no. I.

A serological study of the organism proved it to be a bacillus aertrycke (typhi murium, Group B).\*

A water extract was prepared by grinding and mixing some of the food in sterile water. The extract was divided into two portions. One of them was filtered through a fine Berkefeld candle. The sterile filtrate was fed to three rhesus monkeys in amounts of twenty, twenty-five and thirty cubic centimeters. The animals were observed for twenty-four hours, during which time no symptoms whatsoever developed.

To detect the possibility of a poison added accidentally or with criminal intent, the unfiltered extract was injected through a catheter to the stomachs of five guinea pigs, the amounts varying from 1 to 5 cubic centimeters. Two guinea pigs were also injected subcutaneously with 1 cubic centimeter of the sterile extract without apparent symptoms. White rats and mice also ate some of the food subjected to examination without the production of any symptoms.

The feces of the cooks and persons who had to do with the handling of the food were examined bacteriologically with negative results.

Special field investigations were carried out in the neighborhood of the Goyco School to discover if rat viruses of any kind had been used to exterminate rodents, with negative results.

### THE RIO PIEDRAS FISH POISONING OUTBREAK

On April 27 and 28, 1933, there occurred outbreaks of food poisoning among thirty-nine persons living in different houses and residential sections of the town of Río Piedras.

The epidemiological studies<sup>†</sup> revealed that the only item of the diet which was common to all the persons involved was fish. Nine families prepared fish for dinner on two separate days, and out of forty-two

<sup>\*</sup> In the bacteriological and serological studies performed to type these organisms we have followed the classification advocated by the Salmonella Subcommittee, appointed during 1034 by the Nomenclature Committee of the International Society of Microbiology.<sup>7-9</sup>

<sup>†</sup> The epidemiological investigation was carried out by Dr. E. Martínez Rivera, at that time, Director of the Health Unit of Río Piedras.

persons who ate the fish, thirty-nine were taken sick with symptoms of intoxication (Table No. 2). The fish involved appeared to be a large salt water fish which had been caught in Fajardo on the morning of April 25 and sold in Río Piedras on the 27th and the portions left over sold on the 28th. The fish was kept in the market of Río Piedras in an ice box with other kinds of fish. It appears that only persons who ate from the particular fish (Mero-*Epinephelus morio*), developed symptoms, while those who ate of other types of fish did not. A similar outbreak of food poisoning produced by fish caught in salt water in the neighborhood of the same town was described by us in 1928.<sup>2</sup> At that time the outbreak occurred in the City of San Juan and an organism resembling bacillus enteritidis, in cultural and agglutinating properties was isolated. In the present outbreak of Río Piedras the symptoms of poisoning appeared anywhere from three to seventeen hours after the ingestion of the fish. Some of the patients were extremely ill.

Portions of the fish (fried) consumed by one of the families (No. 1, Table 2) who developed symptoms were kept in the ice box and brought to our laboratory for bacteriological examination. All the fish at the market of Río Piedras was confiscated and samples

All the fish at the market of Río Piedras was confiscated and samples from four different species (Pargo-Naomaenis analis-, Picúa-Syphyreana-, Colorado-Neomaenis vivanus-, and Kingfish or Sierra-Scomberomorus cavalla-) were brought to our laboratory for examination. None of the original fish which was supposed to have caused the outbreak was left over.

The fried fish eaten by Family No. 1 was examined bacteriologically. A pigmented (yellow) hemolytic staphylococcus, a bacillus proteus, and an organism with cultural characteristics, similar to the Salmonella Group (Table 4, Culture No. 2), were isolated. This organism was identified by us as belonging to the enteritidis group (Group D), but being in doubt as to its exact typing, we sent a subculture to the Ministry of Health in London where it was identified as belonging to Group D with a somatic No. IX antigen and an unidentified flagellar antigen. The staphylococcus was sent to Professor Jordan at the University of Chicago where its toxicity was tested on monkeys with negative results.

A water extract prepared from the fish was filtered through a fine Berkefeld candle and the sterile filtrate fed to monkeys in amounts of 25, 30 and 35 cc. No symptoms were observed. The four portions of the fresh fish confiscated by the Health Department were examined bacteriologically and no organisms of the Salmonella Group were iso
 TABLE II

 Relation of Families Involved in the Río Piedras Outbreak, Number Who Developed Symptoms and Other

 Epidemiological Findings.

