APPLICATION OF THE
HAZARD ANALYSIS CRITICAL CONTROL POINT
(HACCP) SYSTEM

for the

IMPROVEMENT OF FOOD SAFETY

WHO-Supported Case Studies on Food prepared in
Homes, at Street Vending Operations, and in Cottage Industries

WORLD HEALTH ORGANIZATION
FOOD SAFETY UNIT
1993
Introduction

Foodborne diseases, i.e. illnesses due to contaminated food, are one of the most widespread health problems of the contemporary world, and an important cause of reduced economic productivity. In addition to human suffering, foodborne diseases cause substantial economic losses. These include loss of income, loss of manpower, medical care costs, loss of food, and a decrease in tourism and food export opportunities. Unfortunately, foodborne diseases are increasing in number and frequency all over the world, and in spite of the efforts that are being made the problem is likely to continue to grow if new strategies are not applied.

The logical conclusion is that the traditional approaches, for both food control and for prevention of foodborne diseases, are insufficient. This is true even in industrialized countries with a sophisticated and costly formal food safety infrastructure. Therefore, a concerted approach to food safety is needed. The World Health Organization's suggestion for this concerted approach is to combine an effective food safety infrastructure with an adequate educational programme. The design of this programme should be based on a combination of two types of information: (a) the information on the sociocultural and economic situation; and (b) the technical information related to food preparation and food habits obtained through the application of the Hazard Analysis Critical Control Point system (HACCP).

HACCP is a rational method, relatively new, for the prevention of foodborne diseases. It consists in the identification of hazards associated with any stage of food production, processing or preparation, the assessing of the related risks, and the determination of the operations where control procedures will be effective.

Through its Food Safety unit at Geneva and its six Regional Offices, the World Health Organization, convinced of the utility and importance of HACCP for improving the safety of foods, has been encouraging the development and application of this system in the integrality of the food chain, from production until consumption.

In particular, as pointed out above, WHO believes that health education in food safety has to be developed in line with the results obtained through the application of HACCP during the preparation and storage of foods in homes, food service establishments, cottage industries, and street markets. For this reason, some years ago WHO promoted and financed the practical application of HACCP during the preparation of foods in homes, by street vendors and in cottage industries in selected developing countries. Intensive courses on HACCP were also organized in these countries in order to discuss the results obtained with national experts, and to motivate local teams to continue such studies. Several papers and articles giving the results of the above-mentioned studies have been published in internationally-renowned journals.

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The purpose of this document is to still further disseminate the principal articles and papers published with WHO support, bringing them together in one volume. Particular attention is given to the experimental studies performed in Peru, the Dominican Republic and Pakistan.

The support of the German Agency for Technical Cooperation (GTZ) and the Industry Council for Development (ICD) is specially acknowledged, particularly with regard to the research work undertaken in Pakistan. We would also like to express our thanks to the editors of the journals in which the papers were originally published for their permission to reproduce them in this document.
Hazard Analyses of Foods Prepared by Inhabitants Along the Peruvian Amazon River

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ABSTRACT

Hazard analyses of food preparation practices were conducted in two households in Indiana (a settlement along the Peruvian Amazon River), in a household in a cluster of about a half dozen houses up river, and in three households in Belen (a district near Iquitos), Peru. These analyses consisted of watching all steps of the operation, recording temperatures throughout all these steps, and collecting samples of food and testing them for common foodborne pathogens and indicator organisms. Foods prepared included rice, plantains, yuca, dry fish, fresh fish, beef, and chicken. During cooking, foods attained temperatures of at least 93.3°C; they usually boiled. Such time-temperature exposure would kill vegetative forms of foodborne pathogenic bacteria, but not heat-resistant spores. When cooked foods were leftover, they were kept either on tables or on the unheated stoves or grills on which they were cooked. During this interval, at the prevailing ambient temperature and high humidity of the jungle region, conditions were such that considerable microbial growth could occur. Time of exposure, however, limited counts to the 10^5 - 10^6 level. In the evening, foods were only mildly reheated, if reheated at all, so temperatures were not attained in the center regions of the food that would have killed microorganisms that had multiplied during the holding period. Hence, the primary critical control point is holding between cooking and serving, but cooking and reheating are critical control points also.

The hazard analysis critical control point (HACCP) approach consists of (a) determination of hazards and assessment of their severity, and the risks they pose; (b) identification of critical control points required to prevent or control significant hazards; (c) establishment of preventive and control measures and criteria; (d) monitoring of each critical control point, and (e) implementation of appropriate and immediate corrective action whenever the criteria are not met. Hazards relate to contamination of foods by microorganisms and to their survival during processing and multiplication during storage. A critical control point is an operation (location, practice, procedure, or process) that if not under control could lead to unacceptable contamination, survival or growth of undesirable microorganisms. Monitoring is the checking that the process or handling procedure at the critical control points is properly carried out.

This approach is applicable to evaluate food safety in homes as well as food processing and foodservice establishments (16). Furthermore, a FAO/WHO Expert Committee on Food Safety recommended that the HACCP approach be used in homes in developing countries to get a greater insight into hazards associated with food preparation and applicable preventive measures (7).

The feasibility of using this approach in households was tested in Peru because of the country's diverse geographic and climatic conditions and the different cultural groups that live there. A determining factor for these diversities and differences is the Andes mountain chain which creates three natural regions: the coastal, the mountains and the jungle. Hazard analyses reported here were conducted in households along the Peruvian Amazon. Food-preparation hazards in households in the other regions were described in two related papers.

MATERIALS AND METHODS

Description of the region, population and diet

The forested eastern slopes of the Andes and the jungle beyond covers more than half of Peru. Many rivers descend from the mountains through the high jungle to form the Amazon basin in the lower jungle. Vegetation is extensive, but the soil is poor for agriculture. The climate is warm and humid, and there is a rainy season.

The Department of Loreto, which contains much of the lower jungle, is inhabited by only 2.7% of the population of Peru, mostly
Description of families

Two families from Indiana were surveyed. Family 1 consisted of nine persons who lived in a thatch-roofed (palm) open-sided wooden hut on stilts. It was located on high ground above the Amazon River. Water was obtained in buckets from the river; it was allowed to settle but it was not boiled before drinking. There was no latrine. A meal of cooked dry-salted fish and plantains was prepared at the time of the survey; all foods were eaten. Cooking was done outside on a wire grill on bricks over a wood fire.

Family 2 consisted of five persons (children 8, 4, and 2); the youngest had diarrhea. They lived in a two-room, wooden house with an attached open kitchen; floors were of wood and the roof of tin. It was located on a street along with several other houses well away from the river. The family kept chickens which roosted in trees and scavenged in the yard for food. A drop privy was located in the backyard above an intermittently-wet creek. Water for drinking and cooking was carried to the house in buckets or pans from the Amazon River. Drinking water was boiled and cooking done on a kerosene stove. Oats, rice, plantains, and fried eggs were prepared at the time of the survey. Leftover rice and a mixture of rice, boar meat and plantains were kept at ambient temperature overnight in the kitchen.

A two-family group (Family 3) of eight persons lived in a small settlement of four houses near Timicuro, about 20 min by boat from Indiana. The family was selected for the analysis because the baby of 11 months had suffered from vomiting, diarrhea, and dehydration for 8 d and had been hospitalized and treated with intravenous saline solution for this condition. The house was constructed of wood with open sides. It was on stilts and had a tin roof. Water was obtained in buckets from the Amazon River; it was not boiled before drinking. Human waste were excreted nearby under cover of the bush. Chickens, ducks and dogs were kept around the house, and they would come into the house at will to drink water from the family water vessel. Cooking was done either in aluminum pans on a wire grill on bricks or directly on the grill over a wood fire. Oats, salt-dried fish, plantains, and yuca were prepared. Eating was done by either metal utensil or by hand. The baby was fed oats, fish and plantain mixture, and sugar water in addition to nursing. Leftover unwanted foods were thrown from the house to the yard for the animals.

A three-family household in Belen was also surveyed. This group lived in a wooden house which was on tall stilts. It had a tin roof and one side was open. One bedroom was partitioned off, but the rest of the house was shared by the three families, at least 16 people. Municipal water was piped just behind the house, but it was only available for approximately an hour in the evening. At this time, residents collected water in pans and buckets and transferred it to a 55-gal. drum (200-L) or to other receptacles. A private latrine was nearby. Garbage was piled at a nearby road and a large number of vultures scavenged the area. Each family had a separate kerosene stove. Family 4 prepared plantains, rice, and meat; family 5 prepared beans, rice, plantains, and fish stew; family 6 prepared plantains, rice, and potatoes with chicken stew at the time of the visit. In all instances the foods were cooked in either aluminum or enamel pans and served hot for lunch and then held without heat until the evening meal (19:00 to 19:30 h) at which time they were either eaten cold or re-heated.

Hazard analyses

The hazard analyses were performed in January (summer) and consisted of collecting specimens and samples, measuring temperatures, and watching food preparation. Dropings from animals that frequented the houses or were kept in the immediate environment of the houses were also collected. Samples of drinking water and sometimes of water used for food preparation or utensil washing were collected. Samples of foods were taken either after cooking, during holding, or after reheating, as appropriate to evaluate a potential hazard. Ingredients (e.g., spices) were sometimes sampled. Temperatures of the interior of foods were taken throughout preparation (cooking, holding, after preparation, and reheating, as appropriate by inserting a thermocouple (type-T with either a bayonet-type sensor of appropriate length or a sensor with wires twisted a fused at the end) into the approximate geometric center of the food being measured. It was difficult, however, to maintain this location throughout the duration of measurement. The thermocouples were plugged into a type-T data logger (potentiometer) (MLX Minilogger, A. D. Data Systems, Inc., Rochester, N.Y.). Time at which temperature measurements were taken was given by the data logging instrument. Observations were made of likely sources of contamination and opportunities for cross contamination. Samples were tested for aerobic mesophilic colony count (AMCC), Salmonella, Shigella, Staphylococcus aureus, Clostridium perfringens, Bacillus cereus and Escherichia coli biotype 1, as applicable, depending on the nature of the samples and the organisms that would be likely contaminants.

Data were evaluated by diagramming the steps of food preparation. Potential sources of contamination (raw foods, equipment and utensils, and persons preparing the food) are noted on the diagram. Likelihood of survival or destruction and likelihood of microbial growth are also shown. Critical control points of the operations that require monitoring are indicated. Graphs were made of the time-temperature exposures of each food prepared during the observations.

Housewives or other persons who prepared foods were requested to do so in their usual manner and were not given any special instructions. Operations were observed to identify sources and routes of contamination by the person preparing the foods, utensils, equipment surfaces, animals, foods of animal origin or by any other circumstances which could have led to contamination. Members of the household were asked if there were cases of diarrhea, especially in children.
Sampling procedure

Initial samples of cooked and uncooked foods were collected with sterile metal spoons or forks and aseptically put into sterile plastic bags. For samples taken later, the previously used units were immediately precooled either under cold water or in ice. They were kept in contact with ice in a covered styrofoam box.

Samples of water were poured aseptically into either sterile plastic bags or sterile flasks with screw caps. Samples from tables, cutting boards, or knives were taken by rubbing the surface with a sterile polyurethane sponge (13), previously moistened with 0.1% peptone water and aseptically put into a wide mouth flask containing the same diluent. Samples of droppings from animals were taken from the yard or floor with a sterile spatula and aseptically put into the bags.

Samples and specimens were taken to the laboratory on the day of collection and either examined on that day or kept refrigerated overnight and tested the following day.

Laboratory procedures

For most of the foods 10 g or 10 ml were homogenized with 0.1% peptone water in screw-capped flasks containing glass beds by means of horizontal and vertical manual agitation for a few seconds. AMCC were made by the pour plate method. Plate count agar (Difco) with the addition of triphenyltetrazolium chloride (TTC) to give a final concentration of 0.01% was used. Incubation was at 35°C for 48 h.

For water samples, the filtration method was used (membrane Sartorius pore size 0.45 μm). Amounts of 100 ml of water, when available, were filtered. The medium for AMCC was as stated above.

E. coli biotype 1 was enumerated by the Anderson Baird-Parker (1) method in tryptone bile agar (prepared from ingredients) by using cellulose acetate filter membranes (pore size 0.45 μm; 85 mm diameter [Oxoid] or 90 mm Gelman). A loopful of solution from samples was streaked onto the surface of the membrane which had previously been placed on top of the same medium. Further confirmation of E. coli biotype 1 was by the IMVIC tests.

Presumptive B. cereus was enumerated by spreading 0.25 ml of each homogenate and of their dilutions on each of four Petri dishes containing phenol-red-egg-yolk-polymyxin (Merck). Incubation was at 35°C for 24 h (11). Starch, citrate, Voges-Proskauer and motility-nitrate tests were used for confirmation.

S. aureus was enumerated by spreading 0.25 ml of the homogenates and their dilutions on each of four Petri dishes with Baird-Parker agar (Oxoid). These were incubated at 35°C for 48 h (6). Further confirmation was on the basis of the results of DNAse test (DNAse agar, Difco), coagulase production (rabbit plasma EDTA, Difco), thermostable nuclease production (medium made from ingredients) following ICMSF (11) procedures. Haemagglutination (staphylslide test, bio-Merieux) was also performed.

C. perfringens was enumerated by using ICMSF (11) procedures (method 2) modified by inoculating 5 ml of the homogenates and their dilutions into sulfite cycloserine agar (prepared from ingredients) in roll tubes. Gelatin-lactose and motility-nitrate tests were used for confirmation.

For isolation and confirmation of Salmonella, procedures recommended by ICMSF (11) were followed. Nutrient broth (Merck) was used as a non-selective pre-enrichment medium, and selenite (Difco), tetraphionate brilliant green (Difco) and Rappaport broths (prepared from ingredients) were used as selective enrichments. Incubation was at 43°C for selenite and tetraphionate broths and 35°C for Rappaport broth. Isolations were made at 24 and 48 h by streaking on brilliant green, bismuth sulfite, Salmonella-Shigella and desoxycholate citrate agar (all from Difco). Animal dropping specimens were put directly onto plating media as well as into selective broths. Polyvalent OMA and OMB antisera (Pasteur Institute) were used for grouping.

For isolation of Shigella, procedures of ICMSF (11) were used. Samples of 25 g were enriched in gram-negative broth (prepared from ingredients), and xylose lysine desoxycholate (Difco), Hektoen (Oxoid) and MacConkey agars (Difco) were used as selective isolation media. Membranes used to filter water samples were put into GN broth after filtration.

RESULTS

Flow processes, hazards and critical control points of preparation of rice, pango (fish and plantains), beef with sauce, fish, and chicken stew, respectively, are presented in Fig. 1-5. Figures 6-11 illustrate time-temperature exposures of all food cooked in each household during the hazard analyses. Results of testing for Salmonella and bacterial counts of foods prepared in the households are summarized in Table 1. Potential sources of foodborne pathogenic microorganisms observed at the time of the analyses are summarized in Table 2.

Family 1. Dried, salted fish (boquichico) boiled and eaten by the time its temperature dropped to 71.1°C (160°F) (Fig. 6). The temperature that plantains reached during cooking was not determined; they were below 48.9°C (120°F), however, for less than 20 min during holding after cooking. No foods were left over.

Neither Salmonella nor Shigella was isolated from the foods (Table 1) or river water before and after settling. AMCC and E. coli biotype I count were less than 1/ml in water-diluted grapefruit juice. Salmonella was also not isolated from a sample of water used to wash fish nor from a sponge-wipe sample from the surface of a table used for preparing food.

Family 2. Rice, oats, and plantains reached 100°C (212°F) during cooking and were served at temperatures above 73.9°C (165°F)(Fig. 7). Leftover rice and rice-boar meat-plantain mixture were held overnight in the kitchen at ambient temperature. AMCCs were 1.6x10^9/g or greater.

Neither Salmonella nor Shigella was isolated from the foods (Table 1), Amazon River water or stream water. Salmonella was not isolated from water that had been previously boiled and kept in a covered pan. It was also neither recovered from shells of several eggs nor from chicken feces. Family 3. During cooking, foods reached at least 93.3°C (200°F), often 100°C (212°F). After the flame died down,
Figure 1. Preparation of rice in households along the Amazon River (Families 2 and 3) and in Belen (Families 4.5, and 6).

Figure 2. Preparation of fish with plantains in households along the Amazon River (Families 1 and 3).

food temperatures dropped rather rapidly to 48.9°C (120°F) and remained between this temperature and 26.7°C (80°F) for 285 to 405 min (Fig. 8). It is quite probable that these foods remained within this range for an additional 120 min before the evening meal was eaten, but the survey was terminated before this time.

Neither Salmonella nor Shigella was isolated from the foods (Table 1), a fecal specimen from the baby, or from a sample of river water. Salmonella was not isolated from uncooked or cooked rice, dish-washing water, surfaces of a knife and a table surface, feces from dogs or feces from chickens and ducks.

Family 4. Both rice and beef boiled during cooking; they remained at this temperature for more than 25 min (Fig. 9). The meat remained between 48.9°C (120°F) and 32.2°C (90°F) for 330 min, the rice for 285 min, and plantains for at least 375 min before reheating. During reheating meat only-reached 43.8°C (111°F) and rice only reached 48.3°C (119°F). Plantains were not reheated.

AMCC of papaya juice was 2x10^3/ml. E. coli biotype I was less than 1/ml. AMCC of uivos (Spondia sp.), a jungle
HAZARD ANALYSIS OF HOME-PREPARED FOODS

Figure 3. Preparation of beef with sauce in household in Belen (Family 4).

Figure 4. Preparation of fish stew in household in Belen (Family 5).
Figure 5. Preparation of chicken stew in household in Belin (Family 6).

Figure 6. Temperature of internal portions of food during preparation in household of family 1, along the Amazon River, Peru.

Figure 7. Temperature of internal portions of foods during preparation in household of family 2, along the Amazon River, Peru.
HAZARD ANALYSIS OF HOME-PREPARED FOODS

Figure 8. Temperature of internal portions of foods during preparations in household of family 3, along the Amazon River, Peru.

Figure 9. Temperature of internal portions of foods during preparation in household of family 4, in Belen, Peru.

Figure 10. Temperature of internal portions of foods during preparation in household of family 5, in Belen, Peru.

Figure 11. Temperature of internal portions of foods during preparation in household of family 6, in Belen, Peru.

TABLE 1. Results of testing for Salmonella and bacterial counts of foods cooked in households along the Amazon River and in Belen.

<table>
<thead>
<tr>
<th>Family</th>
<th>Food Sample</th>
<th>Time of day foods sampled</th>
<th>AMCC/g</th>
<th>E. coli bio1 type</th>
<th>S. aureus/g</th>
<th>B. cereus/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dried, salted uncooked fish</td>
<td>16.30</td>
<td>&gt;1.6x10⁵</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cooked dried salted fish</td>
<td>17.00</td>
<td>3.2x10⁵</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cooked plantains</td>
<td>20.00</td>
<td>&gt;1.6x10⁵</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Rice-boar meat-plaitain dish leftover from lunch</td>
<td>20.00</td>
<td>1.2x10⁵</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cooked oats</td>
<td>20.00</td>
<td>&lt;10</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rice leftover from evening meal held at kitchen</td>
<td>06.00</td>
<td>&gt;1.6x10⁵</td>
<td>&lt;10</td>
<td>-</td>
<td>2.0x10²</td>
</tr>
<tr>
<td></td>
<td>temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Dried, salted fish left-</td>
<td>08.00</td>
<td>&gt;1.6x10⁵</td>
<td>&lt;10</td>
<td>1.0x10³</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>over from previous day</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

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was 1.3x10^5/g, longer holding periods could result in higher counts and associated enterotoxin. Critical control points for fish and pango (fish and plantains) are cooking and the interval between cooking and serving. Reheating becomes an additional critical control point, if practiced.