Family	Persons who ate of meal	Number that ate fish	Number who became sick	Day fish was eaten	How was fish prepared	Range of time of appearance of symptoms	Section of town where family lived	Symptoms	Duration of sympioms
1 2 3 4 5 6 7 8	8 7 14 9 6 6 4 7	7 2 7 8 1 5 4	7 2 5 8 1 5 3 6	Thurs. Thurs. Fri. Thurs. Fri. Thurs. Thurs.	Fried Salad Fried Fried Fried Fried and Sauce Fried	3-15 Hrs. 3-8 Hrs. 3-10 Hrs. 3-13 Hrs. 3 Hrs. 3-17 Hrs. 3-6 Hrs. 31-2-5 Hrs.	Best Poor Best Poor Med. Poor Best Poor	The first symptoms noticed were abdom- inal discomfort and malaise. Symptoms varied in severity from mild to very marked. Symptoms in order of impor- tance were: Abdominal pain	One to 5 days. Some complain- ed of weakness and pain in joints for 15 days. Some cases were vio- lently sick on verge of col- lapse.
9 TOTAL	5	2 42	2 39	Thurs.	Fried	3–5 Hrs. 3–17 Hrs.	Poor —	Nausea Vomiting Diarrhea (blood in 1 case) Headache Dizziness Numbness in extrem- ities Pains in the joints and long bones	

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lated; however, bacterial contamination of the fish was abundant, the predominant organism being bacilli of the Coli group and a bacillus proteus.

The feces of several of the individuals who were taken sick were examined bacteriologically for organisms of the Salmonella Group with negative results.

### BARRANQUITAS OUTBREAK

On April 16, 1937, several physicians were invited to the country residence of a colleague, located in the town of Barranquitas, where lunch was served about two o'clock. From a total of twenty-six persons who partook of the same food, twenty-one were taken sick with symptoms of poisoning. The menu served consisted of olives, salami, stuffed turkey (trufé turkey) chicken salad, rice with chicken, anchovies, caviar, dessert and in one case, steak. The party ended around six o'clock in the afternoon (about three to four hours after the luncheon). About one o'clock that night, i.e., ten hours after lunch, symptoms of poisoning began to appear in some of the guests. Between ten and eighteen hours after lunch, twenty-one out of the twenty-six persons who partook of the lunch were taken with severe symptoms of poisoning. The stuffed turkey which was left over was eaten for dinner around eight o'clock by eight different persons who did not attend the party and out of these, six were also taken ill, making a total of twenty-seven ill and seven who were not affected at all. A complete epidemiological study was made and it became evident that the stuffed turkey was the probable incitant of the poisoning, inasmuch as the persons most severely affected were those who had eaten the largest amount of it, and many of those who were not affected, had not eaten it. Some of those intoxicated were severely ill with acute abdominal pain, vomiting, diarrhea, and a peculiar nervous condition. The percentage of occurrence of each symptom of those affected is as follows:

Abdominal pain100%	Dizziness 22%
Diarrhea 92%	Feeling of weakness
Malaise 25%	Aching joints 11.1%
Nausea 22%	Vomiting 3%
Tenesmus	0.3%

The symptoms began to subside within about twelve hours, with complete recovery within forty-eight hours, except in two cases who remained sick for about a week. The symptoms appeared in the majority of the cases 10 to 15 hours after eating, the shortest incubation period being two hours; the longest, eighteen. In Table No. 3 is shown the relation of the different items of the diet eaten with the development of symptoms.

A bacteriological examination of the stuffed turkey was performed in our laboratory and an organism belonging to the Salmonella Group was isolated. It was found to have the biochemical and serological characteristics of bacillus enteritidis (Table No. 4, Culture 3), and was typed in Group D. A bacillus of the Coli Group was also isolated.

		19.45		Barr	anquit	as Or	utbrea	ık				
	112											
Number of Cases	Olives	Salami	Stuffed turkey	Salad	Chicken and rice	Anchovies	Caviar	Crackers	Steak	Dessert	Developed Sympioms	
15 6 2 1 1 1 1 2 2 2	+ 1 + 1 1 + + + + 1 1	+ + + + + + + + + + + + + + + + + + + +	+++++++    +	++++1     +++++	+ 1 + 1 + + + + + + + 1	+       + +   + + +	+ 1 1 + + 1 + + 1 1	+ + + + + + + + + + + + + + + + + + + +	+	+++++++++++++++++++++++++++++++++++++++	+++++	

TABLE III Relation of the Presence of Symptoms with the Items of Diet Eaten, Barranquitas Outbreak

The turkey was fed to rats and mice and an aqueous extract was prepared and filtered through a fine Berkefeld candle. The filtrate was injected subcutaneously and fed to guinea pigs, rats and mice without the production of symptoms of any kind.

### MUNICIPAL HOSPITAL OUTBREAK

On January 4, 1939, an outbreak of food poisoning occurred in the Municipal Hospital of San Juan with a total of one hundred and three cases.

Lunch was served at the hospital at about 11:30 A.M., to the employees, nurses, patients on regular diet, and other individuals from the outside who ate of the same food. In the afternoon, at five o'clock, dinner was served as usual. The lunch consisted of boiled codfish with oil and vinegar, vegetables (ñame, yautía and potatoes), rice, beans and coffee. In the afternoon the dinner consisted of soup, meat, rice and white beans. At about five o'clock symptoms of poisoning began to appear in a number of the individuals who had eaten at the Municipal Hospital. By seven o'clock a very large number began to be brought to the hospital in ambulances and private automobiles with symptoms of poisoning, so severe that they had to be carried to beds in the hospital.

One hundred and three individuals suffered with symptoms of poisoning; of these, 36.3% were patients of the hospital who were on a solid diet; 45.1% were employees of the hospital; 15.7% were nurses, and 2.9% were outsiders. The onset of the poisoning was abrupt-the incubation period varying anywhere from two to twenty-six hours: in 14.7% of the cases it was between two and four hours; in 69.6% between 5 and 81/2 hours; in 11.2% from 9 to 12 hours; and in 3.7% more than twelve hours. The poisoning was initiated with abdominal pain which was the first and most prominent symptom; it was acute, generalized over the abdomen, colicky in nature and in the majority of the cases excruciating-some of the patients were doubled up in an attempt to diminish the severity of the pain. Ninety-nine percent of the persons affected suffered with this pain which lasted from six to twelve hours or more. Diarrhea was the next most prominent symptom, occurring in 98% of those affected. The stool was watery in character with no mucus and contained blood in 8.8% of the cases. Nausea and vomiting were constant symptoms, occurring in 96 and 93.1% respectively. The vomiting was severe, occurring at frequent intervals and accompanied by considerable retching. In 91.2% there was a clammy perspiration with a feeling of chilliness over the whole body, which in some cases resembled a real chill; 89.2% of the patients felt dizziness.

A complete epidemiological survey<sup>\*</sup> was carried out. All those affected were located but it was impossible to find those who had eaten at the hospital and remained unaffected. A survey was made of all the patients, including the age, occupation, sex, and the different items of the lunch and dinner, together with the symptoms suffered. This revealed that 51% of the patients were males and 49 females; 59.8% were white, 19.6 black and 20.59% mulattoes. The percentage distribution according to age groups was 8.33 from 7-14 years; 29.17 from

<sup>\*</sup> The survey was in charge of Dr. S. Riera López, Epidemiologist of the Department of Health.

15-19 years; 35.42 from 20-30 years and 27 over 30 years. The only item of food that was eaten by all, save one, was the codfish, 99% of those affected having eaten it. Fifty and nine-tenths percent ate yautía, 58.8%, ñame; 85.3%, rice, and 70.6%, beans. An official of the institution ate codfish and no symptoms appeared, but the meal was prepared in a different kitchen from that of the hospital. The epidemiological survey revealed that the codfish eaten at lunch was the item of food which probably caused the outbreak; the food eaten at dinner was eliminated as a possible factor of the outbreak as a very small number of those affected partook of this meal.

Most of the patients recovered within two or three days although there were several who remained in a very serious condition for many days; some as long as two or three weeks.

Samples of the food served at lunch were collected and sent to the laboratory for examination; also samples of the vomitus, urine and feces of a number of the patients affected.

A study was made of the sanitary conditions of the kitchen of the Municipal Hospital. Although the kitchen was neither fly nor rat proof, a fact which made possible the contamination of the food by vermin, conditions in the kitchen were good and the food was prepared with care and cleanliness. The codfish had been placed in water the previous night and kept thus at room temperature until next morning, when the water was drained and replaced with fresh water and boiled. The codfish was then removed, cleansed of bones and skin by the employees and placed in a pail, where vinegar and oil were added. This procedure was performed between 7:30 and 8:00 A.M. The other food was prepared during the morning that the outbreak occurred. The codfish was served between 11:30 to 12:00 o'clock, having had ample time and opportunity for the development and growth of bacteria since the time of its preparation.

No arsenic or other inorganic or organic poisons were detected by chemical examination either in the food, in the vomitus or in the feces examined.\* A careful examination of the beans employed in the luncheon revealed that these did not contain the poisonous Burma or Rangoon<sup>1</sup> types.

All the refuse of food of the hospital is collected in garbage cans and distributed to certain individuals who use it as food for pigs. One of the distributed pans was located, brought back to the hospital and

<sup>\*</sup> Chemical examinations were performed in the Chemical Laboratory of the Department of Health of Puerto Rico under the direction of R. del Valle Sárraga.

samples for the various examinations obtained from it.

Another can had already been used, two individuals had eaten of it and dumped the remainder to three pigs, the former were taken sick with symptoms and brought to the Municipal Hospital while nothing whatsoever happened to the three pigs which ate a considerable amount of the food.

### ANIMAL EXPERIMENTATION

The food as such was fed to a dog, a cat, four white rats and four white mice without the production of symptoms of poisoning.

A water extract was prepared by grinding the food thoroughly, especially the codfish, with about five times its volume in sterile water. The liquid extract was filtered through filter paper first and then through a fine Berkefeld candle and the filtrate fed to two rhesus monkeys in amounts of 20 and 30 cc., and to white rats and mice. No symptoms of any kind were evident. The filtrate was also injected subcutaneously into white rats and mice with no untoward effects whatsoever.