Critical control points for stews and other dishes made from beef, fish, and chicken are (a) cooking, (b) holding between cooking and eating and (c) reheating. Making sure that foods containing liquids boil and simmer during cooking and reheating, determining whether cooked foods are eaten promptly and observing time between cooking and serving at subsequent meals are other means of monitoring these critical control points that can be used by the person who prepares the foods.

Means by which hazards, critical control points, preventive measures, and practical monitoring procedures can be communicated to the inhabitants of the jungle become a major obstacle to food safety. Alerting public health and clinical personnel in health centers so that they can pass information to families of persons who come for treatment of diarrhea can be helpful. Adequately trained local medical practitioners can also be used for this purpose. The “Voice of the Jungle” radio could be used to transmit messages about food hazards and their prevention, but presentations would have to be done in local dialects. Long range solutions to achieve food safety rests in education of children in school and training of teachers.

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REFERENCES

Hazard Analyses of Foods Prepared by Migrants Living in a New Settlement at the Outskirts of Lima, Peru

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ABSTRACT

Hazard analyses of food preparation practices were conducted in three households in a new settlement in the rocky, dusty hillside at the outskirts of Lima, Peru. These analyses consisted of watching all steps of preparation, recording temperatures throughout these steps, and collecting samples of the food and testing them for common foodborne pathogens and indicator organisms. The residents had migrated from different regions of the country; consequently, they prepared different foods. These included soya cereal, milk formula, rice and carrots for feeding a baby who had diarrhea, foot soup, beans, rice and a mixture of beans and rice. The common foodborne pathogens and indicator organisms. The residents at the outskirts of Lima, Peru. These analyses consisted of watching all steps of preparation, recording temperatures throughout these steps, and collecting samples of the food and testing them for common foodborne pathogens and indicator organisms. The residents had migrated from different regions of the country; consequently, they prepared different foods. These included soya cereal, milk formula, rice and carrots for feeding a baby who had diarrhea, foot soup, beans, rice and a mixture of beans and rice. The temperatures attained were high enough to kill vegetative forms of foodborne pathogens, but not their spores. During the interval between cooking in the morning and serving at either lunch or supper time, foods were held either on unheated ranges or in unheated ovens. This interval was long enough to permit some bacterial multiplication, but apparently not to massive quantities. Just before the evening meal, foods were reheated to temperatures that usually exceeded 70°C. Rice, however, was either served cold or if reheated, the center temperature rose a few degrees only. Critical control points for preparation of family meals are cooking, holding between cooking and serving, and reheating. Critical control points for milk formula for babies are using recently-boiled water for the formula, cleaning and boiling bottles and nipples, and, of particular importance, time of holding at room temperature.

The hazard analysis critical control point (HACCP) approach consists of (a) determination of hazards and assessment of their severity and the risks they pose, (b) identification of critical control points required to prevent or control significant hazards, (c) establishment of preventive and control measures and criteria, (d) monitoring of each critical control point, and (e) implementation of appropriate and immediate corrective action whenever the criteria are not met. Hazards relate to contamination of foods by microorganisms and to their survival during processing and multiplication during storage. A critical control point is an operation (location, practice, procedure, or process) that if not under control could lead to unacceptable contamination, survival or growth of undesirable microorganisms. Monitoring is the checking that the process or handling procedure at the critical control points is properly carried out.

This approach is applicable to evaluate food safety in homes as well as food processing and foodservice establishments (9). Furthermore, a FAO/WHO Expert Committee on Food Safety recommended that the HACCP approach be used in homes in developing countries to get a greater insight into hazards associated with food preparation and applicable preventive measures (3).

The feasibility of using this approach in households was tested in Peru because of the country’s diverse geographic and climatic conditions and the different cultural groups that live there. A determining factor for these diversities and differences is the Andes mountain chain which creates three natural regions: the coastal desert, the mountains and the jungle. Hazard analyses reported here were conducted in households of migrants in a new settlement at the outskirts of Lima in the coastal desert. Food-preparation hazards in households in the other regions are described in two related papers.

MATERIALS AND METHODS

Description of the region and neighborhood

Lima is located in the coastal desert region on the shore of the Pacific Ocean; foothills of the Andes begin at its outskirts. Rain seldom falls in this region.

Many Peruvians have left poverty-stricken rural regions in search of employment and a better life in the city. Because of this migration and urbanization, Lima has grown considerably in the past decades. In 1958 its population amounted to about 1 million inhabitants, currently it is approximately 5.5 million.
Chichen meat, liver, gizard, heart

CCP: temperature (boil)

Cook

Hold on range at ambient temperature

Reheat

Serve

Water

Potatoes

Squash

Carrots

Rice

Leeks

Salt

Cut

Peel

Cut

Cut

Cut

Cut

Legend

Δ Initial contamination likely

Δ Contamination from equipment/utensil surfaces likely

△ Contamination from persons handling foods likely

CCP Critical control point

X Inactivation of vegetative forms of bacteria likely

O Survival of vegetative forms of bacteria likely

+ Propagation of bacteria likely

S Spores

Figure 1. Preparation of chicken-vegetable-rice soup in household of migrants to Lima (Family 1).

Many of the migrants live in houses or shanties constructed of brick, concrete block or woven mat in new settlement (Pueblos Jóvenes shanty settlements of squatters) located in rocky, dusty hill-sides in areas surrounding Lima. Some of these settlements lack running water and sewerage, while in others these facilities have recently been installed or are being constructed.

The community chosen for this investigation was one of such settlements. Dirt roads led to houses other than those on the hillside, which tended to be of poorer construction than those at road level. Water and sewerage were being installed, but at the time of the survey, water was delivered by truck and sewage disposal was on an individual basis. This Pueblo Jóvenes had a health center, primary school, nearby open market and bus service. The foods eaten by the inhabitants varied according to their ethnic background, place of origin, economic status, and food-preparation facilities. The National Institute of Nutrition had a nutrition-improvement program which provided food supplements to mothers of young children in the settlement. Three families and a Mother's Club where bulk foods were prepared were evaluated.

Description of the families surveyed

Family 1 lived in a three-room concrete block house with attached store, wash room and animal-pen patio. The floor was earth. Water was stored in drums and a tank. Chickens, turkeys, a cat and a dog were kept in the patio and occasionally came into the house. The family which originally came from the mountain region consisted of parents and four children, the youngest (11 months) had diarrhea. The baby was fed a mixture of evaporated milk and water, soya-flour cereal that was fortified with vitamin A and calcium, soup and starch cooked off a mixture of rice and carrots for the treatment of diarrhea. A soup of carrots, potatoes, chicken meat, liver, gizard and heart was the primary food prepared (Fig. 1). It was served hot at noon and held on the unheated range for serving at the evening meal. Cooking was done on a gas stove. A refrigerator was used to store cold water, raw perishable foods, soft drinks, and leftover cereal. A thermos was used to store boiled water for milk-formula preparation (Fig. 2) during the night and to hold liquid foods (e.g., starch preparation) for the baby during the day.

Family 2 had migrated from the jungle region and lived in a two-family cement block house with concrete floor and tin roof. Twelve persons lived in five rooms of the double house, which consisted of a dining and water storage room, kitchen, small bedroom, "dormitory" room, and sewing room with a bed for an elderly person. An enclosed yard contained a pen for a pig, a larinje, a laundry area, and an area with some flowers. One woman had a...
Figure 2. Preparation of milk-water-sugar formula for feeding baby (Family 1), near settlement, Lima, Peru.

Figure 3. Preparation of mashed potatoes with spinach and milk in a household of migrants to Lima (Family 2).

Figure 4. Preparation of beans and rice in household of migrants to Lima (Family 3).

4-year-old child, and a baby of 11 months who had diarrhea. The other woman had a 3-year-old child. Water from a 55-gal. (200-L) drum outside the house was brought into the house by buckets and stored in several other drums. The women prepared foods together. Foods were cooked on two stoves, a kerosene stove and a gas stove. A refrigerator located in the dining room was used to store water and fresh vegetables. Food prepared on the day of the survey were a salad of carrots, beets and lettuce with home-made mayonnaise; a soup containing spaghetti, milk, onions, oil, paprika, garlic and other seasonings; rice with oil, garlic and salt; and mashed potatoes containing spinach and milk (Fig. 3). These foods were prepared in the morning, served hot for lunch and held on top of the stove without heat until the evening meal (at approximately 20:00 h) when they were reheated before serving.

Family 3 lived in a one-room mat house with earth floor and no windows. The family, which had migrated from another city in the coastal desert region, included four children, the youngest, who was still nursing, had loose stools. Water from a 55-gal. (200-L)
drum was carried up hill to the house and stored in another drum and in pans. The family had two dogs as pets. Potatoes and corn were stored on the floor. Cooking was done on a kerosene stove. Oats with peeled apples were served at breakfast and fed to the baby. Beans (Fig. 4), rice (Fig. 4) and cow's foot soup (Fig. 5) were prepared in the morning and served hot at lunchtime and then held at ambient temperature until supper when they were reheated. A salad of tomatoes and onions was also prepared.

The Mother’s Club had facilities for preparing, cooking, dispensing and serving foods in a structure made of concrete block with a concrete floor. It was located at the back of the school. Club members prepared different menu items 6 days a week. Preparation started at about 10:00 h, and foods were ready for sale around noon; most of the foods were sold by 14:00 h. Local residents usually purchased a container of food and took it home, although a few persons ate at the facility. On the day of the visit, Cau-Cau (a stew made of tripe and potatoes) and rice were prepared. Two samples of each were collected at approximately 13:30 h. One of each was taken to the laboratory; the other was kept at room temperature to simulate holding in homes and temperatures were measured periodically until 19:30 h. At that time, sub-samples were collected and refrigerated until delivery to the laboratory.

Hazard analyses

The hazard analyses were performed in January (summer) and consisted of collecting specimens and samples, measuring temperatures, and watching food preparation. Fecal material from babies having diarrhea was collected. Droppings from animals that frequented the houses or were kept in the immediate environment of the houses were also collected. Samples of drinking water and sometimes of water used for food preparation or utensil washing were collected. Samples of foods were taken either after cooking, during holding, or after reheating, as appropriate to evaluate a potential hazard. Ingredients were sometimes sampled. Temperatures of the interior of foods were taken throughout preparation (cooking, holding after preparation, and reheating, as appropriate) by inserting a thermocouple (type-T with either a bayonet-type sensor of appropriate length or a sensor with wires twisted and fused at the end) into the approximate geometric center of the food being measured. It was difficult, however, to maintain this location throughout the duration of measurement. The thermocouples were plugged into a type-T data logger (potentiometer) (MLX Minilogger, A. D. Data Systems, Inc., Rochester, N. Y.). Time at which temperature measurements were taken was given by the data logging instrument. Observations were made of likely sources of contamination and opportunities for cross contamination. Samples were tested for aerobic mesophilic colony count (AMCC), Salmonella, Shigella, Staphylococcus aureus, Clostridium perfringens, Bacillus cereus and Escherichia coli biotype 1, as applicable, depending on the nature of the sample and the organisms that would be likely contaminants.

Data were evaluated by diagramming the steps of food preparation. Potential sources of contamination (raw foods, equipment and utensils, and persons preparing the food) are noted on the diagram. Likelihood of survival of destruction and likelihood of microbial growth are also indicated. Critical control points of the operations that require monitoring are indicated. Graphs were made of the temperature-time exposures of each food prepared during the observations.

Housewives or other persons who prepared foods were re-
quested to do so in their usual manner and were not given any special instructions. Operations were observed to identify sources and routes of contamination by the person preparing the foods, utensils, equipment surfaces, animals, foods of animal origin or by any other circumstances which could have led to contamination. Members of the household were asked if there were cases of diarrhea, especially in children.

**Sampling procedures**

Initial samples of cooked and uncooked foods were collected with sterile metal spoons or forks and aseptically put into sterile plastic bags. For samples taken later, the previously used sampling utensils were washed and then dipped into 95% alcohol and flame, the latter two steps being repeated three times each. The bagged samples taken from cooking or hot-holding units were immediately precooled either under cold water or in ice. They were kept in contact with ice in a covered styrofoam box.

Samples of water were poured aseptically into either sterile plastic bags or sterile flasks with screw caps. Samples from tables, cutting boards, or knives were taken by rubbing the surface with a sterile polyurethane sponge (7), previously moistened with 0.1% peptone water and aseptically put into a wide mouth flask containing the same diluent.

Fecal-material specimens from babies were collected by rubbing a sterile swab or sponge across the anal region or from fecally-stained diapers and placed into a sterile plastic bag with 0.1% peptone water. Samples of droppings from animals were taken from the yard or floor with a sterile spatula and aseptically put into the bags.

Samples and specimens were taken to the laboratory on the day of collection and either examined on that day or kept refrigerated overnight and tested the following day.

**Laboratory procedures**

For most of the foods, 10 g or 10 ml were homogenized with 0.1% peptone water in screw-capped flasks containing glass beads by means of horizontal and vertical manual agitation for a few seconds. AMCCs were made by the pour plate method. Plate count agar (Difco) with the addition of triphenyltetrazolium chloride (TTC) to give a final concentration of 0.01% was used. Incubation was at 35°C for 48 h.

For water samples, the filtration method was used (membrane Sartorius pore size 0.45 µm). Amounts of 100 ml of water, when available, were filtered. The medium for AMCC was as stated above.

E. coli biotype I was enumerated by the Anderson Baird-Parker (1) method in tryptone bile agar (prepared from ingredients) by using cellulose acetate filter membranes (pore size 0.45 µm; 85 mm diameter [Oxoid] or 90 mm Gelman). A loopful of solution from samples was streaked onto the surface of the membrane which had previously been placed on top of the same medium. Further confirmation of E. coli biotype I was by the IMVIC tests.

Presumptive B. cereus was enumerated by spreading 0.25 ml of each homogenate and of their dilutions on each of four Petri dishes with phenol-red-egg-yolk-polymixin (Merck). Incubation was at 35°C for 24 h (5). Starch, citrate, Voges-Proskauer and motility-nitrate tests were used for confirmation.

S. aureus was enumerated by spreading 0.25 ml of the homogenates and their dilutions on each of four Petri dishes with Baird-Parker agar (Oxoid). These were incubated at 35°C for 48 h (5). Further confirmation of the presence of S. aureus were carried out on the basis of the results of DNase test (DNase agar, Difco), coagulase production (rabbit plasma EDTA, Difco), thermostable nuclease production (medium made from ingredients) following ICMSF (5) procedures. Hemagglutination (staphyloide test, bio-Merieux) was also performed.

C. perfringens was enumerated following ICMSF (5) procedures (method 2) modified by inoculating 5 ml of the homogenates and their dilutions into sultone cycloserine agar (prepared from ingredients) in roll tubes. Gelatin-lactose and motility-nitrate tests were used for confirmation.

For isolation and confirmation of Salmonella, procedures recommended by ICMSF (5) were followed. Nutrient broth (Merck) was used as a non-selective pre-enrichment medium, and selenite (Difco), tetrasionate brilliant green (Difco) and Rappaport broths (prepared from ingredients) were used as selective enrichments. Incubation was at 43°C for selenite and tetrasionate broths and at 35°C for Rappaport broth. Isolations were made at 24 and 48 h by streaking on brilliant green, bismuth sulfite, Salmonella-Shigella and desoxycholate citrate agars (all from Difco), Polyvalent OMA and OMB antisera (Pasteur Institute) were used for grouping before sending the strains for serotyping to the Centers for Disease Control, Atlanta.

For isolation of Shigella, procedures of ICMSF (5) were used. Samples of 25 g were enriched in gram-negative (GN) broth (prepared from ingredients), and xylose lysine desoxycholate (Difco), Hektoen (Oxoid) and MacConkey agars (Difco) were used as selective isolation media. Membranes used to filter water samples were put into GN broth after filtration.

**RESULTS**

Flow processes, hazards, and critical control points (based on authors' observations) of the preparation of chicken-vegetable-rice soup, milk formula, mashed potatoes with spinach, beans and rice, and cow's foot soup, respectively, are illustrated in Fig. 1-5. Results of testing for Salmonella and bacterial counts of foods are summarized in Table 1. Table 2 summarizes potential sources of foodborne pathogenic microorganisms observed. Figures 6-8 illustrate time-temperature exposures of all cooked foods in each household during the hazard analyses. Figure 9 illustrates likely temperatures of foods purchased from the Mothers' Club if they had been held at room temperature until the usual time of eating the evening meal.

**Family 1.** Soya-meal, rice with carrots and chicken-vegetable-rice soup reached boiling or near-boiling temperatures during preparation (Figure 6). Broth of the soup boiled for approximately 45 min. Temperatures of the soya meal remained above 73.9°C (165°F) for 40 min; rice and carrots exceeded this temperature for 50 min. Soya-meal was within the temperature range of 21.1-48.9°C (70-120°F) for 235 min, even though it was refrigerated after 160 min in this range. Rice and carrots would have remained in this temperature range for 420 min, the broth soup for 245 min, and chicken in soup for 290 min, if the foods were held until 19.30 h before reheating.

Salmonella anatum was isolated from one of two specimens of fecal material from the baby. Another isolation of Salmonella was made from water used for washing raw vegetables and chicken, but the serotype was Salmonella thompson. Salmonella, however, was not isolated from food (Table 1), water from 55-gal (200-L) storage drum, cutting security.
### TABLE 1. Results of testing for Salmonella and bacterial counts of foods cooked in households in a new settlement, Lima, Peru.