A bacteriological examination was made of the food which was collected, employing the usual routine followed in our laboratory. A hemolytic staphylococcus aureus was isolated from many plates; also bacilli of the coli and proteus groups. Several of the colonies selected for study turned out to be organisms with morphological and biochemical reactions, resembling the Salmonella Group (Table 4, Culture 4), which serologically was found to be a bacillus aertrycke (typhi murium), belonging to Group B.

The feces of twenty-three of the patients were examined bacteriologically and in three an organism of the Salmonella Group identical culturally and serologically to the one isolated in the food was found.

#### OUTBREAKS PRODUCED BY STAPHYLOCOCCI

Since Barber<sup>15</sup> reported the first instance of food poisoning produced by a staphylococcus albus occurring in the udder of a healthy cow and transmitted to various individuals, it took a good many years before staphylococci were definitely connected as causative agents in the production of food poisoning.

In 1933 Jordan and his associates showed the relation between certain staphylococci and food poisoning and demonstrated that a filtrable toxic product (enterotoxin) was formed as a result of the growth of staphylococci in food, toxin which was capable when fed to human vol-

#### TABLE IV

Cultural and Biochemical Characteristics of Salmonella Organisms Studied

and the second se				and the second		
	1	2	3	4	5	6
Motility	+	+	+	+	+	+
Milk	A	A	A	A	A	
Indol	-	-	-	-	-	+
Hydrogen Sulphide	+	+	+	+	+	+
Gelatin	-	-	-	-	-	-
Glucose	Ð	$\oplus$	Ð	$\oplus$	Ð	Ð
Lactose	-	-		-	-	-
Sucrose	-	-	-	-		-
Mannite	Ð	$\oplus$	$\oplus$	$\oplus$	Ð	0
Maltose	⊕	$\oplus$	$\oplus$	$\oplus$	$\oplus$	0
Xilose	$\oplus$	$\oplus$	Ð	Ð	Ð	0
Salicin	-	-	-	-	-	⊕ .
Sorbitol	Ð	$\oplus$	$\oplus$	Ð	$\oplus$	Ð
Rhamnose	$\oplus$	$\oplus$	$\oplus$	$\oplus$	Ð	Ð
Arabinose	$\oplus$	<b>⊕</b>	Ð	Ð	Ð	0
Dulcitol	$\oplus$	Ð	Ð	Ð	$\oplus$	0
Trehalose	$\oplus$	$\oplus$	Ð	$\oplus$	$\oplus$	Ð
Inosite	+	+	+	+	+	+
Levulose	0	$\oplus$	Ð	$\oplus$	$\oplus$	Ð
Galactose	0	⊕.	Ð	$\oplus$	0	Ð
Dextrin	Ð	$\oplus$	Ð	$\oplus$	Ð	$\oplus$
Tartrate	+	+	+	+	-	+

⊕ Acid and gas

+ Positive or acid

No change
 A Alkaline reaction

unteers and monkeys, of producing gastro-intestinal manifestations such as vomiting and diarrhea.<sup>16-18</sup>

### POISONING BY CHEESE

Cheese appears to be a rather frequent cause of food poisoning in Puerto Rico, the following four outbreaks being traced to cheese apparently contaminated with staphylococcus.

The first of these outbreaks occurred in Sabana Grande during May, 1933. Five people were involved. During the lunch hour, native cheese was served as one of the items of a meal partaken of by five members of a family, and was the only item of the diet which was eaten by all.

From three to six hours later symptoms began to appear, manifested by severe vomiting, marked dizziness and abdominal pains of varying degrees. There was no diarrhea or other manifestations. The symptoms lasted from eight to twelve hours, subsiding slowly to recovery.

A portion of the cheese was sent to our laboratory where a bac-

teriological examination revealed the presence of a hemolytic staphylococcus aureus. A water extract of the cheese was made by grinding it in about five times its volume in water. The suspension was filtered through cotton and part of it through a fine Berkefeld filter candle. Two rhesus monkeys were then fed with the filtered extract, one with 15 cc., the other with 30 cc. and placed under observation for twentyfour hours. No vomiting or diarrhea was noticed, however, about one to one and a half hours after the feeding, both monkeys became restless and took to a corner of the cage where they became so weak and dizzy that they fell and had to lie down on their sides, without changing their posture. After remaining in this state for about an hour they began to recover slowly, raising their heads and sitting up, and in about two hours were in perfect condition again.

The second outbreak of cheese poisoning occurred on December 31, 1933, in the town of Río Piedras. On the previous evening native cheese had been served at dinner at a private residence. At lunch time, that is, eighteen hours later, a heavy meal was eaten consisting of chicken, yautía, chayote, potatoes, plantain, ham and bacon. No symptoms whatsoever were noticed.

Three hours later the fourteen persons who had partaken of the lunch ate some of the native cheese, which was left over from the previous evening, and three to six hours after eating the cheese, symptoms of intoxication began to appear in thirteen out of the fourteen.