<table>
<thead>
<tr>
<th>Family</th>
<th>Food Sample</th>
<th>Time of day foods sampled</th>
<th>Temperature °F/°C</th>
<th>AMCC/g</th>
<th><em>Escherichia coli</em> biotype I/g</th>
<th><em>Bacillus cereus</em> g</th>
<th><em>Staphylococcus aureus</em> g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soya meal fortified</td>
<td>0715</td>
<td>79/26</td>
<td>3.3x10³</td>
<td>-</td>
<td>1x10²</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Soya-meal-water mixture after cooking</td>
<td>0748</td>
<td>168/76</td>
<td>&lt;30</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Milk-water mixture made from water left in thermos overnight</td>
<td>0800</td>
<td>-</td>
<td>&gt;1.6x10⁵</td>
<td>4.0x10⁴</td>
<td>&lt;10</td>
<td>6.0x10⁵</td>
</tr>
<tr>
<td></td>
<td>Rice starch after cooking</td>
<td>1210</td>
<td>134/57</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rice with carrots after cooking</td>
<td>1210</td>
<td>134/57</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rice with carrots after holding</td>
<td>1930</td>
<td>86/30</td>
<td>&lt;30</td>
<td>-</td>
<td>1x10²</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chicken-vegetable-rice soup after cooking</td>
<td>1214</td>
<td>163/73</td>
<td>-</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chicken-vegetable-rice soup after holding</td>
<td>1930</td>
<td>86/30</td>
<td>3.9x10⁴</td>
<td>1x10²</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Soya meal</td>
<td>79/26</td>
<td>79/26</td>
<td>&lt;10</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Milk from baby bottle</td>
<td>1700</td>
<td>-</td>
<td>-</td>
<td>1x10²</td>
<td>1x10²</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Milk from baby bottle³</td>
<td>1900</td>
<td>-</td>
<td>&gt;1.6x10⁵</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Canned, condensed milk (evaporated)³</td>
<td>1045</td>
<td>79/26</td>
<td>8.3x10⁴</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Milk, water and spinach mixture³</td>
<td>1200</td>
<td>-</td>
<td>2.4x10⁵</td>
<td>&lt;10</td>
<td>3.0x10</td>
<td>3.8x10</td>
</tr>
<tr>
<td></td>
<td>Mashed potatoes³</td>
<td>1139</td>
<td>114/45</td>
<td>-</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mashed potatoes with milk and spinach³</td>
<td>1226</td>
<td>181/83</td>
<td>&lt;30</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Mashed potatoes with milk and spinach before reheating³</td>
<td>1950</td>
<td>87/31</td>
<td>2.0x10⁴</td>
<td>&lt;10</td>
<td>1.0x10²</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Rice served at lunch</td>
<td>1338</td>
<td>140/60</td>
<td>1.6x10⁵</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rice before reheating³</td>
<td>1950</td>
<td>89/32</td>
<td>5.1x10⁵</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Vegetable salad³</td>
<td>1338</td>
<td>85/29</td>
<td>8.0x10²</td>
<td>&lt;10</td>
<td>1.0x10²</td>
<td>1.8x10²</td>
</tr>
<tr>
<td></td>
<td>Oats with apples³</td>
<td>1100</td>
<td>-</td>
<td>1.3x10⁹</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mashed garlic³</td>
<td>-</td>
<td>84/29</td>
<td>3.4x10⁵</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Beans after cooking, served at lunch</td>
<td>1230</td>
<td>210/99</td>
<td>&lt;10</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Beans before reheating³</td>
<td>1808</td>
<td>99/37</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Beans and rice³</td>
<td>1827</td>
<td>155/68</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rice after cooking, served at lunch</td>
<td>1230</td>
<td>187/86</td>
<td>&lt;10</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rice before reheating³</td>
<td>1810</td>
<td>96/36</td>
<td>&lt;10</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cow’s foot soup after cooking, served at lunch³</td>
<td>1220</td>
<td>167/75</td>
<td>&lt;10</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cow’s foot soup before reheating foot³</td>
<td>1655</td>
<td>85/29</td>
<td>8.0x10²</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cow’s foot soup after reheating³</td>
<td>1800</td>
<td>209/98</td>
<td>5.0x10²</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mothers’ Rice Club</td>
<td>1930</td>
<td>81/27</td>
<td>3.2x10⁴</td>
<td>6.0x10⁵</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Cau Cau</em> (soup containing tripe, potatoes and other vegetables)</td>
<td>1930</td>
<td>80/27</td>
<td>4.0x10³</td>
<td>6.0x10⁵</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Salmonella* not isolated.

³Per ml.
TABLE 2. Potential sources of contamination observed during hazard analyses performed in households in a new settlement, Lima, Peru.

<table>
<thead>
<tr>
<th>Source</th>
<th>Family 1</th>
<th>Family 2</th>
<th>Family 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby with diarrhea</td>
<td>X</td>
<td>X</td>
<td>X/</td>
</tr>
<tr>
<td>Chickens in or near house</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigs near house</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs/cats in household</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Enteric microorganisms from inadequate sewage disposal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No privy</td>
<td>b/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteric microorganisms from water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drums and other water vessels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subject to contamination</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Enteric microorganisms from raw foods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handling raw meat or poultry</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Spores from foods/environment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw vegetables/potatoes</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Cereal (rice, soya-meal, barley, etc.)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Earth floor</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Spices used</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Vectors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No screens</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Flies obvious</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

*a* Source present

*b* Questionable

*b* Not easily accessible.

board and knife, dish and utensil-washing pan, cleaning and washing cloth, or composite of chicken, turkey, dog, and cat feces. Shigella was neither isolated from the baby’s feces nor from drinking water. The AMCC was greater than 1.6 x 10³ ml, the E. coli biotype 1 count was 40/ml, and the S. aureus count was 60/ml in the baby’s formula. A sample of water had an AMCC greater than 5 x 10³/ml and the E. coli biotype 1 count of 40/ml.

Family 2. Internal temperature of whole potatoes during cooking reached 90.6°C (195°F) (Figure 7). Potatoes were subject to contamination afterwards during peeling, mashing, or adding ingredients. After the addition of spinach and evaporated milk, mashed potatoes were reheated to a temperature of 91.1°C (196°F) and they remained above 73.9°C (165°F) for 50 min. Carrots and beets reached 94.4°C (202°F) during cooking and remained above 73.9°C (165°F) for 25 min. However, they were subject to contamination from hands, knife and cutting areas during peeling and slicing. Rice and soup containing spaghetti reached boiling temperatures. Rice exceeded a temperature of 73.9°C (165°F) for 80 min; soup exceeded this temperature for 75 min. Any surviving spores, however, could germinate and resulting vegetative cells multiply during the interval of almost 5 h that the foods were below 48.9°C (120°F), until reheating. During reheating, mashed potatoes reached 96.1°C (205°F). The soup reached 72.2°C (162°F) and exceeded 65.5°C (150°F)
for over 20 min and 60°C (140°F) for over 30 min. Portions of rice attained a temperature of only 48.3°C (119°F) during reheating.

Salmonella was not isolated from foods (Table 1). It was also not isolated from drinking water (boiled the day before), water from a thermos (boiled the day before), cleaning cloth, a swab from a cutting board, two specimens from the baby's feces or from pig's feces. Shigella was neither recovered from the baby's feces nor from the drinking water. The drinking water had an AMCC that exceeded 5 x 10^3/ml; E. coli biotype I was not found.

Family 3. Cow's foot soup, beans, and rice were all boiled (Fig. 8). The soup remained above 93.2°C (200°F) for 70 min and above 73.9°C (165°F) for more than 85 min, the beans remained above 93.2°C (200°F) for over 70 min and above 73.9°C (165°F) for over 130 min, and rice remained over 93.2°C (200°F) for 20 min and above 73.9°C (165°F) for over 40 min. The soup remained in the temperature range of 21.1-48.9°C (70-120°F) for 215 min before reheating. Beans stayed in this temperature range for 325 min, and rice remained in this range for 295 min. During reheating, the soup (which was heated longer and earlier than usual because the meat was still tough) boiled for over 20 min. One batch of beans and rice mixture was reheated to 100°C (212°F); another batch was reheated to 72.8°C (163°F).

Salmonella was not isolated from foods (Table 1), drinking water, vegetable-washing water, dish and utensil-washing, water from the thermos, or swab of a cutting board. It was also neither isolated from a fecal specimen from the baby nor from a dropping from one of the dogs. Shigella was neither isolated from the baby's feces nor from the drinking water. Drinking water had an AMCC of 30/ml and an E. coli biotype I count of 30/ml. Water from the thermos had an AMCC of 1.0 x 10^3/ml.

Mothers' Club. The simulation study of foods prepared by the Mother's Club showed that the foods could be in a temperature range 21.1-48.9°C (70-120°F) that would promote rapid microbial growth for over 7 h if they were purchased and held at room temperature until the evening meal (Fig. 9). At 19:30 h and with product temperatures of 26.6-27.2°C (80-81°F) the Cau-Cau had an AMCC of 4.0 x 10^3/g and the rice an AMCC of 3.2 x 10^3/g; both had a B. cereus count of 60/g (Table 2).

**DISCUSSION**

The hot-food preparation practices observed were rather similar in the three households, even though the families were of different ethnic backgrounds and from different regions of the country. Foods for lunch and supper were prepared in the morning, served for lunch to those persons at home, and held without heat on the top of stoves until supper time when they were reheated and served.

During cooking, foods either were boiled or reached temperatures that would be lethal to vegetative forms of pathogenic bacteria, but heat-resistant spores would survive. AMCCs were often less than 10/g after cooking. Most pans of foods were covered with lids during and after cooking, so contamination would most likely occur just during serving. Vegetative cells that reached food by this means would probably perish during the interval that many foods were at temperatures that exceeded 54.4°C (130°F) during holding. Therefore, spore forms (e.g., B. cereus and C. perfringens) are of particular concern in reference to food safety. After cooking, foods were sometimes held at ambient temperatures long enough (e.g., 5-7 h) to allow considerable increase in cells resulting from germinating spores. High AMCCs, however, were not always encountered and B. cereus did not exceed 6 x 10^3/g. This is surprising and more work is needed before these data can be assumed to be typical. With additional holding time at ambient room temperature higher counts would be expected.

Temperatures achieved during reheating were often, though not always, high enough to kill vegetative forms of pathogenic bacteria. They were not, however, high enough to inactivate heat-resistant emetic toxin of B. cereus (4).

In reference to care and feeding of the babies, some hazards were noted. Babies sometimes crawled, played or slept in areas accessible to animals or where animal feces sometimes fell. Mothers did not always wash their hands after changing the babies' diapers, and when they did it was often in the same pan of water used for food preparation or utensil washing. Although water was boiled and kept in a thermos, the thermos and baby bottles were neither effectively washed nor boiled. The bottles were often subjected to contamination during filling. AMCC above 1.6 x 10^3/ml and presence of S. aureus and B. cereus in milk from baby bottles in households for Family 1 and 2 corroborate these observations. Also, a high AMCC was obtained from a sample of canned unsweetened evaporated milk opened the day before and kept under refrigeration (Family 2). Critical control points would be to boil water and to disinfect thermos and bottles before filling, feed the baby promptly after preparation of formula, and keep canned milk after opening and prepared milk formula in a refrigerator if available; if not, the formula should be given to the baby promptly after preparation. It is usually recommended that baby bottles be boiled for 5 min (2).

Weaning foods are often contaminated, and bacterial contaminants multiply during holding between preparation and feeding (8). Therefore, whenever practicable, infants...
should be fed weaning foods soon after preparation. If refrigerators are available, they should be used to hold formula or weaning foods between preparation and feeding if there is any delay.

The source of *Salmonella anatum* that infected the baby of Family 1 could not be ascertained. The infection occurred some days before the visit. Potential sources of the organism, however, were present in the household. Chickens and turkeys are often infected and their feed contaminated (6). These fowls sometimes came into the house, and they had access to the shed where utensils were washed and stored. *Salmonella thompson* was recovered from wash water in a pan in the kitchen, showing that *Salmonella* was present in the environment. A family member or the dog could have also been a reservoir. Other sources could have been raw poultry, raw meat, and eggs that reached the kitchen. These were handled, cut or sometimes washed by the mother before she prepared the baby’s formula or otherwise cared for the baby. Hand washing by the mother was often done in the same pan used for washing raw foods or kitchen utensils.

Critical control points for soups are cooking, holding between cooking and serving, and reheating. Critical control points for oats, mashed potatoes, rice, and beans -- foods that are likely to contain spores which are probably not killed during cooking -- are (a) holding between cooking and serving and (b) reheating.

Monitoring of cooking and reheating must be done in ways that are practical to perform in households of persons with limited resources, facilities, and technical information. For example, the preparer can observe whether liquid foods and those containing liquids boil to monitor the effectiveness of cooking and reheating. Monitoring of foods after cooking can be done first to see whether all the foods are eaten at the first serving. For those that are left, the lapse of time between cooking and either serving or reheating can be monitored. When financial resources and preparation circumstances permit, safe practices would be to eat cooked foods promptly after cooking or to feed leftovers to animals. If refrigerators are available, foods can be held between meals and overnight in them. If so, foods being refrigerated should be stored in shallow layers. Foods can also be held safely for short periods at room temperature or held at hot (55°C/131°F) temperatures for up to a few hours. Monitoring of any sort must be integral to food safety education of the public.

Many persons in the new settlements do not have refrigerators. Some who do, either do not know that cooked foods should be stored in them during the interval between meals or it is their usual practice not to refrigerate cooked foods. To initiate change, efforts to educate persons to refrigerate foods properly could be made by health educators and other health-agency personnel (e.g., nutritionists) who visit households. Furthermore, manufacturers of refrigeration equipment could attach stickers to their units, include notices in packets of information inside the units, and describe recommended food-storage practices in their manuals. Processed foods with labels could include instructions for refrigerating foods after they are opened, thawed, cooked, rehydrated, as appropriate for the type of product.

Customs, facilities and the time required for food preparation do not always permit the utilization of safe practices. Mothers, who are the usual preparers of foods, have considerable demand on their time in addition to the preparation of food. Therefore, foods are frequently held from one meal to the next and perhaps overnight at temperatures that allow bacteria to multiply.

Habits are difficult to change, but they do change and evidence of this was obvious in the new settlements. Residents had left their homes and resettled, thus undergoing many changes in lifestyle. Radios, television, thermos containers, gas stoves and electric refrigerators were used whenever financial resources permitted. There was also evidence of the impact of health education on treatment of diarrheal disease and on improved nutritional practices. For example, articles about diarrheal disease problems and their early treatment frequently appeared in Lima newspapers, and on radio and television. Nutritionists worked with the Mothers’ Club members to improve nutrition. Similar approaches could be initiated to cope with hazards associated with home-prepared foods. Critical control points and practical means of prevention and monitoring practices could be specified.

Once the problems and solutions are identified and cultural patterns and social structures understood, appropriate educational materials must be either selected or developed and training and educational efforts implemented to stimulate behavioral change that translates into safer food preparation and storage practices. Educational activities to meet this end include: (a) modifying curricula at universities; (b) training professionals who are presently working in the field of public health; (c) presenting health education information to adults during home visits by public health professionals; (d) passing information along when foods are distributed to low-income families; (e) holding discussions with groups such as Home-Town and Mothers’ Clubs; and (f) teaching children in school.

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REFERENCES


Hazard Analyses of Foods Prepared by Inhabitants Near Lake Titicaca in the Peruvian Sierra

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ABSTRACT

Hazard analyses of food preparation practices were conducted in two households in an Andean Indian Pueblo near Puno, Peru and in a house on the outskirts of this city. These analyses consisted of watching all steps of preparation, recording temperatures throughout all these steps, and collecting samples of food and testing them for common foodborne pathogens and indicator organisms. Only cereal-potato soup (a very popular and inexpensive food in the region), kidney stew, and parched cereal were prepared during the survey. The soups boiled during cooking and most of them were eaten during the first serving. Vegetative forms of pathogenic bacteria would have been killed during cooking, but heat-resistant spores would have survived. Leftovers in the pueblo homes, when there were any, remained without heat on the clay stoves on which they had been cooked until eaten or reheated. In the other household, cooked foods were moved from the stove to an earthen floor and kept there until reheating. Under this condition, cooling was more rapid than when left on stoves. The interval of time that cooked foods were between 49°C (120°F) and 21°C (70°F) during holding was less than 4 h, thereby limiting spore germination and bacterial multiplication. In the only household in which foods were reheated, they boiled. Critical control points for food preparation and reheating in homes are cooking, holding between cooking and serving, and reheating.

The hazard analysis critical control point (HACCP) approach consists of (a) determination of hazards and assessment of their severity, and the risks they pose; (b) identification of critical control points required to prevent or control significant hazards; (c) establishment of preventive and control measures and criteria; (d) monitoring of each critical control point, and (e) implementation of appropriate and immediate corrective action whenever the criteria are not met. Hazards relate to contamination of foods by microorganisms and to their survival during processing and multiplication during storage. A critical control point is an operation (location, practice, procedure, or process) that if not under control could lead to unacceptable contamination, survival or growth of undesirable microorganisms. Monitoring is the checking that the process or handling procedure at the critical control points is properly carried out.

This approach is applicable to evaluate food safety in homes as well as food processing and foodservice establishments (11). Furthermore, a FAO/WHO Expert Committee on Food Safety recommended that the HACCP approach be used in homes in developing countries to get a greater insight into hazards associated with food preparation and applicable preventive measures (5).

The feasibility of using this approach in households was tested in Peru because of the country's diverse geographic and climatic conditions and the different cultural groups that live there. A determining factor for these diversities and differences is the Andes mountain chain which creates three natural regions: the coastal desert, the mountains and the jungle. Hazard analyses reported here were conducted in households in an Andean Indian Pueblo near Puno and at its outskirts in the Sierra. Food-preparation hazards in households in the other regions are described in two related papers.

MATERIALS AND METHODS

Description of the region, population and diet

The Sierra Region of Peru is high land of gentle slopes, plateaus, canyons, volcanoes and towering groups and ranges of mountains. Ten mountains exceed 6,000 m (19,685 ft). It covers about one-fourth of Peru's land area, but approximately one-half of the country's (17 million) population live there, an extremely high
density for such agriculturally-poor land. Most of the inhabitants of the region are Andean Indians and many speak either Quechua or Aymara rather than Spanish. Furthermore, many of them are virtually outside the money economy.

Puno, the capital of a Department in the high-plains region in the south-eastern part of the sierra, is at an elevation of 3,855 m (12,648 ft) along the shore of Lake Titicaca. This lake is the largest in Peru and the highest in the world. It greatly influences the climate of the region by creating temperate conditions. Furthermore, it also influences the formation of springs and is an important source of fish and reeds.

Ichuilla is a hillside village also on the shore of Titicaca Lake about 20-30 km (12.5-18.8 miles) from Puno. Potatoes and cereals are grown in terraces, and animals are grazed.

In general, the diet of persons who live in the region consists of a breakfast of herbal tea and chairo. Chairo is a soup consisting of potatoes (sometimes two types, a white or yellow variety and a processed potato chuño negro), cereal (often quinoa or wheat), and other ingredients (see Fig. 1). Chuño negro is processed by setting potatoes on the ground for 2-3 d in the cold season, where they freeze. Next, they are stepped on to remove water and part of the peel, and left in the cold air in sunlight to dry (10). Thus they are freeze-dried. They are washed and peeled before adding to the soup. Lunch usually consists of parched cereal or potatoes or both. Supper often consists of parched cereal, leftover chairo, and perhaps chuño.

The diet is high in carbohydrates. Quinoa (Chenopodium quinoa) provides the major protein source; it contains between 10-12% protein. Chuño negro contains 4% protein and the common white and yellow potatoes have approximately 2% protein (2). Intake of fat is low. A nutritional survey in the region showed that the average calories per capita was 1,860 (3). Another survey taken just after harvest when foods were plentiful and income at its peak, gave caloric intake of people in rural areas of Puno to be 2,990 and 57 g of total protein. For Peruvians, this is considered as 77% of the caloric and 100% of protein requirements (7).

The infant mortality rate in the region was 127/1,000 live birth compared to 99/1,000 live birth for the entire country (2,3). Gastroenteritis is the third highest cause of death only exceeded by respiratory diseases and perinatal deaths.

**Figure 1. Preparation of Chairo (e.g. Quinua soup) in households along Lake Titicaca and in the outskirts of Puno (Families 1,2,3).**

1,2,3 relate to practices carried out in specific households, numbers correspond to Family numbers.
Description of families surveyed

Three families were surveyed: two in Ichuralla in the Chucuito District and one at the outskirts of the capital. Family 1 from Ichuralla consisted of a mother and six children, ages 13 years to 9 months; the father was hospitalized. Food was prepared in an adobe hut with earth floor and a thatched roof made of reeds. The hut was separate from the living quarters and a small store. Cooking was done on a baked-clay stove made close to the floor. Sticks, dried leaves and dried dung were used as fuel. Guinea pigs were raised and kept inside the hut. Water was obtained from a spring about 100 m from the house. There was no close-by source of pollution, but a few privies were located up-hill and defecation by humans and animals could occur sporadically in the area. Chickens and cats were kept in the yard, and they often drank from pans containing the family’s water supply.