The symptoms were severe, manifested by abdominal pain, nausea, vomiting and diarrhea in practically all of them, clammy perspiration, muscle cramps, headache, malaise and dizziness were seen, and bloody diarrhea occurred in one. Within twenty-four hours all the symptoms had disappeared in all except one who remained sick for five days with prostration, diarrhea with blood and extreme nervousness. The epidemiological investigation pointed to the cheese as the inciting agent, because the one who escaped such symptoms did not eat cheese.

Portions of the remaining cheese were sent to the laboratory and a hemolytic staphylococcus aureus was isolated in the bacteriological study. An extract was made from the cheese, filtered through a fine Berkefeld candle and fed to two monkeys (in amounts of 20 and 30 cc.) which within one to one and a half hours later developed vomiting, restlessness and finally took to a corner where they remained quietly for about one or two hours.

The third outbreak occurred on March 15, 1936, in the town of Isabela. The investigation reveals that severe gastro-intestinal symp-

toms were produced in three children from two to four hours after eating native cheese. The symptoms were characterized by severe abdominal pain, nausea and slight diarrhea, symptoms which lasted for twenty-four hours. A bacteriological examination of the cheese revealed the presence of a hemolytic staphylococcus aureus and a bacillus coli. No animal experimentation was done in this case.

The fourth outbreak occurred in Santurce, in Barrio Obrero, on Monday, January 16, 1939. Five persons partook of a lunch which consisted of vegetables, lamb stew, rice, and beans. All five ate native cheese which was bought in its original container from a public vendor. About five o'clock one of the persons began to feel ill, and at about seven o'clock, two more. The other remained well.

The symptoms were headache with dizziness and abdominal pain. One of the persons had diarrhea. None had vomiting. The pain was of a colicky nature over the whole abdomen. All the persons intoxicated believed that the cheese was responsible for the outbreak and gave some to a cat, and claimed that in a very short time the cat vomited.

The cheese was brought to our laboratory for examination. A bacteriological examination revealed the presence of a hemolytic staphylococcus aureus. Experimentation in animals with an aqueous extract fed to rats and white mice gave no results whatsoever. A filtrate sterilized through a fine Berkefeld candle was fed to monkeys and injected into rats and mice. Rats, mice and monkeys were given portions of the cheese which they ate promptly without symptoms of poisoning. Besides the staphylococcus aureus the bacteriological examination also revealed the presence of a coliform bacillus.

#### POISONING WITH CANDY

During June, 1937, an outbreak of food poisoning occurred in a well-to-do family. It appears that during lunch there was served to seven persons some candied egg yoke with wine.

Anywhere from five to thirteen hours after lunch the seven persons eating the candy, (the only item of the diet in common to all), developed symptoms of poisoning. The symptoms were manifested by acute abdominal pain, very severe nausea, vomiting, diarrhea, malaise and feeling of weakness. Within forty-eight hours all had completely recovered.

A bacteriological examination of the candy revealed the presence of an organism of the bacillus coli group and a hemolytic staphylococcus albus. A water extract of the candy was prepared and injected into

white rats and guinea pigs, both subcutaneously and into the stomach through a rubber catheter without the production of symptoms of any kind. Due to the lack of monkeys at the time we were unable to test the ability of the staphylococcus to produce an enterotoxin.

### POISONING WITH FISH

During the month of August, 1938, some fish was received at the Biological Laboratory with the request that it be investigated chemically and bacteriologically since it was supposed to be the causative agent of the poisoning of several persons in the Municipality of Maunabo. The fish was served in different places and in different forms and from four to eight hours after its ingestion it gave rise to acute abdominal pain, nausea and vomiting which subsided after twenty-four hours, with complete recovery of everyone.

A bacteriological examination of the fish revealed the presence of a hemolytic staphylococcus albus and a bacillus coli. Due to the lack of monkeys the toxicity of the staphylococcus was not tested.

Feeding experiments with rats and mice, and the injection into the stomach and subcutaneously of a water extract of the food, did not produce symptoms in the animals.

### UNDETERMINED ETIOLOGICAL AGENTS

It is a well known fact that there are outbreaks of food infections and intoxications, in which a careful epidemiological study points to a definite food as the inciting agent and yet it is impossible to determine the exact etiological agent in spite of bacteriological or chemical examinations, or through animal experimentation. Under undetermined etiology we are including three outbreaks in which the exact causative factor could not be determined.

### FOOD POISONING AT CAMP HICACO

During the month of October, 1936, there occurred at one of the C.C. Camps (Hicaco at Luquillo) of the Forest Service of the United States in Puerto Rico, a very severe outbreak of food poisoning.

On Friday, October 23, 1936, dinner was served to all the occupants of Camp Hicaco and within five to eight hours, thirty people became sick with symptoms of food poisoning. Next day, that is, Saturday, a few hours after lunch, fifty of the occupants of the camp were taken sick with similar symptoms to that of the previous day. In other words, within a period of twenty-four hours about eighty people who partook of the food prepared under the supervision of the camp authorities,

were taken sick with symptoms of food poisoning. The following is a description of the symptoms.\* The onset was ushered by sudden, severe, crampy epigastric pain, followed shortly by nausea and vomiting and later on by violent diarrhea, at times with mucus and blood. The duration of the symptoms fluctuated between 24 to 72 hours, being much worse for the first few hours after the onset. Some cases came pretty close to collapsing. The pulse was rather slow and easily compressible. During the first few hours after the onset, the patients looked sick and were evidently in agonizing distress; the skin was cold with profuse clammy sweat and dehydration in the severe cases. Physical examination was irrelevant except for epigastric tenderness with some voluntary muscular protection. An average leucocytosis of 10,000 was present with an increase in the granulocytes (neutrophilic) to about 75. In one case a count of 24,200 with 95% neutrocytes was noted. There was also noted in the cases studied, an increase in the sedimentation time of the erythrocytes. There was also found in many cases a rise in the eosinocytes due to the presence of uncinariasis.

A complete epidemiological examination of this outbreak was not possible at the time as a good many of the individuals affected were transported to different hospitals and others went home. The menu served on both days consisted of codfish, soup, beans, rice, meat, oil which was used with the codfish, coffee and sugar, bread, water, milk and vegetables.

None of the actual food consumed at the camp on both occasions was available for examination. Samples of the food in stock, from which both meals were prepared, were sent to our laboratory and subjected to bacteriological studies, which revealed a hemolytic staphylococcus albus from the rice, coffee, meat and codfish. A non-hemolytic staphylococcus was isolated from the codfish, rice, coffee, and beans. From none of the food examined was any organism of the Salmonella Group found. The food was fed to laboratory animals (rats and mice) without any untoward effect. A water extract was prepared and was injected subcutaneously and into the stomach of rats and guinea pigs without any results.

A bacteriological examination was performed on the feces of forty-six of the eighty men affected, but results were essentially negative except in three cases; in one, a bacillus of the dysentery group, Flexner strain was isolated; in two, an organism with biochemical characteristics re-

<sup>\*</sup> Information supplied by Dr. C. Muñoz McCormick, Chief Medical Officer of the Forest Service of Puerto Rico.

sembling the Salmonella Group, one a bacillus paratyphosus B (Table 4, Culture 5), and the other an unidentified species with certain of the characteristics of the salmonella bacilli (Table 4, Culture 6). The latter was inagglutinable with the sera of salmonella organisms which we have in our possession.

### POISONING WITH CHOCOLATE CANDIES

This small outbreak occurred in the town of Orocovis during January, 1934. Nine individuals, laborers, in apparent good health had their lunch at their homes, none of the food being common to any two of them. Two hours later, while at work, they all ate some chocolate candy ("kisses"), obtained from an original box and each candy wrapped separately in lead paper.

Within a very short time (two to five hours) all nine persons became sick with slight nausea, severe abdominal pain, diarrhea, and vomiting which persisted anywhere from three to ten hours. The only item of food which was common to all nine individuals was chocolate candies and naturally they were blamed.

What was left of the chocolate candies was sent to the Biological Laboratory at San Juan for examination. Unfortunately none of the food which was eaten at the lunch hour by any of these individuals could be obtained for examination.

In the laboratory the candy was subjected to a bacteriological examination which did not reveal any bacteria. Chemical examination did not reveal the presence of poisonous substances. Animal experimentation was done, utilizing rhesus monkeys, guinea pigs, white mice and rats. Guinea pigs and white rats were fed into the stomach through a rubber catheter with a water extract of the candies without the appearance of symptoms of any kind. A water extract was made with the candies and filtered through a fine Berkefeld candle and fed to monkeys which developed no symptoms of any kind. The filtrate was also injected subcutaneously to white mice and white rats.

#### POISONING WITH FISH

During the month of March, 1936, there occurred in the City of San Juan several cases of food poisoning distributed among two different families.

The epidemiological examination revealed that the only item of food eaten by the individuals affected was fried fish, served during the lunch hour. Four hours after eating the fish, symptoms of acute in-

toxication began to appear, manifested by severe nausea and vomiting, and abdominal pains localized throughout the abdomen and in the form of cramps. In one of the two families there were four cases, three adults and a young child six years old, in the other there were two adults and two children. All the cases recovered promptly. Everyone affected ate some of the fish which was supposed to be the inciting agent of the poisoning. None of the original four outbreaks of food poisoning pro-duced by bacilli of the Salmonella Group are presented. One of the outbreaks, that of the Municipal Hospital, is perhaps the largest out-break of food poisoning on record in Puerto Rico, as far as we have been able to determine been able to determine.

### DISCUSSION

In the four outbreaks produced by organisms of the Salmonella Group it is interesting to note that in two the epidemiological evidence pointed to codfish as the possible vehicle that transported the specific bacilli to the gastro-intestinal tract.