Herbal tea with sugar and bread was served at breakfast. Cachaí containing potatoes, carrots, onions, oregano, beef fat, and oil was prepared and served at lunch and kept unheated until the evening meal. Barley was parched and eaten sporadically throughout the day. The baby was fed breast milk and given herbal tea. The bottle used for the tea was frequently on the floor.

Family 2, also from Ichuralla, consisted of a mother and eight children, ages 20 to 2 years. The house, high on the hillside, was of adobe with earth floor and thatched roof made of reeds. The kitchen, cooking facilities and menu were similar to that of Family 1. Tableware and pans were washed by pouring boiled water over them and after a few minutes rubbed by hand. Boiled water was also used to rinse the dishes just before serving. Several sheep were kept in a pen just off the patio; guinea pigs were raised and kept in the kitchen, and there was a pet cat. Water was obtained from an unprotected spring several hundred meters from the house; human waste were excreted along the hillside.

Family 3 lived in a two-story adobe house with tin roof. The family consisted of mother, father and eight children, whose ages ranged from 23 to 2 years. Two pigs and their nursing young were kept in a pen next to the kitchen. Chickens roamed freely in the yard, and the family also had a large dog. Water was from a spring, but it was piped into the yard and controlled by a faucet. There was no latrine. Cooking was done on a single kerosene camp-type heater in a separate adobe hut with tin roof and earth floor. Food, animal feed and water were stored in this room. Cachaí, (see Fig. 1) and a kidney stew (see Fig. 2) were prepared. The soup was prepared at 11:00 h, eaten between 14:00 and 14:30 h and the leftover portion was reheated at supper time. Pans containing leftovers were kept on the floor until reheating.

Hazard analyses

The hazard analyses were performed in January (summer) and consisted of collecting specimens and samples, measuring temperatures, and watching food preparation. Droppings from animals that frequented the houses or were kept in the immediate environment of the houses and dried dung used as fuel were also collected. Samples of drinking water and sometimes of water used for food preparation or utensil washing were collected. Samples of foods were taken either before cooking, during holding, or after reheating, as appropriate to evaluate a potential hazard. Ingredients were sometimes sampled. Temperatures of
the interior of foods were taken throughout preparation (cooking, holding after preparation, and reheating, as appropriate) by inserting a thermocouple (type-T with either a bayonet-type sensor of appropriate length or a sensor with wires twisted and fused at the end) into the approximate geometric center of the food being measured. It was difficult, however, to maintain this location throughout the duration of measurement. The thermocouples were plugged into a type-T data logger (potentiometer) (MLX Minilogger, A. D. Data Systems, Inc., Rochester, N. Y.). Time at which temperature measurements were taken was given by the data logging instrument. Observations were made of likely sources of contamination and opportunities for cross contamination. Samples were tested for aerobic mesopholic colony count (AMCC), Salmonella, Shigella, Staphylococcus aureus, Clostridium perfringens, Bacillus cereus and Escherichia coli biotype 1, as applicable, depending on the nature of the samples and the organisms that would be likely contaminants.

Data were evaluated by diagramming the steps of food preparation. Potential sources of contamination (raw foods, equipment and utensils, and persons preparing the food) are noted on the diagram. Likelihood of survival or destruction and likelihood of microbial growth are also shown. Critical control points of the operations that require monitoring are indicated. Graphs were made of the time-temperature exposures of each food prepared during the observations.

Housewives or other persons who prepared foods were requested to do so in their usual manner and were not given any special instructions. Operations were observed to identify sources and routes of contamination by the person preparing the foods, utensils, equipment surfaces, animals, foods of animal origin or by any other circumstances which could have led to contamination. Members of the household were asked if there were cases of diarrhea, especially in children.

**Sampling procedures**

Initial samples of cooked and uncooked foods were collected with sterile metal spoons or forks and aseptically put into sterile plastic bags. For samples taken later, the previously used sampling instruments were washed and then dipped into 95% alcohol and flamed, the latter two steps being repeated three times each. The bagged samples from cooking or hot-holding units were immediately precoured either under cold water or in ice. They were kept in contact with ice in a covered styrofoam box.

Samples of water were poured aseptically into either sterile plastic bags or sterile flasks with screw caps. Samples from tables, cutting boards, or knives were taken by rubbing the surface with a sterile polyurethane sponge (9), previously moistened with 0.1% peptone water and aseptically put into a wide mouth flask containing the same diluent. Samples of droppings from animals were taken from the yard or floor with a sterile spatula and aseptically put into the bags.

Samples and specimens were taken to the laboratory on the day of collection and either examined on that day or kept refrigerated overnight and tested the following day.

**Laboratory procedures**

For most of the foods 10 g or 10 ml were homogenized with 0.1% peptone water in screw-capped flasks containing glass beads by means of horizontal and vertical manual agitation for a few seconds. AMCC were made by the pour plate method. Plate count agar (Difco) with the addition of triphenyltetrazolium chloride (TTC) to give a final concentration of 0.01% was used. Incubation was at 35°C for 48 h.

For water samples, the filtration method was used (membrane Sartorius pore size 0.45 μm). Amounts of 100 ml of water, when available, were filtered. The medium for AMCC was as stated above.

**E. coli** biotype 1 was enumerated by the Anderson Baird-Parker (1) method in tryptone bile agar (prepared from ingredients) by using cellulose acetate filter membranes [pore size 0.45 μm, 85 mm diameter (Oxoid) or 90 mm Gelman]. A loopful of solution from samples was streaked onto the surface of the membrane which had previously been placed on top of the same medium. Further confirmation of **E. coli** biotype 1 was by the IMVIC tests.

Presumptive **B. cereus** was enumerated by spreading 0.25 ml of each homogenate and of their dilutions on each of four Petri dishes with phenol-red-egg-yolk-polymyxin (Merck). Incubation was at 35°C for 24 h (8). Starch, citrate, Voges-Proskauer and motility-nitrate tests were used for confirmation.

**S. aureus** was enumerated by spreading 0.25 ml of the homogenates and their dilutions on each of four Petri dishes with Baird-Parker agar (Oxoid). These were incubated at 35°C for 48 h (8). Further confirmation was on the basis of the results of DNase test (DNase agar, Difco), coagulase production (rabbit plasma EDTA, Difco), thermostable nuclease production (medium made from ingredients) following ICMSF (8) procedures. Haemagglutination (staphylyslide test, bio-Merieux) was also performed.

**C. perfringens** was enumerated by using ICMSF (8) procedures (method 2) modified by inoculating 5 ml of the homogenates and their dilutions into sulfite cycloserine agar (prepared from ingredients) in roll tubes. Gelatin-lactose and motility-nitrate tests were used for confirmation.

For isolation and confirmation of **Salmonella**, procedures recommended by ICMSF (8) were followed. Nutrient broth (Merck) was used as a non-selective pre-enrichment medium, and selenite (Difco), tetrahydrate brilliant green (Difco) and Rappaport broths (prepared from ingredients) were used as selective enrichments. Incubation was at 35°C for selenite and tetrahydrate broths and 35°C for Rappaport broth. Isolations were made at 24 and 48 h by streaking on brilliant green, bismuth sulfite, Salmonella-Shigella and desoxycholate citrate agars (all from Difco). Animal dropping specimens were put directly onto plating media as well as into selective broths. Polyvalent OMA and OMB antisera (Pasteur Institute) were used for grouping.

For isolation of **Shigella**, procedures of ICMSF (8) were used. Samples of 25 g were enriched in gram-negative broth (prepared from ingredients), and xylose lysine desoxycholate (Difco), Hektoen (Oxoid) and MacConkey agars (Difco) were used as selective isolation media. Membranes used to filter water samples were put into GN broth after filtration.

**RESULTS**

Flow processes, hazards and critical control points of preparation of **chaireo** (quinoa soup, wheat soup) and kidney stew are presented in Fig. 1 and 2, respectively. Figures 3-5 illustrate time-temperature exposures of all foods cooked in each household during the hazard analyses. Bacterial counts
of foods are summarized in Table 1. Table 2 summarizes potential sources of foodborne pathogenic microorganisms that were observed.

Family 1. Wheat soup reached the boiling point (88.3°C/191°F at the altitude of the house surveyed) several times during cooking, and exceeded 73.9°C (165°F) for 110 min (Fig. 3). While the soup remained on the stove after fuel burned out, the temperature fell from 48.9°C (120°F) to 21.1°C (70°F) in 225 min. The soup had an AMCC of 8.1 x 10⁶/g immediately after cooking; this increased to 2.5 x 10⁹/g at 15:30 h (Table 2). Neither Salmonella nor Shigella was isolated from foods (Table 1), spring water for drinking, spring water for washing dishes, a cleaning cloth, sponge wiped across earth floor frequented by guinea pigs, or dried feces used as fuel.

Family 2. Quinoa soup boiled (87.2°C/189°F at the altitude of the house surveyed) for approximately 1 h, and the temperature exceeded 73.9°C (165°F) for 140 min (Fig. 4). While the soup remained on the stove after fuel burned out, the temperature fell from 48.9°C (120°F) to 21.1°C (70°F) in 245 min. Immediately after cooking the soup had an AMCC of 3.0 x 10⁶/g; this count increased to 3.3 x 10⁹/g at 14:00h (Table 1).

Neither Salmonella nor Shigella was isolated from quinoa soup, surface of a cutting board and knife, spring water or spring water which had been previously boiled for drinking purposes and kept in pans in the house. Furthermore, Salmonella was not recovered from either a stone-mortal used to grind the pepper or from dried dung.

Family 3. Kidney stew and quinoa soup with potatoes and meat were boiled during cooking (Fig. 5). After cooking, the soup remained at boiling point for about 50 min; the kidney stew stayed at this temperature for about 20 min. The stew remained between 48.9°C (120°F) to 21.1°C (70°F) for 195 min; the kidney stew remained between these temperatures for 90 min before reheating. Both foods boiled during reheating.

Neither Salmonella nor Shigella was isolated from foods (Table 1), spring water from the faucet, spring water stored in a covered earthenware pot, feces from pigs or feces from chickens. The spring water contained 10 E. coli biotype 1/ml.

**DISCUSSION**

Vegetative forms of pathogenic bacteria would have been killed during cooking of the foods, but heat-resistant spores would have survived. AMCC were less than 10⁵/g. Sporeforming organisms were isolated from spices and barley, which is expected. Contamination could have occurred while portions of the hot foods were dispensed for eating, but other contamination was unlikely because lids were kept on pans. Vegetative cells that may have reached the food after cooking were likely to have died because temperatures remained in lethal ranges (above 54.4°C/130°F) for intervals that usually exceeded 1 h; and above 73.9°C (165°F) for a considerable time. Therefore, spores of B. cereus and C. perfringens are the pathogens of primary concern. Contamination with Salmonella or other enteric pathogen from dried dung is a possibility, however, the period of time during which temperatures remained between 21.1°C (70°F) and 48.9°C (120°F) was usually less than 4 h, thereby limiting but not preventing spore germination and bacterial multiplication. No change in AMCC was observed in one household;
HAZARD ANALYSIS OF HOME-PREPARED FOODS

TABLE 1. Results of testing for Salmonella and bacterial counts of foods cooked by inhabitants near Lake Titicaca in the Sierra of Peru.

<table>
<thead>
<tr>
<th>Family</th>
<th>Food/Sample</th>
<th>Time of food sample</th>
<th>Temperature °F/°C</th>
<th>AMCC/g</th>
<th>Escherichia coli biotype I/g</th>
<th>Bacillus cereus/g</th>
<th>Clostridium perfringens/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wheat (Triticum aestivum) soup ¹</td>
<td>10.30</td>
<td>173/78</td>
<td>8.1x10³</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wheat, dry</td>
<td>07.30</td>
<td>62/17</td>
<td>1.6x10³</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Barley (Hordeum vulgare) parched soup</td>
<td>13.00</td>
<td>62/17</td>
<td>7.0x10²</td>
<td>-</td>
<td>2.0x10³</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Quinua soup ¹</td>
<td>09.30</td>
<td>67/19</td>
<td>3.0x10³</td>
<td>-</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Quinua soup ¹</td>
<td>14.00</td>
<td>60/16</td>
<td>3.3x10³</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Onion, oil, red pepper mixture ²</td>
<td>-</td>
<td>-</td>
<td>8.7x10³</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Barley, parched</td>
<td>14.00</td>
<td>60/16</td>
<td>&lt;10</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Quinua soup ¹</td>
<td>14.30</td>
<td>63/17</td>
<td>&lt;10</td>
<td>-</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Quinua soup ¹</td>
<td>18.00</td>
<td>65/18</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Kidney stew ²</td>
<td>18.20</td>
<td>130/54</td>
<td>5.0x10³</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Commercial seasoning</td>
<td>-</td>
<td>-</td>
<td>8.2x10³</td>
<td>&lt;10</td>
<td>4.0x10³</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Quinua, dry</td>
<td>11.20</td>
<td>62/17</td>
<td>1.6x10³</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Quinua, after washing</td>
<td>11.30</td>
<td>62/17</td>
<td>4.4x10³</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Caihua (Chenopodium pallidicaule) (cereal) parched and ground</td>
<td>-</td>
<td>-</td>
<td>1.0x10³</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

⁴Salmonella not isolated.
⁵Staphylococcus aureus 10/g.

TABLE 2. Potential sources of contamination observed during hazard analyses of families.

<table>
<thead>
<tr>
<th>Source</th>
<th>Family 1</th>
<th>Family 2</th>
<th>Family 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feces</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Chicken in or near house</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Pigs near house</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sheep near house</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Guinea pigs in kitchen</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Dogs/cats in household</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Dung fuel and handling</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Enteric microorganisms from inadequate sewage disposal</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Nolatrine</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Enteric microorganisms from water</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Spring water (unprotected)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Water vessels subject to contamination</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Enteric microorganisms from raw foods</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Handling raw meat or poultry</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Spores from foods/environment</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Raw vegetables/potatoes</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cereal (quinua, barley, caihua, rice)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Earth floor</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Spices used</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Vectors</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>No screens</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

X = Source present.

Critical control points for the preparation of chairo (quinua and wheat soups) and stews are (a) cooking, (b) holding between serving at lunch and supper, and (c) reheating. Cooking and reheating by the food preparer can be monitored by observing that the foods boil. Monitoring of holding can be done by observing whether the foods are eaten promptly after cooking or by observing the duration of holding. Although only limited information was obtained on this subject, the practice of removing pans of foods from cooking units and putting them on the floor offered some protection. Temperatures in the high-plains region tend to be cool, even in the warm season, particularly at night. In Household 3, for instance, a covered heavy-gauge metal pan, which was kept on the floor near a wall, was used to store raw meat and other perishable items. Raw meat in this pan was found to be at a temperature less than 15.6°C (60°F) at midday. Air temperatures, recorded near the cooking area, showed that the temperature of the room was rather cool during periods when cooking was not done. Food areas were even cooler. Hence, foods might be kept between serving at lunch and reheating at supper time in covered pans either on one and two orders of magnitude increase in AMCC occurred in the other households. At the time of year of the survey, the ambient temperature in the kitchen huts was rather low (usually near 16°C/60°F at day time and cooler at night) other than intervals during which cooking was done.

Reheating practices were not evaluated in two households, but in the third, the reheated foods boiled. Therefore, vegetative cells (which would have accumulated as a result of multiplication of cells from germinating spores) would have been killed. Heat-stable emetic toxin of B. cereus, if present, would survive the reheating (6). Hazards would exist if the holding time after cooking was prolonged (e.g., overnight) and the reheating inadequate.
the floor (or even perhaps in a depression constructed for this purpose) out of sunlight and away from any heat source or outside in the shade either on a protected shelf or hung in some fashion when the weather is cool. Such practices should result in more rapid cooling than keeping foods on unheated stoves and thus decrease microbial multiplication. Applied research could test this hypothesis.

Although the families surveyed in Ichuralla had limited financial means and lacked facilities and supplies such as electricity and soap, several safe practices were observed. For example, in Household 2, foods were boiled during cooking, drinking water was boiled, boiled water was used to wash dishes and to pour over dishes just before serving. In the household at the outskirts of Puno, cooked foods were stored on the cool surface of the floor and foods were boiled during reheating. No doubt, during a more extensive survey, other safe practices might be observed. This kind of safe community practices should be taken into account by food scientists and public health personnel and encouraged where appropriate. On the other hand, hazardous practices - such as holding foods without refrigeration between meals and perhaps overnight - exist and should be discouraged. The safest practices need to be selected and if necessary modified to ensure greater safety. The public should then be alerted about the hazards and appropriate preventive measures.

The challenge is to devise a means to communicate this sort of information to the public. The information can be presented to Pueblo “chiefs” and other leaders to alert them to the health risks and get their advice about how to communicate the information and as well as their assistance in carrying out the task. Leaders of the Mothers’ Club could be contacted and advised on safe procedures. They could pass along key information during their discussions with other women in the Pueblo. Midwives and “medicine men” could communicate this information if they were trained in these matters and motivated. Nutritionists and personnel at the local health center could also provide advice in food safety, as well as on their primary interest matters (nutrition, birth control, immunization, first aid, and hygiene). To do this, however, they need training in food safety with particular attention to hazards associated with local food-preparation methods and practical control measures.

Mass media (such as radio, television and newspapers) may also be of value, particularly in cities such as Puno. However, there are obvious limitations of these media in remote villages. Presentations would have to be done in Quechua and Aymara as well as in Spanish.

Long range solutions rest with education of children. Information about hazards associated with preparation of popular foods in a region and safe practices, as well as personal hygiene and education about good nutrition should be incorporated into the curricula of primary and secondary schools. For this to be effective, however, teachers should be educated and trained in these matters and provided with appropriate training aids to effectively communicate the information.

ACKNOWLEDGMENTS

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REFERENCES

Critical Control Points of Street-Vended Foods in the Dominican Republic

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Abstract

Hazard analyses were conducted at four street-vending stands in the Dominican Republic. Temperatures of foods were measured during cooking, display (holding), and reheating (when done). Samples were taken at each step of the operation and at 5 to 6-h intervals during display. Foods usually attained temperatures that exceeded 90°C at the geometric center during cooking and reheating. At three of the stands, foods (e.g., fish, chickens, pork pieces) were fried and held until sold. Leftovers were held overnight at ambient temperatures in the home of the vendor or in a locked compartment of the stand. They were usually reheated early in the morning and displayed until sold. During the interval of holding, aerobic mesophilic counts progressively increased with time from about 10³ to 10⁹/g. The higher counts were usually associated with holding overnight. Escherichia coli (in water, milk and cheese samples), Bacillus cereus (in bean and rice samples), and Clostridium perfringens (in meat, chicken and bean samples) were isolated, but usually in numbers less than 10³/g. At the other stand, foods (e.g., beans, rice, meat and chicken) were cooked just before serving as complete meals. There were no leftovers. This operation was less hazardous, although there were many sanitary deficiencies. Recommendations for prevention and control of microbial hazards (mainly reducing holding time, periodic reheating and requesting reheating just before purchasing) are given. The need and suggestions for implementing educational activities to alert and inform those concerned about hazards and preventive measures are presented.

Street vendors of foods are a common part of the lifestyle in countries in which there are high unemployment, low salaries, limited work opportunities, limited social programs and urbanization. Persons who sell foods on streets benefit from a positive cash flow, are often free from taxes, can sell what they want, are able to set their own work hours (but which are often long), are frequently free from regulation because the few existing regulations on the subject are usually not enforced, and can get into business with a minimal outlay of capital. Street vendors provide an essential service to factory, construction and office workers, shoppers, persons in transit, persons with low income and others by selling complete meals, refreshments and snacks at a relatively low price without delay for preparation. The long distance between home and work place forces many workers to eat away from home. Facilities that meet modern standards of sanitation and provide food at low prices, however, are not always located near work places, markets, schools, parks, recreation events or bus stops.

Because of these situations and of the crowds of people at these locations, street vendors often congregate. Purchasers of street-vended foods are often preoccupied with food prices and convenience rather than with food safety, quality and hygiene.

There are many concerns about the sanitation of street vending operations. For example, stands and carts used by vendors are often of crude construction. Running water is seldom available at the stands. Hand, dish and utensil washing is usually done in one or more buckets or pans of water (sometimes without soap) and disinfection is rarely done. Waste water is usually discarded in streets and garbage is sometimes discarded nearby providing attraction, food and harborage for insects and rodents. Foods are ineffectively protected from flies. Most importantly, safe food storage temperatures are difficult to maintain. Furthermore, toilet and lavatory facilities are not readily available, which forces vendors to eliminate their body wastes in nearby secluded areas, and they often do not properly wash hands afterwards. Foods are either prepared in these itinerant places or in the homes of the vendors, which may also lack sanitary facilities. The situations described are typical of street vending in many places throughout the world as well as the Dominican Republic.

Health risks are associated with initial contamination of raw foods with pathogenic bacteria and subsequent contami-
nation by vendors during preparation from cross contamination, survival of pathogens during preparation, and microbial proliferation during display.

In countries where street vending of foods is common, there is usually a lack of information about the incidence of foodborne diseases, and investigations of outbreaks of these diseases is seldom done. (This is so in the Dominican Republic). Yet, diarrheal diseases are commonly experienced by persons of all ages. The relative importance of street-vended foods in contributing to diarrheal disease, in general, and outbreaks of foodborne diseases, in particular, is undefined. (Also, the situation in the Dominican Republic). Epidemiologic associations between street-vended foods and illnesses, however, have been made (10,17). Furthermore, certain foods (e.g., poultry, pork, beef, fish and rice) that are sold by street vendors are frequently identified during investigation of outbreaks of foodborne disease in countries that have surveillance activities. Pathogens, indicator organisms or groups, and high numbers of aerobic mesophilic microorganisms have been isolated from street-vended foods (8,9,14,16,17). The high counts suggest microbial propagation.

To develop a better understanding of the microbiologic problems associated with street-vended foods, hazard analyses were used to identify hazards and to assess risks associated with street-vended foods in Santo Domingo, Dominican Republic. Critical control points were determined and preventive measures suggested.

**MATERIALS AND METHODS**

**Hazard analyses**

Hazard analyses were made of three street-vending stands along a busy street in Santo Domingo, and a fourth vendor on a neighborhood street was visited twice but only food temperatures were measured. The hazard analyses consisted of observing food preparation and storage practices to identify sources and modes of actual or potential contamination. The hazard analysis critical control points concepts is reviewed in reference (19). Temperatures in internal regions of foods were measured throughout cooking, during holding after cooking, and during reheating (when this was done). Samples were taken of foods at sequential stages of preparation and subsequently tested for aerobic mesophilic colony counts and pathogens of concern.

Food temperatures were measured by inserting a thermocouple (type T) with a needle-type sensor of appropriate length with the point near the geometric center and with the shaft mostly covered by the food. Air temperatures were taken with type-T thermocouples with welded ends. Thermocouples were washed, immersed in 95% alcohol and flamed three times before inserting into foods. The thermocouple leads were plugged into either a battery-powered data logger (MLX Minilogger, A. D. Data Systems, Inc., Rochester, N. Y.) or a hand-held battery-powered potentiometer (Atkins Digital Thermocouple, 497, Atkins Technical, Gainesville, FL.; reference to trade marks does not constitute recommendation by the sponsoring institutions nor by the authors). Time was recorded automatically on the data logger and observed from a wrist watch when the hand-held potentiometer was used.

Observations made at the vending sites were evaluated by diagramming sequential flow of foods during preparation and holding. Potential sources of contamination from raw foods, equipment, utensils, and persons preparing the foods as well as likelihood of microbial survival or destruction and likelihood of microbial multiplication were noted on the diagram. Critical control points that need to be monitored were indicated at appropriate steps of the operations.

Samples of foods that were collected were not the same as those having thermocouples attached, but they underwent the same process. They were taken either after cooking or reheating or during holding, as appropriate, to evaluate either a potential hazard or control measure. These were collected aseptically with either metal spoons or forks which had been cleaned and then inserted into 95% alcohol and flamed. The inserting into alcohol and flaming was repeated three times. Samples were put into sterile Whirl-Pak plastic bags. Food-contact surfaces were sampled by rubbing a sterile swab over them to attempt recovery of Salmonella. If the surface was dry, the swab was first moistened in sterile 0.1% peptone water (Difco). The cotton-tipped end of the swab was broken into a tube of tetraphionate (Difco) brilliant green broth.

Samples were put immediately in an insulated container with ice, and they were taken to the laboratory on the day of collection. Examination started either on that day or samples were kept refrigerated overnight and tested the next morning.

**Laboratory procedures**

Ten g of solid or semi-solid analytical samples were homogenized with 90 ml of 0.1% peptone water in plastic bags by using a Stomacher 400. Liquid or semi-liquid analytical samples were mixed by inversion before 10 ml were taken. Decimal dilution(s) were made according to ICMSF (13). Aerobic mesophilic colony count (AMCC) was made by the pour plate method [ICMSF (13) method 1].

Beans and rice, only, were tested for Bacillus cereus. It was enumerated on phenol-red egg-yolk polymyxin agar (prepared from ingredients) by spreading 0.25 ml of the homogenate on each of four petri plates and 0.1 ml per dilution on duplicate plates. The plate lids were cracked open in an incubator for 15 min at 50°C to prevent spreading before incubation at 35°C for 24 h (13). Motility-nitrate and Voges-Proskauer tests were used for confirmation.

Meat, poultry and beans were tested for Clostridium perfringens. It was enumerated by inculcating 1 ml of the homogenates and their dilutions into tryptose sulfite cycloserine agar without egg yolk (prepared from ingredients) in duplicate plastic pouches prepared according to Bladel and Greenberg (3). Incubation was done at 35°C for 48 h. Gelatin-lactose and motility-nitrate tests were used for further confirmation.

Water, milk and cheese were tested for Escherichia coli. Biovar I was enumerated on tryptone bile agar (prepared from ingredients), by using acetate filter membrane (pore sized 0.45 μm; 85 mm-diameter, Millipore) according to the procedure of Anderson and Baird-Parker (1).

For isolation of Salmonella, analytical samples of 25 g were preenriched in 225 ml of lactose broth (Difco or Merck). Either selenite broth (Difco) or selenite cistine (Merck) and tetraphionate brilliant green broth (Difco) were used as selective enrich-
aments. All media were incubated at 35°C. Isolations were made at 24 and 48 h by streaking half of a plate each of brilliant green agar (Difco) and Salmonella-Shigella agar (Merck). Swab samples in tetrasodium brilliant green broth were incubated at 35°C and streaked at 24 and 48 h on the above mentioned media. Procedures of ICMSF (13) were followed for incubation of these media and for further confirmation of Salmonella. Polyvalent antisera (Fisher Diagnostics) were used for sero-grouping.

Staphylococcus aureus was enumerated by spreading 0.25 ml of the homogenates on each of four petri plates and 0.1 ml per dilution on duplicate plates containing Baird-Parker agar (Oxoid). These were incubated at 35°C for 48 h (13). Further confirmation was done on the basis of coagulase production. Samples of water were tested according to procedures recommended by the World Health Organization (18). Water activity was tested with a hygrometer (Hygrodynamics No. 1.15-3050).

Descriptions of the street vending operations

Several street vending operations were visited before the analysis to select operations that were typical. Others were visited afterwards by teams to confirm that similar procedures were carried out and that similar hazards prevailed.

Street Vendor J was located on a busy street along side other vendors in a factory district near an area where transport vans parked for loading. The vendor's stand consisted of a rectangular metal frame, approximately 1 m x 1 m x 2 1/2 m high having three shelves in the upper portions and a cabinet in the bottom. Cooked foods were put on white paper on the lower two shelves. Sheets of plastic were draped from several stands to a fence on the other side of a side walk to provide some protection from rain and sun.

The stand was operated by the owner and an assistant between 06:00 and 22:00 hours. Most business was done around noon, but sales were made sporadically throughout the day.

Foods served were fried chicken, fried pork ribs with rind (chicharrón), fried fish (Bocito), fried ham and pieces of fried pig's head. Fried pork ribs with rind and pig's head were boiled before frying in lard.

Cooking was done over charcoal in a metal tire-rim (sup-ported by three legs that were welded to the rim) in the street in front of the stand. The cooked foods were displayed on the shelf.

Figure 1. Contamination, survival and growth of microorganisms associated with the preparation and display of fried chicken and fried fish and critical control points of operations and monitoring of Street Vendor J in Santo Domingo, Dominican Republic.
Contamination, survival and growth of microorganisms associated with preparation and display of pork ribs (with attached rind) and pieces of pig's head and critical control points and monitoring of operations of Street Vendor 1 in Santo Domingo, Dominican Republic.

until sold: when sales were made, foods were either reheated or not, as requested by the customer. Requests for reheating, however, were unusual during the time that the survey was made. According to the operator, food leftover at the end of the day was taken to his home and put into a refrigerator. It, however, was either not very cold, the foods were kept in bulk during storage, or the story was untrue because the foods were at ambient temperature when the vendor began operations the following morning. At that time, leftover foods were reheated by deep-fat frying. The operation was observed and temperature measurements were taken from 06:00 until 20:30 one day and from 06:00 until 10:00 the next day. Figures 1 and 2 illustrate the steps of the operation and indicate critical control points.

Street Vendor 2 sold fried pork ribs with rind, pork belly, ham, beef, sausage, cheese, plantains, corn bolillo (corn dough with anise), wheat tornoja (containing salted fish, green pepper and vegetables), and boiled yuca.

Two men operated the stand from about 06:00 until 21:00 or 22:00 hours. The stand was located in a factory district on the same street as Vendor 1 (two blocks away) near a busy intersection and transfer point for taxis, vans and trucks that carried people to various locations throughout the city and suburbs. The stand was crudely made of wood and consisted of a cabinet and overhead framing. A plastic sheet was draped from the framing to a 2 1/2-m high wall in back of the stand to provide some protection from the sun and rain. Another sheet of plastic was put over the food when it was raining.

Cooking was done, by charcoal, in pots supported either on a metal rack a half meter above the ground or on a square metal frame behind the vendor's stand. Foods were prepared in a manner similar to that used by Vendor 1, but they were initially cooked and sometimes reheated in the early afternoon instead of early morning. Cooked foods were displayed on top of the cabinet in a white metal pan. Sporadic sales occurred throughout the day. More foods were leftover than sold, and these were piled into a plastic bucket and put in the cabinet overnight. Reheating...
was sometimes done if requested by a customer.

Vendor 3 was located under a large tree in front of a factory down the street from Vendor 2. Complete meals of rice, beans, combinations of them (moros), meat and chickens were prepared. The vendor arrived at the stand around 07:30 hours and left between 13:00-14:00 hours. A large number of customers ate food at the stand at noon time. Most of them brought their own plate, bowl or pots and eating utensils, but a few plates and utensils were available for those who did not. These and cooking utensils were rinsed in a plastic pan of water and rubbed by hand followed by rinsing with water in a plastic bucket.

Equipment consisted of a large wooden table with a slatted top, a small metal-topped table, and several tire-rim cooking stands. The end slab on the large table (which had numerous ticks and grooves) was used for cutting meat; the metal-topped table was used to cut up chickens and other ingredients.

Pre-soaked beans and raw ingredients were brought to the stand in a cart. Raw meat and chickens were cut on the tables. Ingredients as shown in Figures 6-8 were added and cooking was done in pots heated by charcoal. The sauce was made of garlic, salt, fresh green pepper, onion, tomato paste, vinegar, oil, flavor cube and whole pepper. These were ground, if necessary, and mixed and fried. Foods were dispensed by large metal spoons from the pots in which they were cooked; none were leftover.

Vendor 4 was visited on two occasions: once during cooking and once during display. A large variety of fried foods and mixed foods dishes were prepared. Cooking was done outside in back of three rooms which were used for preparation, storage of raw and leftover foods and for eating. Cooked foods were displayed in a glass-encased cabinet on a sidewalk in front of the

Figure 3. Temperatures of foods prepared by Street Vendor 1, Santo Domingo, Dominican Republic.

Figure 4. Documented (from Table 1) and extrapolated time-temperature exposures of foods prepared and displayed by Street Vendor 1, Santo Domingo, Dominican Republic.
cooked beans in her home the day before leaving them at ambient temperature overnight. This process obviously permitted considerable multiplication of microorganisms, because the AMCC of the beans before cooking on the day of final preparation was $1.8 \times 10^9$ (Table 1). During cooking at the vending site, the beans were boiled for approximately 1 h (Fig. 9), yet the AMCC was still quite high ($9.2 \times 10^9$) just before serving. Spore formers were probably present, but *B. cereus* and *C. perfringens* were not recovered.

Rice was heated to near-boiling (Fig. 9). Shortly after cooking, the AMCC was $1.0 \times 10^9$, most of the organisms were *B. cereus* (Table 1).

Chicken attained a temperature of $82^\circ$C ($180^\circ$F) during cooking (Fig. 9); the AMCC after cooking was $5.0 \times 10^9$. A surprisingly high AMCC ($9.0 \times 10^9$) was found in the recently cooked meat.

*Salmonella* (polyvalent II) was isolated from a swab sample taken from the wooden table used to cut meat. *Entrobacteriaceae* (but no *Salmonella*) was isolated from the cooked beans. *Salmonella* was neither isolated from wash water from raw chicken nor from swabs rubbed over raw chicken skin, raw meat, or the table on which chicken was prepared.

The water activity was 0.95 for a sample of fried ham, 0.88 for fried sausage, 0.93 for *bolito*, 0.92 for *torreja*, 0.82 for fried fish and 0.83-0.94 for fried chicken.

During display of foods by Vendor 4 at 17:00 hour, food temperatures ranged from 27-41°C (80-150°F); the temperature of the air in the cabinet was 27°C (80°F). Foods would have remained within this temperature range until either they were sold or until the shop closed, and longer for foods left overnight.

A few samples of foods were collected from other vendors. Two raw milk samples had AMCC of $1.0 \times 10^9$ and $9.9 \times 10^9$; *S. aureus* counts of $2.5 \times 10^9$ and $4.4 \times 10^9$, respectively; *E. coli* biovar I was <10/g in both. A mixture of concentrated milk and orange juice (pH 3.5) had an AMCC of $2.7 \times 10^9$. *Pastel de hoja* (minced meat in mashed plantains wrapped in banana leaves) had an AMCC of $1.0 \times 10^9$ and a *B. cereus* count of $1.5 \times 10^9$ when collected at 18:00 hours (59°C/138°F).

**DISCUSSION**

Cooking is a critical control point relative to vegetative forms of pathogenic bacteria (e.g., *Salmonella*) that may be on raw meat, raw poultry and raw fish, or that may reach foods during preparation. Foods cooked by Street Vendors 1 and 3 attained temperatures that should be sufficiently high to kill large numbers of vegetative cells, but not all spores, of pathogenic bacteria. Yet, some foods had rather high aerobic mesophilic colony counts afterwards. The high count found in cooked beans at Vendor-3's operation could be explained either by survival of spores which could have come initially from the beans, garlic, coriander or pepper or by reduction of, but not elimination of, a very large number of vegetative cells that propagated during soaking overnight. In regard to