All four of these outbreaks and others which we have previously reported have been produced by meat products, in none of these cases have we found any outbreak produced by cheese. In their epidemi-ological characteristics the four outbreaks were very similar although the severity and percentage appearance of the symptoms varied to a certain extent.

One of the outbreaks occurred chiefly among children of school age, one among people of high social and professional standing and the other two among people of different social and economic status.

The onset in all four outbreaks was sudden, the period of incubation was as a rule short, from four to ten hours. Abdominal pain was a char-acteristic and rather predominant symptom among the individuals af-fected. Nausea was also a predominant symptom occurring in most of those affected. Vomiting was absent in one of the outbreaks. Diarrhea of those affected. Vomiting was absent in one of the outbreaks. Diarrhea was a fairly constant symptom although it was not characterized by the appearance of mucus, blood being found in a small percentage of the cases. All four outbreaks were found to be produced by organisms of the Salmonella Group; two by bacillus aertrycke (typhi murium, Group B); one by a bacillus enteritidis (Group D) and the other by a very interesting organism of Group D with a somatic O antigen No. IX and an unidentified flagellar antigen. Dr. W. M. Scott<sup>10</sup> of the Min-istry of Health in London who typed this organism for us, states that there are several such group D new types in existence. Epidemiological characteristics were more or less similar in all four

outbreaks and resembled in every respect those described for outbreaks occurring in the temperate zone.11 Although some observers are inclined to consider the presence of the specific organism in the feces of the agglutinins in the blood of the individuals affected as the most important finding, we do not quite agree. The demonstration of a specific organism of the Salmonella Group in the food eaten, when backed by conclusive epidemiological evidence, is in our opinion more important and convincing than the mere presence of the organism in some of the feces, as it is quite difficult to isolate the inciting bacilli in all the feces of those affected. Likewise the presence of agglutinins in the blood is no conclusive criterion, inasmuch as there is considerable cross agglutination among the many members of the Salmonella Group. Vaccination against typhoid and paratyphoid A and B are very extensively employed nowadays and the presence of agglutinins in the serum might lead to erroneous conclusions. The fact that these individuals recover promptly and become dispersed very soon after the outbreak makes it quite difficult (except in special instances) to collect feces for examination or blood for agglutination reactions when the latter has to be obtained ten to twelve days after the outbreak is over, and when the patients are feeling well again.

In all four of the outbreaks we were able to isolate the inciting bacilli from the offending food.

In previous investigations which we have carried out we have studied outbreaks of food poisoning produced by organisms of the Salmonella Group. In 1928<sup>1-2</sup> we reported an outbreak of food poisoning produced by fish in which a bacillus enteritidis was isolated from the food; another outbreak (produced possibly by lobster), to which we ascribe bacillus suipestifer (cholera suis) as its cause since we isolated said organism from the feces of the individuals affected. We also studied a small outbreak in which seven individuals were affected and in which bacillus enteritidis was isolated from the stools of the majority of them. In the last two instances no food was obtained for examination.

Bacilli of the Salmonella Group (aertrycke, enteritidis and an atypical organism with some biochemical characteristics resembling the group, but which serologically could not be placed) have been isolated by us<sup>12-13</sup> from the feces and internal organs of wild rats captured in the commercial and residential sections of San Juan.

Morales Otero<sup>14</sup> studied an extensive outbreak of diarrhea among pigs which occurred in one of our government institutions, and isolated from the feces, blood, and from the organs of affected animals a bacillus of the Salmonella Group (cholera suis or suipestifer of Group C). The

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disease very much resembled hog-cholera, had a high mortality and was septicemic in nature, but experimental investigation with filtrates did not produce the disease; while the feeding of isolated cultures to young pigs reproduced the disease with identical symptomatology.

Six outbreaks, in which there were experimental or epidemiological evidence or suspicious findings to incriminate staphylococci (enterotoxin) as their cause, are presented. Of these, four were produced by cheese, one by candied egg yoke and one by fish. Of those produced by cheese, in two the feeding of a sterile aqueous filtrate to monkeys produced symptoms indicating the possible presence of a filtrable toxic substance. The type of food poisoning produced by the toxic products (enterotoxin) elaborated by staphylococci is very similar in its epidemiologic characteristics and symptomatology to that caused by bacteria of the Salmonella Group. All the experimental evidence at hand leads to the presumption that the implication of staphylococci in outbreaks of food poisoning is at times rather difficult, inasmuch as one has to depend upon feeding experiments with human volunteers (which are not always available) or on monkeys which may at times be rather unreliable as to the manifestations resulting from the feeding of the filtrates.

Three outbreaks are described in which the epidemiological evidence points to a food infection but further studies could not detect a definite etiological agent. Since portions of the original food consumed could not be obtained for examination, we had to depend more on the bacteriological examination of the feces in one outbreak, the larger, and results were essentially negative. The epidemiological characteristics of the three outbreaks were similar in many respects to those of cases brought about by food contaminated with bacilli of the Salmonella Group or with staphylococci capable of liberating an enterotoxin.

### SUMMARY

 We have presented thirteen outbreaks of food poisoning studied epidemiologically with special reference to their etiology, occurring since 1933, when we published a résumé of our studies and observations on a number of similar outbreaks together with a historical review of these conditions since the early days of the colonization of our Island.
 Although we have been unable to detect any cases of botulism,

2. Although we have been unable to detect any cases of botulism, we believe that the condition must exist in our Island, inasmuch as conditions here are favorable for the contamination of the food with bacillus botulinus and the development of its exotoxin in its growth in the food. 3. In our studies we have included four outbreaks of epidemic form produced by members of the Salmonella Group; two by bacillus aertrycke (Group B; typhi murium), one by bacillus enteritidis (Group D), one by an organism belonging to Group D, (somatic O antigen IX with an unidentified flagellar antigen, apparently a new species), and six outbreaks in which there was found sufficient epidemiological evidence or suspicious findings in the experimentation with animals to lead us to believe that they were produced by toxic products (enterotoxin) elaborated by the staphylococcus. In three outbreaks although the epidemiological characteristics pointed to poisoning by food, no definite etiological agent could be ascertained.

4. Bacilli of the Salmonella Group have been isolated from the organs and feces of wild rats captured in the commercial and residential sections of San Juan, Puerto Rico, and also associated with a fatal septicemic disease in pigs.

5. Food infections are rather prevalent in Puerto Rico although the cases on record are few because the majority pass unnoticed as they are not seen by physicians, and those that receive medical attention are not always reported to the proper health authorities.

6. The epidemiology of food infections and intoxications in Puerto Rico, a small but densely populated tropical island with limited economic resources, follows more or less the same trends as in the Temperate Zones. Our climate is a favorable factor for the growth and development of bacteria with which food may become infected before, during, or after its preparation for human consumption.

#### REFERENCES

- Costa Mandry, O. Food Poisoning in Puerto Rico. P.R. Jr. P.H. & T. Med. 9:44. 1933.
- Costa Mandry, O. Food Infections in Puerto Rico. A Bacteriological and Epidemiological Study of Three Outbreaks. P.R. Review of Public Health and Tropical Medicine. 4:128. 1928.
- 3. Costa Mandry, O. Envenenamientos por substancias alimenticias. Bol. Asoc. Méd. de P.R. 21:31. 1928.
  - Bol. de la Rev. de Hig. y T.B.C. de Valencia, España. Núm. 171:28, Núm. 172:38, Núm. 173:42. 1930.
- Costa Mandry, O. and Garrido Morales, E. Food Poisoning in a Porto Rican Family due to Arsenic. Bol. Asoc. Méd. de P.R. 22:200. 1930.
- Costa Mandry, O. Infecciones intestinales por ingestión de alimentos proteicos contaminados. Bol. Asoc. Méd. de P.R. 23:414. 1931.
- 6. Costa, Mandry, O. Envenenamientos por substancias alimenticias. P.R. y su Enfermera 8: Núms. 26 y 27. 1932.
- 7. The Genus Salmonella Lignières. 1900. Salmonella Subcommittee of the

Nomenclature Committee, International Society for Microbiology. Jr. Hyg. 34:333: 1934.

- 8. Jordan, E. O., and Burrows, William. A textbook of General Bacteriology. 12th Edition, 1938. W. M. Saunders Co., Philadelphia and London.
- White, P. Bruce. Further Studies of the Salmonella Group. Privy Council, Medical Research. Council Special Report Series 103. H.M. Stationery, London, 1926.
- 10. Scott, W. A. (Personal written communications.)
- 11. Jordan, E. O. Food Poisoning and Food-Borne Infections. University of Chicago Press. 1931.
- 12. Costa Mandry, O. The Rat as a Carrier of Organisms of the Food Poisoning Group in Puerto Rico. P.R. Health Bulletin. 2:75. 1938.
- Costa Mandry, O. Notes on the Intestinal Flora of Wild Rats in San Juan, P.R. P.R. Health Bulletin. 2:297. 1938.
- 14. Morales-Otero, P. (Personal communication.)
- 15. Barber, M. A. Milk Poisoning Due to Staphylococcus albus Occurring in the Udder of a Healthy Cow. Phil. Jr. Sc. 9:515. 1914.
- Jordan, E. O. The Production by Staphylococcus of a Substance Producing Food Poisoning. Jr. A.M.A. 94:1648. 1930.
- 17. Jordan, E. O. Staphylococcus Food Poisoning. Jr. A.M.A. 97:1704. 1931.
- 18. Jordon, E. O., and Burrows, William. Further Observations on Staphylococcus Food Poisoning. Am. Jr. Hyg. 20:604. 1934.