![Figure 5. Temperatures of foods during holding by Street Vendor 2, Santo Domingo, Dominican Republic.](image-url)
<table>
<thead>
<tr>
<th>Vendor product</th>
<th>Sampling time</th>
<th>Temperature (C/F)</th>
<th>AMCC/g</th>
<th>Clostridium perfringens/g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bocito (Fried fish)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left overnight</td>
<td>1 06:20</td>
<td>25/77</td>
<td>8.5x10⁶</td>
<td></td>
</tr>
<tr>
<td>After reheating</td>
<td>1 06:45</td>
<td>99/210</td>
<td>5.3x10⁵</td>
<td></td>
</tr>
<tr>
<td>After 7 h holding</td>
<td>1 14:00</td>
<td>29/84</td>
<td>8.6x10⁵</td>
<td></td>
</tr>
<tr>
<td>After additional 5 h holding</td>
<td>1 19:45</td>
<td>26/79</td>
<td>1.9x10⁵</td>
<td></td>
</tr>
<tr>
<td>After overnight holding</td>
<td>2 06:30</td>
<td>19/66</td>
<td>9.8x10⁴</td>
<td></td>
</tr>
<tr>
<td>After reheating and 3 h holding</td>
<td>2 10:00</td>
<td>29/84</td>
<td>4.0x10⁴</td>
<td></td>
</tr>
<tr>
<td><strong>Fried chicken</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left overnight</td>
<td>1 06:20</td>
<td>25/77</td>
<td>4.4x10⁷</td>
<td>2.2x10²</td>
</tr>
<tr>
<td>After reheating</td>
<td>1 06:30</td>
<td>97/206</td>
<td>1.1x10⁴</td>
<td>&lt;10</td>
</tr>
<tr>
<td>After 7 h holding</td>
<td>1 14:00</td>
<td>28/82</td>
<td>1.0x10⁵</td>
<td>&lt;10</td>
</tr>
<tr>
<td>After additional 5 h holding</td>
<td>1 19:35</td>
<td>26/79</td>
<td>1.3x10⁴</td>
<td></td>
</tr>
<tr>
<td>After overnight holding</td>
<td>2 06:30</td>
<td>20/68</td>
<td>3.7x10⁴</td>
<td></td>
</tr>
<tr>
<td><strong>Fried pigs head pieces</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left overnight</td>
<td>1 06:20</td>
<td>25/77</td>
<td>6.3x10⁸</td>
<td>3.5x10²</td>
</tr>
<tr>
<td>After reheating and 7 h holding</td>
<td>1 14:00</td>
<td>27/80</td>
<td>4.4x10⁴</td>
<td>2.9x10²</td>
</tr>
<tr>
<td>After additional 5 h holding</td>
<td>1 19:35</td>
<td>28/82</td>
<td>9.1x10⁴</td>
<td></td>
</tr>
<tr>
<td>After holding overnight</td>
<td>2 06:30</td>
<td>20/68</td>
<td>4.2x10⁴</td>
<td></td>
</tr>
<tr>
<td>After reheating and 3 h holding</td>
<td>2 10:00</td>
<td>30/86</td>
<td>6.7x10⁴</td>
<td></td>
</tr>
<tr>
<td><strong>Fried ham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left overnight</td>
<td>1 06:20</td>
<td>25/77</td>
<td>6.9x10⁸</td>
<td>3.5x10²</td>
</tr>
<tr>
<td>Chicharron (fried pork ribs with rind attached)</td>
<td>1 14:00</td>
<td>30/86</td>
<td>3.4x10⁶</td>
<td>&lt;10</td>
</tr>
<tr>
<td>After cooking and 5 h holding</td>
<td>1 19:20</td>
<td>28/82</td>
<td>5.2x10⁴</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Left overnight</td>
<td>2 06:20</td>
<td>20/68</td>
<td>5.8x10⁶</td>
<td></td>
</tr>
<tr>
<td>Drinking water</td>
<td>1 14:00</td>
<td></td>
<td>1.5x10¹⁰</td>
<td></td>
</tr>
<tr>
<td><strong>Fried corn bollito (history unknown)</strong></td>
<td>1 08:13</td>
<td>28/82</td>
<td>7.3x10⁴</td>
<td></td>
</tr>
<tr>
<td>After additional 12 h holding</td>
<td>1 20:18</td>
<td>23/73</td>
<td>9.0x10⁴</td>
<td></td>
</tr>
<tr>
<td>After left overnight</td>
<td>2 08:30</td>
<td>25/77</td>
<td>4.6x10⁶</td>
<td></td>
</tr>
<tr>
<td><strong>Fried wheat torreja (history unknown)</strong></td>
<td>1 08:15</td>
<td>28/82</td>
<td>7.5x10⁴</td>
<td></td>
</tr>
<tr>
<td><strong>Fried sausage (history unknown)</strong></td>
<td>1 08:10</td>
<td>31/87</td>
<td>4.2x10²</td>
<td>5x10²</td>
</tr>
<tr>
<td>After additional 6 h holding</td>
<td>1 14:45</td>
<td>25/77</td>
<td>1.2x10⁵</td>
<td>&lt;10</td>
</tr>
<tr>
<td>After additional 5 h holding (cooked 1400-1500)</td>
<td>1 20:15</td>
<td>23/73</td>
<td>8.0x10⁵</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Left overnight</td>
<td>2 08:24</td>
<td>25/77</td>
<td>3.5x10⁹</td>
<td></td>
</tr>
<tr>
<td><strong>Fried beef (history unknown)</strong></td>
<td>1 08:04</td>
<td>28/82</td>
<td>4.6x10⁸</td>
<td>&lt;10</td>
</tr>
<tr>
<td>After cooking</td>
<td>1 14:50</td>
<td>25/77</td>
<td>9.0x10⁸</td>
<td>&lt;10</td>
</tr>
<tr>
<td>After 5.5 h holding</td>
<td>1 20:13</td>
<td>23/73</td>
<td>7.9x10⁸</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Left overnight</td>
<td>2 08:27</td>
<td>25/77</td>
<td>3.6x10⁹</td>
<td></td>
</tr>
<tr>
<td><strong>Fried ham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left overnight, not reheated</td>
<td>1 08:05</td>
<td>28/82</td>
<td>1.4x10⁹</td>
<td>3.5x10²</td>
</tr>
<tr>
<td>After cooking or reheating</td>
<td>1 14:45</td>
<td></td>
<td>1.5x10³</td>
<td>&lt;10</td>
</tr>
<tr>
<td>After 5 h holding</td>
<td>1 20:10</td>
<td>23/73</td>
<td>1.3x10⁶</td>
<td></td>
</tr>
<tr>
<td>Left overnight</td>
<td>2 08:23</td>
<td>25/77</td>
<td>1.1x10⁶</td>
<td></td>
</tr>
<tr>
<td><strong>Fried cheese (history unknown)</strong></td>
<td>1 08:15</td>
<td>28/82</td>
<td>4.5x10³⁺²⁺</td>
<td>&lt;10</td>
</tr>
<tr>
<td>After heating</td>
<td>1 14:50</td>
<td>25/77</td>
<td>5.0x10³⁺</td>
<td></td>
</tr>
<tr>
<td>After 5 h holding</td>
<td>1 20:14</td>
<td>23/74</td>
<td>1.7x10³⁺⁴⁻</td>
<td></td>
</tr>
<tr>
<td>Chuleta (Fried pork belly) (history unknown)</td>
<td>1 20:15</td>
<td>23/73</td>
<td>4.5x10⁴</td>
<td></td>
</tr>
<tr>
<td>At least 5 h holding</td>
<td>2 08:37</td>
<td>25/77</td>
<td>2.8x10⁶</td>
<td></td>
</tr>
<tr>
<td>Chicharron (fried pork ribs with rind attached) (history unknown)</td>
<td>1 20:20</td>
<td>23/73</td>
<td>7.3x10⁴</td>
<td></td>
</tr>
<tr>
<td>Left overnight</td>
<td>2 08:23</td>
<td>25/77</td>
<td>5.7x10⁵</td>
<td></td>
</tr>
<tr>
<td><strong>Beans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precooked at house: left overnight</td>
<td>1 08:53</td>
<td>25/77</td>
<td>1.8x10⁶</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Cooked and at end of serving</td>
<td>1 12:57</td>
<td>27/80</td>
<td>9.2x10⁵</td>
<td></td>
</tr>
<tr>
<td><strong>Rice</strong></td>
<td>1 11:15</td>
<td>72/162</td>
<td>1.0x10⁴</td>
<td></td>
</tr>
</tbody>
</table>

JOURNAL OF FOOD PROTECTION, VOL. 51, MAY 1988
**Moro (rice and beans)**

- After cooking: 1 11:35 98/208 $5.0 \times 10^{2}$
- Chicken After cooking: 1 11:35 67/153 $5.0 \times 10^{3}$
- Meat After cooking: 1 11:25 55/131 $9.0 \times 10^{5}$
- Water: 1 08:53 - $9.0 \times 10^{1}$

*Salmonella* was not isolated from any food sample.

*Escherichia coli* <10/ml, AMCC results/ml.

*Bacillus cereus* <10/g.

*Staphylococcus aureus* <10/g.

*C. coli* 7.4x10/g.

*B. cereus* 1.0x10/g.

*B. cereus* 2.0x10/g.

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**Figure 6.** Contamination, survival and growth of microorganisms associated with preparation and display of chicken and a mixture of chicken and rice and critical control points and monitoring of operation of Street Vendor 3 in Santo Domingo, Dominican Republic.

The latter possibility, growth of *B. cereus* in uncooked beans during prolonged soaking has been observed (4). Either raw meat or raw poultry or spices could have accounted for the presence of heat-resistant spores in the meat dish. The high count in the cooked meat at Vendor-3's operation could have been due to a combination of survivors and contaminants from utensils. Large numbers of microorganisms could have been initially on the meat or introduced during cutting the raw meat on the wooden table slab or during other preparation methods.

The finding of *B. cereus* cells in rice and moro (which contains rice) shortly after cooking suggests that their spores survived cooking (4,11). Although vegetative cells can multiply in moist rice which is held for sufficient time within a temperature range that permits multiplication (12), high *B. cereus* counts were not recovered from the few samples obtained. This bacterium, however, has been responsible for outbreaks of foodborne illness when similar foods were held under similar conditions for several hours (6,11). Additional testing is needed to fully evaluate this situation for foods that are sold by street vendors.

Holding cooked foods at ambient temperature for 13 h or longer, as done by Vendors 1, 2 and 4 is quite hazardous. It is the major critical control point of the street-vending (cook/hold) operations surveyed. Increases in AMCC were observed as the duration of holding was prolonged, indicat-
Figure 7. Contamination, survival and growth of microorganisms associated with preparation and display of beef stew and critical control points and monitoring of operations of Street Vendor 3 in Santo Domingo, Dominican Republic.

Figure 8. Contamination, survival and growth of microorganisms associated with preparation and display of beans, rice and moro and critical control points and monitoring of operations of Street Vendor 3 in Santo Domingo, Dominican Republic.
Critical control points for foods prepared in households in which babies had salmonellosis

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2 Pan American Health Organization and Food Safety Consultation and Training, Tucker, GA, U.S.A.,
3 Dominican Institute of Industrial Technology, Santo Domingo, Dominican Republic,
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Sixteen babies undergoing rehydration therapy were examined for enteric pathogens. Salmonella agona was isolated from four, Salmonella enteritidis from two, Shigella boydii from one; neither Campylobacter nor Yersinia were recovered from any of the babies. Three households in which Salmonella group B (S. agona) was isolated from the babies were selected for hazard analysis of food preparation practices. In one house, S. agona was recovered from the feces of the mother and grandmother of the baby and from a kitchen knife, a blender, malagueta (spice) used to favor milk, a mop and flies. All foods were cooked to 100°C, and many were eaten a short time afterwards. Some foods were held at ambient room temperature until the arrival of an absent family member or kept overnight. During the holding interval large numbers of microorganisms accumulated in the foods, often exceeding 10^7/g. Bacillus cereus was recovered from 7 of 16 samples of cooked foods. A sample of 'moro' (rice and beans mixture) had a count of 1.5 x 10^8/g 5 h after cooking. Fecal coliforms were isolated from 11 of 12 foods samples; six samples of milk exceeded 10^7/g. Staphylococcus aureus was isolated from 11 samples; a sample of milk had a count > 10^9/g. Critical control points for milk formula were heating, holding after heating, cleaning and disinfecting bottles, nipples and pans used to store milk, and utensils used to dispense the milk.

Key words: Hazard analysis critical control point; Hazard analysis; Salmonellosis; Critical control point

Introduction

Diarrhea is a common cause of morbidity in the Dominican Republic, and babies are frequently treated for dehydration in hospitals, a situation found in many developing countries. To gather information on the possible role that foods fed to babies and also eaten by other family members have in the causation of diarrhea, hazard analyses were conducted in households in which babies were suffering from salmonellosis (Salmonella agona).

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Methods and Materials

Selection of households

Physicians in the rehydration ward of the pediatric hospital in Santo Domingo were requested to take rectal swabs from babies being given rehydration therapy and to gather information from the mother about feeding practices and the household situation. Furthermore, they were asked to describe the nature of the diarrhea, dates of onset and whether antibiotics were given. Questions asked of the mother concerned: number of household members and of bedrooms; source of water and whether it is available on the premises; whether water given to the baby is boiled; type of milk and food given to the baby (e.g., breast milk only, formula only, breast milk and formula, breast milk and family food, family food only); whether there is a refrigerator; and whether the family would permit a survey to be conducted within its house. This information, along with isolations made from fecal swabs, was used to choose three households for further study.

Description of households

Household 1 consisted of 18 persons in San Cristobal, a town about 20 km west of Santo Domingo. The family lived in half of a double house constructed of stucco-covered concrete block, concrete floor, concrete slab roof and wooden jalousie windows. There was a small sitting room, three bedrooms, a kitchenette, and a bathroom. Most of the cooking was done outside using charcoal in a metal tire-rim mounted on three metal legs, but some cooking was done on a small charcoal burner in one of the bedrooms. Running water from a community supply was available in the house. The family did not keep animals, but chickens frequently came through the yard to scavenge for food.

Household 2 consisted of 15 persons in Santo Domingo. The house was constructed of concrete block covered with stucco; the windows were of the jalousie type, the floor was of concrete, and the roof was of corrugated tin. It was the largest house in the survey and consisted of a long living/dining room, three bedrooms, a kitchen and a bathroom. A concrete block wall enclosed a large back yard in which a dog, a cat and chickens were kept. Although the house was plumbed for running water and there was a flush-type toilet, water was obtained from a tap about a block away. A drum of water was located in the kitchen and additional water was kept in approx 5 l plastic jugs. Cooking was done on a four-burner gas range and oven. Leftover foods were kept either in a refrigerator or in the oven. Milk prepared for the baby was not refrigerated.

Household 3 consisted of a baby and its parents and grandmother, also in Santo Domingo. The small house was constructed of concrete block and had wooden jalousie windows, a tin roof and a concrete floor. Living quarters consisted of two bedrooms and a kitchen. In the kitchen, there was a table holding a small three-burner gas stove; another holding dishes, a dish pan and covered dishes of cooked foods; and a 200 l drum of water. These items occupied most of the (2 x 1.5
m) floor space. Outside, beyond a board-covered window, was a concrete block area in which garbage was kept. A bathroom with flush-type toilet (flushed by siphon action) and a water tap and drain were located outside the house.

Hazard analyses

Hazard analyses consisted of observing food preparation and storage practices and watching for sources and modes of contamination. Temperatures in internal regions of foods were measured throughout cooking, holding after cooking and during reheating (if done). Samples were taken of foods at various stages of preparation and tested subsequently for aerobic mesophilic colony count and pathogens of concern.

Temperatures were measured by inserting a thermocouple (type T) capable of measuring temperatures from $-40^\circ C$ to $232^\circ C$ and having a needle-type sensor of appropriate length so as to be mostly covered by the food with the point near the geometric center. Air temperatures were taken with sensors with welded ends without tips. The thermocouple leads were plugged into either a battery-powered data logger (MLX Minilogger, A.D. Data Systems Inc. Rochester, NY, U.S.A.) or a hand-held battery powered potentiometer (Atkins Digital Thermometer 497, Atkins Technical, Gainesville, FL, U.S.A.). Time was recorded automatically on the data logger and observed from a wrist watch when the hand-held potentiometer was used. Time-temperature data were interpreted in reference to whether foodborne pathogens would survive cooking and reheating and serving. Observations made in the households were evaluated by making a diagram of the flow of the foods during preparation and holding. Potential sources of contamination from raw foods, water, equipment, utensils and persons preparing the foods were noted, as was the likelihood of survival or destruction and likelihood of microbial multiplication. Critical control points and factors that need to be monitored were indicated at appropriate steps of preparation.

Sampling foods

Samples of foods were taken either after cooking or reheating or during holding, as appropriate to evaluate either a potential hazard or control measure. Sample units were collected aseptically with either metal spoons or forks which had been cleaned and then inserted into 95% alcohol and flamed (three times). These were then put into sterile (whirl-pak) plastic bags.

Sampling food-contact surfaces

Food contact surfaces were sampled by rubbing a sterile swab over surfaces touched by foods. If the surface was dry, the swab was first moistened in sterile 0.1% peptone water (Difco). The cotton-tipped end of the swab was broken into a tube of tetrathionate brilliant green broth (Difco).
Collecting fecal specimens

Rectal swabs which were taken by physicians at the hospital were put into tubes of Cary-Blair transport medium (Difco). The tubes were refrigerated until analyses were performed.

In homes, fecal specimens from babies were collected by dabbing and twisting two swabs into fecal material on a soiled diaper. One swab was put into a tube of Cary-Blair transport medium and the other was put into a tube of tetrathionate brilliant green broth (Difco). Other persons from which specimens were collected, were each given a large plastic cup (which was labeled with their names), a paper towel and rubber band. They were instructed to defecate into the cup or otherwise put some of their feces into the cup, cover it with the towel, and secure the towel with the rubber band.

Laboratory procedures

Swabs taken from Cary-Blair transport medium were inoculated into several media in attempts to isolate Salmonella, Shigella, Yersinia and Campylobacter. For the isolation of Salmonella and Shigella, MacConkey (Gibco), bismuth sulfite (Gibco) and xylose lysine deoxycholate (XLD) (bio Mérieux) agar plates were streaked and selenite broth (Difco) was inoculated. All media except bismuth sulfite agar were incubated overnight at 37°C for 48 h. Bismuth sulfite and XLD agars were also streaked from the inoculated selenite broth. Up to the three colonies suspected of being pathogens per plate were picked to triple sugar iron agar (Difco), lysine iron agar (Difco), Simmon's citrate agar (Difco), hydrogen sulfide, indole and motility medium (SIM, Gibco), motility, ornithine decarboxylase and indole medium (Mio, Difco) and urea broth (Gibco). Salmonella polyvalent antisera I (A-E and Vi) and II (F-I) (Fisher Diagnostics) were used for grouping. Positive strains were sent to the National Institute of Microbiology, Carlos G. Malbrán, Buenos Aires, Argentina for serotyping. Shigella antisera groups, A, B, C and D (Fisher Diagnostics) were used for typing.

Cefsulodin-irgasan-novobiocin (CIN) agar (Oxoid) was used for the isolation of Yersinia enterocolitica. Incubation was at room temperature (approx. 29°C) and at 37°C for 24 h. Cold enrichment was also performed by holding the Cary-Blair tubes at 4°C for a week before streaking onto CIN agar as described above. Biochemical identification was performed with the same media as was used to characterize Salmonella and Shigella.

For the isolation of Campylobacter, blood agar base No. 2 (Oxoid) with the addition of the Campylobacter-selective supplement (Blaser-Wang) (Oxoid) and ovine blood (defibrinated) was used. Incubation was done in a jar with a gas-generating envelope for Campylobacter (Oxoid) at 42–43°C for 48 h. A Gram stain with carbofuchsin (in place of safranin) was performed on suspected colonies. All colonies with spirillum-shaped organisms were confirmed by determining their sensitivity to nalidixic acid and cefalotin and by performing oxidase, catalase, and hippurate hydrolysis tests.
10 g of solid or semi-solid analytical samples of foods were homogenized with 90 ml of 0.1% peptone water (Difco) in plastic bags using a stomacher. Liquid or semi-liquid analytical samples were mixed by inversion before 10 ml were taken. Decimal dilution(s) and aerobic mesophilic colony counts (AMCC), pour plate method 1, were done using plate count agar (Difco) according to ICMSF methods (1978).

*Bacillus cereus* was enumerated with phenol-red egg-yolk polymyxin agar (prepared from ingredients) by spreading 0.25 ml of the homogenate on each of four Petri plates and 0.1 ml of each dilution on duplicate plates. This procedure and confirmation followed those given by ICMSF (1978). Samples of raw rice, corn starch, cubes of chicken soup, raw beans and dried milk were screened for spores of *B. cereus* by putting 25 g of the food into 50 ml of tryptic soy broth (Difco). These samples were blended (if necessary), heated at 70°C in a water bath for 15 min, and then cooled in an ice bath before incubating overnight at 30°C. A loopful of the incubated broth was streaked onto phenol red-egg-yolk polymyxin agar which was incubated overnight at 30°C.

Fecal coliforms were enumerated by the most probable number (MPN) method using E.C. broth (Difco) incubated at 44.5°C in a water bath for 24 h if gas was present. Samples without gas were incubated for an additional 24 h.

*Staphylococcus aureus* was enumerated by spreading 0.25 ml of the homogenate on each of four Petri plates and 0.1 ml of dilutions on duplicate plates containing Baird-Parker agar (Oxoid). Confirmation was by coagulase production. Methods followed procedures in ICMSF (1978).

For the isolation of *Salmonella* from foods, analytical samples of 25 g were pre-enriched in 225 ml of lactose broth (Difco or Merck). Either tetrathionate brilliant green broth (Difco) and selenite broth (Difco) or selenite cysteine broth (Merck) were used as selective enrichments. All media were incubated at 35°C and isolations were made at 24 and 48 h by streaking half a plate of brilliant green (Difco) and *Salmonella-Shigella* agars (Merck). Procedures of ICMSF (1978) were followed for incubation of these media and for further confirmation of *Salmonella*. Polyvalent antisera (Fisher Diagnostics) were used for serogrouping before sending the strains for serotyping. Swab samples from equipment and utensils were inoculated into tetrathionate brilliant green broth which was incubated at 35°C and streaked at 24 and 48 h intervals on the above mentioned media.

For isolation of *Shigella*, analytical samples of 25 g were enriched in 225 ml of Gram-negative (GN) broth (prepared from ingredients) according to ICMSF (1978) and incubated at 35°C overnight; XLD agar (Difco) and *Salmonella-Shigella* agars (Merck, Difco) were used for selective isolation. Procedures followed those of ICMSF (1978).

**Results**

*Salmonella* was isolated from six rectal-swab specimens collected from 16 babies who were being given rehydration therapy at the pediatric hospital. Four of the
<table>
<thead>
<tr>
<th>Household</th>
<th>Description of food</th>
<th>Time sampled</th>
<th>Temp. (°C)</th>
<th>AMCC/g b</th>
<th>B. cereus/g</th>
<th>S. aureus/g</th>
<th>Fecal coliform MPN/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milk formula made from dried milk and corn starch (prepared 07:00 h)</td>
<td>1 18:00</td>
<td>27</td>
<td>$3.8 \times 10^5$</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&gt;1100</td>
</tr>
<tr>
<td></td>
<td>Reconstituted milk-corn starch formulae (prepared 07:00 h)</td>
<td>2 11:45</td>
<td>27</td>
<td>$1.6 \times 10^6$</td>
<td>&lt;10</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>'Moro' (rice and beans)</td>
<td>2 17:25</td>
<td>28</td>
<td>$2.9 \times 10^7$</td>
<td>$1.5 \times 10^6$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Spaghetti with tomato sauce (pH 4.2; cooked 12:00 h)</td>
<td>1 14:00</td>
<td>29</td>
<td>$1.1 \times 10^8$</td>
<td>$1.5 \times 10^5$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Shredded fresh coconut</td>
<td>2 10:00</td>
<td>27</td>
<td>$6.7 \times 10^8$</td>
<td>&lt;10</td>
<td>-</td>
<td>&gt;1100/g</td>
</tr>
<tr>
<td></td>
<td>Coconut milk</td>
<td>2 10:15</td>
<td>27</td>
<td>$4.5 \times 10^9$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rice with coconut milk</td>
<td>2 12:23</td>
<td>77</td>
<td>$8.2 \times 10^4$</td>
<td>-</td>
<td>-</td>
<td>1100/g</td>
</tr>
<tr>
<td></td>
<td>Beans with coconut milk</td>
<td>2 12:30</td>
<td>50</td>
<td>$4.0 \times 10^2$</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Vegetable soup</td>
<td>1 20:00</td>
<td>93</td>
<td>$3.8 \times 10^4$</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dried fish with potatoes and vinegar, pH 4.6</td>
<td>2 -</td>
<td>-</td>
<td>$8.9 \times 10^4$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wash water from dried salted fish</td>
<td>2 12:00</td>
<td>-</td>
<td>$1.9 \times 10^4$</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dried milk</td>
<td>2 -</td>
<td>-</td>
<td>-</td>
<td>Pos.</td>
<td>&lt;10</td>
<td>-</td>
</tr>
<tr>
<td>Item</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Pos.</td>
<td>-</td>
</tr>
<tr>
<td>Dried beans</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Neg.</td>
<td>-</td>
</tr>
<tr>
<td>Water previously boiled</td>
<td>1</td>
<td>18:00</td>
<td>27</td>
<td>$8.5 \times 10^3$</td>
<td>-</td>
<td>-</td>
<td>$2.3 \times 10^3$</td>
</tr>
<tr>
<td>Reconstituted milk</td>
<td>1</td>
<td>20:00</td>
<td>33</td>
<td>$2.8 \times 10^3$</td>
<td>-</td>
<td>$&lt;10$</td>
<td>$&gt;1.1 \times 10^6$</td>
</tr>
<tr>
<td>Reconstituted milk left-over from day before</td>
<td>2</td>
<td>07:00</td>
<td>8</td>
<td>$6.6 \times 10^3$</td>
<td>-</td>
<td>$2.5 \times 10^5$</td>
<td>$1.1 \times 10^6$</td>
</tr>
<tr>
<td>refrigerated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reconstituted milk after boiling with seasoning</td>
<td>2</td>
<td>08:45</td>
<td>82</td>
<td>$3.2 \times 10^3$</td>
<td>-</td>
<td>$&lt;10$</td>
<td>$&lt;3$</td>
</tr>
<tr>
<td>Idem</td>
<td>2</td>
<td>14:45</td>
<td>28</td>
<td>$3.3 \times 10^3$</td>
<td>-</td>
<td>-</td>
<td>$1.5 \times 10^3$</td>
</tr>
<tr>
<td>Rice after cooking</td>
<td>2</td>
<td>12:30</td>
<td>85</td>
<td>$&gt;3.0 \times 10^{11}$</td>
<td>$3.5 \times 10^4$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rice and beans kept in oven</td>
<td>1</td>
<td>19:45</td>
<td>28</td>
<td>$6.5 \times 10^7$</td>
<td>$&lt;10$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Beans leftover and stored in refrigerator</td>
<td>2</td>
<td>11:00</td>
<td>8</td>
<td>$5.9 \times 10^6$</td>
<td>$&lt;10$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Beans</td>
<td>2</td>
<td>12:35</td>
<td>54</td>
<td>$3.1 \times 10^6$</td>
<td>$1.5 \times 10^4$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>'Moro' (ready made meal from neighbor)</td>
<td>2</td>
<td>12:35</td>
<td>-</td>
<td>$5.0 \times 10^5$</td>
<td>$3.5 \times 10^2$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Luncheon meat stew</td>
<td>1</td>
<td>20:30</td>
<td>32</td>
<td>$&lt;10^3$</td>
<td>$&lt;10$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steamed chicken</td>
<td>2</td>
<td>12:30</td>
<td>63</td>
<td>$4.6 \times 10^5$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Plantains</td>
<td>1</td>
<td>20:30</td>
<td>32</td>
<td>$6.0 \times 10^3$</td>
<td>$&lt;10$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yuca</td>
<td>1</td>
<td>20:30</td>
<td>31</td>
<td>$1.3 \times 10^4$</td>
<td>$&lt;10$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water from plastic jugs for drinking</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>$6.0 \times 10^5$</td>
<td>-</td>
<td>-</td>
<td>$&lt;3$</td>
</tr>
<tr>
<td>Water from tap on street</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>$2.5 \times 10^3$</td>
<td>-</td>
<td>-</td>
<td>$&lt;3$</td>
</tr>
</tbody>
</table>
TABLE I (continued)

<table>
<thead>
<tr>
<th>Household</th>
<th>Description of food</th>
<th>Time sampled</th>
<th>Temp. (°C)</th>
<th>AMCC/g</th>
<th>B. cereus/g</th>
<th>S. aureus/g</th>
<th>Fecal coliform MPN/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Raw milk</td>
<td>2 08:00</td>
<td>29</td>
<td>8.0×10^9</td>
<td>–</td>
<td>&lt;10</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>Milk leftover in bottle</td>
<td>2 08:00</td>
<td>25</td>
<td>6.4×10^9</td>
<td>–</td>
<td>7.0×10^2</td>
<td>1.5×10^3</td>
</tr>
<tr>
<td></td>
<td>Reconstituted milk with corn starch, malagueta and cinnamon</td>
<td>2 14:15</td>
<td>29</td>
<td>5.2×10^9</td>
<td>–</td>
<td>&lt;10</td>
<td>&gt;1.1×10^6</td>
</tr>
<tr>
<td></td>
<td>Pork and rice</td>
<td>1 20:30</td>
<td>54</td>
<td>&lt;10</td>
<td>1.5×10^2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Pork and rice leftover</td>
<td>2 07:45</td>
<td>25</td>
<td>5.8×10^7</td>
<td>1.3×10^2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>‘Moro’</td>
<td>2 14:20</td>
<td>54</td>
<td>4.0×10^3</td>
<td>&lt;10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Dried milk</td>
<td>2 –</td>
<td>–</td>
<td>–</td>
<td>Neg.</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Dried beans</td>
<td>2 –</td>
<td>–</td>
<td>–</td>
<td>Pos.</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Bottled water for baby</td>
<td>1 18:00</td>
<td>27</td>
<td>1.1×10^2</td>
<td>–</td>
<td>–</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>Drinking water</td>
<td>1 –</td>
<td>–</td>
<td>–</td>
<td>9.3×10^4</td>
<td>–</td>
<td>9.0</td>
</tr>
</tbody>
</table>

*Salmonella* was not isolated from any food sample.

*Aerobic mesophilic colony count.*
isolates were *Salmonella agona*; the other two were *Salmonella enteritidis*. *Shigella boydii* was isolated from another baby, but neither *Campylobacter* nor *Yersinia* was recovered from the specimens.

*Salmonella agona* was isolated from the baby of Household 1 and from its 11-years-old sister. This serotype was not isolated from any of the samples of food or water (Table I).

At House 2, *Salmonella* was neither isolated from the baby (from which *S. agona* had been isolated at the hospital) nor from the grandmother who did most of the cooking. *Salmonella indiana*, however, was recovered from fecal droppings of the family cat which frequently walked in the area inhabited by chickens. *Salmonella* was not isolated from any of the samples of food or water (Table I).

At House 3, *Salmonella agona* was recovered three times from fecal specimens from the baby whose diarrhea persisted. It was also isolated from stool specimens (solid in consistency) from the mother and grandmother. *Salmonella agona* was also isolated from a kitchen knife, a blender, *malaguea* (*Amomis caryophyllata*) (spice balls used to flavor foods and milk), a pool of approximately 10 flies killed in the room where the baby was kept, and a string from a mop (Table II summarizes results of specimens and samples collected during the hazard analyses in House 3).

---

**Fig. 1.** Contamination, survival and growth of microorganisms associated with preparation and storage of milk formulas, washing and disinfecting bottles and nipples, and critical control points of operations and monitoring procedures in Household 1.
As a result of these findings, three additional samples of malagueta were collected; one from a large supermarket, one from a small store and one from a street vendor of spices. *Salmonella* was not isolated from these samples.

TABLE II
Isolations of *Salmonella* from specimens collected from members of Household 3 and from samples of food, water, utensils and equipment surfaces and the environment

<table>
<thead>
<tr>
<th>Description of specimen/sample</th>
<th>Salmonella serovar</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baby</strong></td>
<td></td>
</tr>
<tr>
<td>Rectal swab at hospital</td>
<td><em>agona</em></td>
</tr>
<tr>
<td>Day 1, swab of loose stool from diaper</td>
<td><em>agona</em></td>
</tr>
<tr>
<td>Day 1, loose stool collected in cup by family</td>
<td><em>agona</em></td>
</tr>
<tr>
<td>Day 2, swab of loose stool from diaper</td>
<td><em>agona</em></td>
</tr>
<tr>
<td>Mother (solid stool)</td>
<td><em>agona</em></td>
</tr>
<tr>
<td>Father (solid stool)</td>
<td>–</td>
</tr>
<tr>
<td>Grandmother (solid stool)</td>
<td><em>agona</em></td>
</tr>
<tr>
<td>Diaper pail</td>
<td>–</td>
</tr>
<tr>
<td>Drain in bathroom</td>
<td>–</td>
</tr>
<tr>
<td>Water from pan outside below supply tap</td>
<td>–</td>
</tr>
<tr>
<td>Water from drum in kitchen used for cooking and cleaning</td>
<td>–</td>
</tr>
<tr>
<td>Water from jug used for drinking</td>
<td>–</td>
</tr>
<tr>
<td>Water, bottled (purchased for baby)</td>
<td>–</td>
</tr>
<tr>
<td>Dried beans</td>
<td>–</td>
</tr>
<tr>
<td>Dried milk</td>
<td>–</td>
</tr>
<tr>
<td>Milk reconstituted before heating</td>
<td>–</td>
</tr>
<tr>
<td>Milk (boiled with malagueta and cinnamon)</td>
<td>–</td>
</tr>
<tr>
<td>Malagueta (<em>Amomis caryophyllata</em>)</td>
<td><em>agona</em></td>
</tr>
<tr>
<td>Apple juice given to baby (pH 3.6)</td>
<td>–</td>
</tr>
<tr>
<td>Pear juice given to baby (pH 3.5)</td>
<td>–</td>
</tr>
<tr>
<td>Rice with pork after cooking</td>
<td>–</td>
</tr>
<tr>
<td>Rice with pork after holding at ambient (room) temperature overnight</td>
<td>–</td>
</tr>
<tr>
<td>Beans and rice after holding at ambient (room) temperature overnight</td>
<td>–</td>
</tr>
<tr>
<td>Kitchen knife</td>
<td><em>agona</em></td>
</tr>
<tr>
<td>Blender</td>
<td><em>agona</em></td>
</tr>
<tr>
<td>Mop string</td>
<td><em>agona</em></td>
</tr>
<tr>
<td>Flies (pool of 10 collected in baby's room)</td>
<td><em>agona</em></td>
</tr>
</tbody>
</table>

-, Salmonella not detected.
Fig. 2. Contamination, survival and growth of microorganisms associated with preparation and storage of 'moro' and critical control points and monitoring procedures in Household 3.

Fig. 3. Contamination, survival and growth of microorganisms associated with preparation and storage of chicken stew and critical control points and monitoring procedures in Household 2.
TABLE III
Temperature attained during cooking and holding of foods \(^a\) in households in the Dominican Republic

<table>
<thead>
<tr>
<th>Household</th>
<th>Food</th>
<th>Time above 74°C (min)</th>
<th>Time between 74 and 54°C (min)</th>
<th>Time between 49 and 21°C (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soup</td>
<td>70</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>tomatoes and peppers</td>
<td>67</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>potatoes and carrots</td>
<td>47</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>spaghetti</td>
<td>3</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>flavor cube</td>
<td>20</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Baby formula</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>water</td>
<td>12</td>
<td>48</td>
<td>&gt; 445 (^b)</td>
</tr>
<tr>
<td></td>
<td>cornstarch</td>
<td>4</td>
<td>42</td>
<td>&gt; 445 (^b)</td>
</tr>
<tr>
<td></td>
<td>dried milk</td>
<td>55</td>
<td>42</td>
<td>&gt; 445 (^b)</td>
</tr>
<tr>
<td></td>
<td>Water used to heat baby bottle</td>
<td>45</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beans</td>
<td>80</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>-</td>
<td>-</td>
<td>&gt; 180 (^b)</td>
</tr>
<tr>
<td></td>
<td>Rice 1</td>
<td>97</td>
<td>22, 28 (^c)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rice 2</td>
<td>&gt; 65</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rice and beans</td>
<td>-</td>
<td>-</td>
<td>&gt; 485 (^b)</td>
</tr>
<tr>
<td>2</td>
<td>Milk</td>
<td>18</td>
<td>55</td>
<td>&gt; 258 (^b)</td>
</tr>
<tr>
<td></td>
<td>Beans</td>
<td>47</td>
<td>42</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>54</td>
<td>31</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chickens</td>
<td>43</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Plantains</td>
<td>66</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Yuca</td>
<td>63</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Luncheon meat stew</td>
<td>23</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>Pork and rice dish</td>
<td>-</td>
<td>-</td>
<td>680 (^d), 975 (^e)</td>
</tr>
<tr>
<td></td>
<td>pork</td>
<td>90</td>
<td>71</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>rice</td>
<td>72</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>14</td>
<td>27</td>
<td>&gt; 307 (^b)</td>
</tr>
<tr>
<td></td>
<td>Beans before rice added</td>
<td>144</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>‘Moro’ (rice and beans)</td>
<td>60, 63</td>
<td>29, 51 (^e)</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) All foods attained 100°C during cooking.
\(^b\) > indicates that foods were still left at room temperature at the completion of the survey.
\(^c\) Two portions handled differently.
\(^d\) If eaten at breakfast time.
\(^e\) If eaten at lunch time.
Food-flow diagrams for typical foods prepared in the homes are illustrated in Figs. 1–3. Hazards, critical control points and approaches for monitoring are indicated. Fig. 1 illustrates the preparation of a milk formula for feeding a baby in Household 1. Similar procedures were used in Households 2 and 3, but cornstarch was not always added by the preparer in House 2, and straining was not always done. The mother of Household 3 boiled the nipple and top but not the bottle (which was only washed) and cinnamon was added rather than brown sugar and lemon. Fig. 2 illustrates the preparation of ‘moro’, a very popular food in the Dominican Republic, in Household 3. Fig. 3 illustrates the preparation of chicken stew in Household 2.

Table III summarizes temperature data that foods attained during cooking and holding after cooking and the duration within certain temperature ranges. During cooking, all foods attained a temperature of 100°C and were usually eaten promptly after cooking. Occasionally, cooked foods were saved for a few hours for persons who were not at home during meal time. They were sometimes held from one meal to the next or overnight. Only one household had a refrigerator; cooked foods in the other households were held at ambient room temperature. Sometimes, as shown in Table I, foods were within a temperature range at which microbial multiplication could occur for long periods.

Aerobic mesophilic colony counts of samples of foods collected in the households during hazard analyses are listed in Table I. Over half of the counts exceeded $10^6$/g. *Bacillus cereus* was isolated from 7 (44%) of 16 samples of cooked food and from 3 of 5 dry food samples. Three samples of cooked foods (beans, rice and ‘moro’) had counts exceeding $10^4$/g. The sample of ‘moro’ had a *B. cereus* count of $1.5 \times 10^6$/g; it had been stored at room temperature for approximately 5 h after cooking.

*Staphylococcus aureus* was isolated from 2 of 11 samples of foods. The count exceeded $10^5$/g in a sample of milk which had been prepared from dried milk the day before.

Fecal coliforms were isolated from 11 out of 12 food samples; 10 had counts that exceeded $10^3$/ml and 6 from milk exceeded $10^5$/g.

**Discussion**

*Salmonella* is a significant cause of diarrhea in babies in the Dominican Republic. Although the number of specimens collected in the pediatric hospital was small, *Salmonella* was isolated from 38%.

The initial source of *Salmonella* that infected the babies was not identified. In Household 3, however, several isolations of *S. agona* were made from which a hypothesis of a common source could be made. The *S. agona* isolated from the knife could have come from raw meat or raw poultry; this would not be surprising. If it had been the initial source that infected the baby, however, it would have had to survive there for several days without having been wiped or washed off.
Flies could have either brought salmonellae into the house or picked them up there. They were seen on soiled diapers in the diaper pan and could have readily picked up organisms from the feces. Flies reflect their environment in regard to the microorganisms that they carry (Greenberg, 1971). Once infected or contaminated, of course, they can play a role in furthering the spread of microorganisms.

It was impossible to determine whether the mother and grandmother of Household 3 were infected prior to or after the baby had become infected because they did not manifest symptoms. Whichever, the initial source was not discovered.

Malagueta was kept in a small jar subject to contamination from the container and other spices, but it was inaccessible to flies. Hands may have contaminated the malagueta if the spiceball had been handled prior to being put into the jar. Spices are subject to contamination with *Salmonella* during harvesting, processing and storage (ICMSF, 1980). If so, malagueta could be the source of *S. agona* for many households because it is commonly used to flavor foods and milk in the Dominican Republic and four babies were infected by this serovar. This hypothesis remains to be tested.

The mop served to spread salmonellae throughout the house. It probably either picked up these organisms in the entrance way where soiled diapers were washed, in the toilet room, or in the kitchen from meat and poultry scraps or drippings that fell to the floor. These organisms apparently survived and possibly multiplied on the damp feces- and food-stained mop. The mop could have transferred these organisms to the hands of the user when wrung-out as well as to the floor and other surfaces that it contacted. Mops should be washed after use and the heads boiled or at least soaked in recently-boiled water periodically and hung so as to dry.

*Bacillus cereus* was often found in cooked products, particularly beans and rice. A sample of 'moro', for example, had $1.5 \times 10^6$ *B. cereus* per g after being left at room temperature for approximately 5 h (see Table III, Household 1). With such a quantity of organisms, emetic or diarrheagenic toxins could generate within a few hours (Gilbert, 1979). Although this high count was only found in a single sample, it shows that high counts of *B. cereus* do develop in rice/beans subject to time-temperature abuse in homes, as has been demonstrated in food-service establishments by Bryan and Bartleson (1985), Bryan et al. (1981). Large numbers of *B. cereus* have been found in weaning and household foods by Barrell and Rowland (1979), Capparelli and Mata (1975) and Rowland et al. (1978). *B. cereus* may play a significant role as a causative agent of diarrhea in developing countries. Foods (rice and beans) commonly eaten and the time-temperature abuse that they are frequently subjected to should cause concern and stimulate further investigation.

*Staphylococcus aureus* was occasionally isolated from home prepared foods. In a sample of reconstituted milk, which had been kept at room temperature for several hours, it exceeded $10^5$ /g (see Table III, Household 2). The milk had been boiled, so contamination, no doubt, occurred afterwards during straining, while pouring, or from pans, cans or jugs used to store the milk. Holding at room temperature for long intervals permitted multiplication of staphylococci and possible formation of enterotoxins. Rowland et al. (1978), reported isolating up to $10^6$/ml *S. aureus* from home prepared milk held for 8 h after preparation.
A reconstituted dried-milk and cornstarch mixture that was kept at room temperature frequently contained large quantities of fecal coliforms (Table I). This finding is consistent with *Escherichia coli* isolations from weaning foods reported by Barrell and Rowland (1979) and Rowland et al. (1978). The numbers increased in proportion to the holding time. Contamination after heating, during straining or contact with improperly cleaned pans and holding at room temperature for several hours are likely to lead to this situation. Large quantities of fecal coliforms were also found in shredded coconut where contamination probably occurred during husking, from the knife, shredder, or hands of the preparer.

The high aerobic mesophilic counts (AMCCs) in many of the samples of foods infer prolonged holding within the temperature range that these microorganisms multiply. This was observed (Tables I and III), for example, in samples of leftover, cooked pork and rice (Household 3), reconstituted milk (at 11:45, 17:15 and 18:00 h in Household 1, and leftovers from the day before in Household 3), and rice and beans (Households 1 and 2).

In the homes surveyed, foods eaten, recipes used, and food preparation practices were similar; differences, when there were any, related to facilities in the kitchens. Hazards observed in the homes were associated with: (1) presence of bacterial spores on rice, beans, spaghetti, spices and raw vegetables and enteric pathogens on raw meat, raw poultry and fresh fish; (b) time-temperature abuse of cooked foods during holding after cooking; (c) cross contamination from raw to cooked foods; (d) improper cleaning of utensils and storage containers, and (e) handling of foods.

Critical control points (WHO/ICMSF, 1982), related to the foods being prepared, could be classified into three categories: (a) milk-based preparations for babies, (b) rice and beans and (c) meat/chicken/vegetable-containing dishes, soups and stews. Critical control points for the milk preparations for babies were heating water and/or milk, holding milk afterwards, and washing and disinfecting bottles and nipples and storage containers. Thorough heating (equivalent to or exceeding time-temperature standards for pasteurization) is essential to kill enteric and other vegetative bacteria that are likely to be present in raw milk or water and which on rare occasions may be found in dried-milk, or that reach the mixture during preparation. Proper holding (e.g., that of short duration; or under refrigeration) of heated milk preparations prevents outgrowth of spores and multiplication of resulting vegetative cells. The spores may come from cornstarch, spices (e.g., malagueta and cinnamon) as well as being present in the milk itself, and some will survive cooking. *Bacillus cereus* has been isolated from dried milk by Torres-Angel et al. (1980); and from milk preparations in homes by Barrell and Rowland (1979).

The critical control point for preparation of rice, beans, and ‘moro’ is holding after cooking. The hazard is that spores survive cooking and following their germination the resulting vegetative cells multiply at room or ambient outside temperature.

Thorough cooking of meat/chicken/vegetable dishes, soups and stews is a critical control point. So too is holding within a temperature range that inhibits the germination of spores and the multiplication of resulting vegetative cells, as well as microbial contaminants that reach the foods after cooking. If the foods are liquid or
predominantly liquid, monitoring of cooking can be done by ensuring that foods boil and then simmer. Monitoring the cooking of solid foods and masses of small particles (e.g., beans, rice) is more difficult. It will probably have to be done on the basis of the cooks' knowledge and experience about the thoroughness of cooking.

Holding cooked foods (e.g., for a person who is not at home at meal times or any other reason) for eating several hours (e.g., overnight) after preparation is a food safety hazard or major concern. This observation was made by Bryan (1978), Bryan et al. (1986), Capparelli and Mata (1975), Roberts (1982), Rowland et al. (1978) and Van Steenbergen et al. (1983). There are a few time-proven ways to eliminate this hazard. Some may be impractical in certain households, however, because of limited financial resources, lack of facilities, or the time persons arrive home relative to food preparation.

The best advice that can be given to households in developing countries were modern facilities for holding cooked foods are lacking is to eat foods promptly after cooking - while they are still hot. Otherwise, they should be prepared as late as feasible before the planned meal time. Whenever feasible, feeding-size portions of weaning foods should be prepared for a single feeding and not held between feedings.

Cooked foods were usually protected from flies or airborne contaminants by lids on pans or by bowls or saucers placed over the dishes. Cooked foods were seldom touched by hand, but they were dispensed with utensils and put into pans, bowls, and dishes that were not washed using hygienic procedures. Thus, kitchen utensils and tableware could play a role in cross contamination and be vehicles by which microorganisms survive, and, when food residues and moisture are present, multiply. From observations made during preparation, utensil surfaces appeared to be a more probable source of fecal coliforms and S. aureus than flies or dust.

Reheating cooked foods, when this is done, may be a critical control point. Many cooked foods, however, were eaten without reheating. If reheated, they should be brought to the boil and simmered or otherwise reach high temperatures during reheating. Monitoring procedures are as with cooking. If large quantities of microorganisms are in foods as a result of temperature abuse during prolonged storage, reheating as commonly done may only reduce the contaminants by 2-3 logs.

Knowing the critical control points in homes directs attention to education. Information about critical control points, their control and monitoring in homes needs to be communicated to persons (e.g., health officials, teachers, and the public) who can put it into effect. In this regard, it is recommended to first train health officials (e.g., epidemiologists, food microbiologists, sanitarians, and perhaps nutritionists) to conduct hazard analyses so as to focus attention on specific food-preparation practices and to uncover factors that contribute to the causation of diarrheal diseases. In this training, some contemporary views about causation of diarrheal diseases may have to be modified, and the belief that foods are safe because they are cooked must be dispelled because many foods are eaten many hours after cooking.

An educational campaign aimed at informing the public and stimulating them to action should follow confirmation of the data by national public health authorities
and appropriate socio-cultural data. Such a campaign should include alerts of hazards associated with the preparation of foods that are frequently eaten in homes. Means to prevent the hazardous situations of effecting control at critical control points, of monitoring critical control points in a practical way and of taking action when the situation is out of control should be the emphasis of the campaign. Information about hazards associated with preparation of popular foods and safe practices, as well as personal hygiene and education about good nutrition, should be incorporated into the curricula of primary and secondary schools. For this to be effective, teachers need to be so educated and provided with training aids to communicate this information effectively.

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References


Critical control points for foods prepared in households whose members had either alleged typhoid fever or diarrhea

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Hazard analysis of food preparation practices were conducted in four households and eleven others were visited to survey both food preparation practices and environmental conditions. Households selected had members who were suffering from either diarrhea of unknown etiology or alleged typhoid fever. Hazard analyses and sanitary surveys included gathering data on time-temperature exposures of foods, collecting samples of food and drinking water, sampling sewage or drains, and obtaining stool specimens from persons with diarrhea and from family controls. Food samples were tested for aerobic mesophilic colony counts and common foodborne pathogens; specimens were tested for Salmonella, Shigella, Campylobacter and Yersinia. Campylobacter was isolated from two persons purported to have diarrhea, but neither Salmonella, Shigella nor Yersinia were recovered from alleged cases or controls. Salmonella agona was recovered from a latrine. Most foods were cooked to internal temperatures to or near to boiling. Those not promptly eaten were held at ambient room or outside temperatures until a subsequent meal, until a family member returned home, or until lunch time when taken to the fields. During these intervals, microorganisms multiplied and mesophilic aerobic organisms increased often reaching $10^8/g$ or greater before consumption. None of these foods were reheated before eating. Bacillus cereus was isolated from 4 of 10 samples; one sample of 'morc' (beans and rice) exceeded $10^6/g$, two other samples exceeded $10^5/g$. Staphylococcus aureus was isolated from 7 of 14 samples, one exceeded $10^5/g$. Fecal coliforms were isolated from 8 of 14 food samples, five exceeded $10^5/g$. Neither Salmonella nor Shigella were isolated from any food, the community water supplies or from vessels of water within houses. Fecal coliform counts of water were less than 3/ml, except one sample from a clay vessel (9/ml). Risks associated with cooked foods which were not promptly eaten appeared to be greater than that associated with water.

Key words: Hazard analysis; Critical control point; Sanitary surveys

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Introduction

Diarrheal diseases are the main causes of morbidity in the Dominican Republic, and they have held this position for many years. Since 1979, the incidence rate of reported clinically diagnosed cases has exceeded 2000 per 100,000 population. From 1980 to 1983, 44% of these cases occurred in children less than 1 year old and only 11% were 15 years old or over. During the interval 1979 to 1983, the incidence rate for clinically diagnosed, laboratory-unconfirmed cases of typhoid and paratyphoid fevers ranged from 15 to 29 per 100,000 population. To illustrate this situation, during 1984, at the Robert Reid Cabral Pediatric Hospital in Santo Domingo, diarrheal diseases were the primary cause of hospitalization and the second highest cause for consultations, for admissions due to communicable diseases, and for deaths as described by González and Mendoza (1986). Official statistical data on foodborne and waterborne diseases are unavailable.

Specific factors that influence transmission of the etiologic agents of these diseases in the Dominican Republic are essentially unknown. To shed some light on this situation, a study was initiated to gather information about food preparation and holding practices in households and sanitary facilities on premises of a few persons who allegedly had either fever or diarrhea.

Methods and Materials

Selection of households

During discussions with health officials in Santiago de los Caballeros, Dominican Republic, it was ascertained that two possible outbreaks of alleged typhoid fever were occurring. One was in Buenos Aires, a crowded suburb of Santiago; the other was in La Delgada, a farm village.

In Buenos Aires, a household (1) in which three persons recently had alleged typhoid fever was chosen for study. A house (2) located behind and downhill from House 1 was also selected because a child who resided there was recovering from alleged typhoid fever.

In La Delgada, a household (3) from which a 13 year-old boy was recovering from alleged typhoid fever and a neighbor who had recently died of the same syndrome (household 4) were selected for study. Upon questioning members of these households, it was discovered that persons residing in 11 neighboring houses were suffering from either ‘the fever’ or diarrhea. These additional households were also included.

Household 1 was located in Buenos Aires where open sewers and streams of sewage emerging from water-carried toilets and overflowing latrines are commonplace. There were six members at this household; parents, three children and a teenaged, live-in maid. House 1 was constructed of concrete blocks; it had wooden jalousie windows, a corrugated-tin roof and a concrete floor. Inside, there was a narrow living/dining room, three small bedrooms, a kitchen and a small room (with
water tap and drum for storing water) that was used as a bath/shower room. A pit latrine with tin shelter and wooden platform and riser was located in a fenced small back yard. The pit was completely filled to the riser. The latrine was a few meters from a concrete block retaining wall separating this property from that of House 2. During rains, surface run-off water (including sewage effluent) flowed over the wall down onto the walk-way adjacent to House 2 and sometimes seeped into this house.

The kitchen of House 1 had a gas range and a refrigerator. The refrigerator, however, was not in working order, but it was used to store milk and leftover food on top and on the shelves inside.

Household 2 consisted of a woman and two children living in a one-room 5.5 X 4 m apartment in the back of a concrete block house with concrete floor and tin roof. Cooking was done on two charcoal burners. Water was obtained from a tap in an outside shower/toilet room, and stored in a clay vessel near the cooking area. This room also contained a syphon-type toilet that was flushed by pouring water into the bowl.

Household 3, consisting of 5 persons, resided in La Delgada, a farm village of 30–40 houses. Living quarters included three wood-framed structures with palm-thatched roofs and earthen floors. One structure had wooden siding and was used for sleeping. Another had siding of thin vertical slats; it was used as a kitchen. The third was mostly open and was used for family activities. Community well water, chlorinated at its source, was piped into the backyard. Nearby was a wooden privy that covered a pit latrine with wooden platform and riser. Chickens, ducks, guinea pigs and two steers were kept in the yard.

Cooking was done in iron pots and aluminium pans in the center of the kitchen on a concrete block structure. Sticks and charcoal were used as fuel. Condiments and leftover foods were stored on shelves in a corner of the hut, and cups, pans and lids were hung on the walls. Water was kept in two large earthenware vessels.

Houses 4–15 were located in the same village. All but two were of similar construction with pit latrines. The exceptions were a house made of concrete blocks with a concrete floor and tin roof, and a wood-framed house with a concrete floor and store attached where staple foods, soft drinks, beer and rum were sold. The store keepers also boiled milk and made an ice-milk product which was also sold in the store. Water was piped to most yards, although some persons collected roof run-off during rains. Both were used for drinking without boiling. Cooking was done either in a separate structure or a short distance from the houses.

Hazard analyses

A microbiological hazard is considered as unacceptable contamination, growth, or survival of microorganisms of concern to food safety or of unacceptable production or persistence in foods of toxic microbial metabolites (WHO/ICMSF, 1982). Hazard analyses and critical control point evaluations were conducted in households from the morning on one day until the afternoon of the next, as described in our previous work by Michanie et al. (1987).
Sanitary survey

The environmental situation within and around the houses was evaluated. Samples of community water were collected from taps at the homes and at the source after allowing the water to flow for about 1 min. Water stored in earthenware vessels or drums were sampled by inserting a cotton pad (tampon) into each vessel and keeping it in place with a string for approximately 18 h. These pads were sometimes tied below leaking faucets in a manner that water ran over them. After sampling, each pad was lowered into a Whirl-pak bag and the string was then cut by a clipper that had just been dipped into 95% alcohol and flamed three times.

Sewage was sampled by dropping a sanitary napkin (pad) attached by a string into a latrine pit, so that it came to rest in or on the sewage. Just prior to removal, the pads were moved about so as to touch various surfaces of the sewage in the pit. These pads were also put into streams of sewage flowing from surface latrines, sewers or drains; they were held in place with wires. Small cotton pads held in place with strings were also put into households drains. All pads were left in place for approximately 18 h.

Collecting food and specimen sample units

As described by Michanie et al. (1987) sample units of foods were taken either after cooking or during holding, as appropriate, to evaluate potential hazards or control measures.

Persons were each given a large plastic cup, a paper towel, and a rubber band. They were instructed to defecate into the cup or otherwise put some of their feces into it, cover it with the towel, and secure the towel with the rubber band. A sterile swab was streaked into the feces several times and transferred to Cary-Blair transport media (Difco) by a member of the investigative team.

Laboratory procedures

Specimens were analysed in the same way as in a previous study by Michanie et al. (1987) to isolate Salmonella, Shigella, Yersinia, and Campylobacter. Aerobic mesophilic colony count (AMCC), Bacillus cereus, Staphylococcus aureus, Salmonella and Shigella were determined in food samples, following the same procedures and culture media described by Michanie et al. (1987).

The sanitary tampon pads and napkins were analysed for attempts to isolate Salmonella and Shigella; they were divided into three equal parts: one each was put into 100 ml of Gram-negative broth (Difco), selenite broth (Difco) and tetrathionate brilliant green broth (BBL). These were incubated at 37°C for 24 h. Bismuth sulfite (Difco) and xylose lysine desoxycholate (XLD, Difco) agars were used for the isolation of Salmonella from tetrathionate brilliant green and selenite broth; XLD and Salmonella-Shigella agars (Merck and Difco) for the isolation of Shigella from Gram-negative broth. Salmonella and Shigella were characterized as described by Michanie et al. (1987).
Results

*Campylobacter* was isolated from a fecal specimen from a 11-month old baby of Household 1. *Salmonella, Shigella, Campylobacter* and *Yersinia* were not recovered from stool specimens from members of the family. *Salmonella agona*, however, was recovered from the latrine. *Salmonella* was neither isolated from a 7-year-old boy who had recently been hospitalized and was recovering from alleged typhoid fever at House 2 nor from a 2-year-old neighbor boy who was suffering from diarrhea and sometimes ate at the house.

At La Delgada, neither *Salmonella, Shigella* nor *Yersinia* were recovered from fecal specimens obtained from 16 persons who purportedly had either diarrhea or alleged typhoid fever or from stool specimens from 20 persons who were purportedly

<table>
<thead>
<tr>
<th>Household</th>
<th>Food</th>
<th>Highest temperature attained (°C)</th>
<th>Time above 74°C (min)</th>
<th>Time between 54 and 74°C (min)</th>
<th>Time between 21 and 49°C (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oats with chocolate</td>
<td>97</td>
<td>20</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>100</td>
<td>75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Fish stew</td>
<td>100</td>
<td>48</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>'Toyota' (vegetable)</td>
<td>98</td>
<td>47</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>'Mondongo' (tripe) stew</td>
<td>98</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Yuca</td>
<td>91</td>
<td>15</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Beans</td>
<td>100</td>
<td>173</td>
<td>29</td>
<td>445&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Rice</td>
<td>100</td>
<td>66</td>
<td>28</td>
<td>410&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Spaghetti</td>
<td>65</td>
<td>0</td>
<td>43,55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
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<td>Spaghetti with sauce</td>
<td>100</td>
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<td>30</td>
<td>0</td>
</tr>
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<td></td>
<td>Spaghetti after sauce added</td>
<td>77</td>
<td>7</td>
<td>9</td>
<td>0</td>
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<td>4</td>
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<td>100</td>
<td>173</td>
<td>7</td>
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<td>Rice, batch 1</td>
<td>100</td>
<td>102</td>
<td>52</td>
<td>34</td>
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<tr>
<td></td>
<td>Rice, batch 2</td>
<td>100</td>
<td>92</td>
<td>11</td>
<td>&gt;160&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Beans and rice</td>
<td>-&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>26-82</td>
</tr>
<tr>
<td></td>
<td>Yuca</td>
<td>97</td>
<td>26</td>
<td>6</td>
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</tr>
<tr>
<td></td>
<td>Cucumber</td>
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<td>5</td>
<td>Beans</td>
<td>100</td>
<td>158</td>
<td>72</td>
<td>&gt;448&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>100</td>
<td>74</td>
<td>67</td>
<td>&gt;375&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> If eaten at 20:00 h as done the day before.
<sup>b</sup> Separated, some left in pan, other portion in bowl.
<sup>c</sup> Foods within temperature range at the completion of the survey.
<sup>d</sup> Not determined.
<table>
<thead>
<tr>
<th>Household</th>
<th>Description of food</th>
<th>Day</th>
<th>Sample hour</th>
<th>(°C)</th>
<th>AMCC/g b</th>
<th>B. cereus/g</th>
<th>S. aureus/g</th>
<th>Fecal coliform (MPN/ml)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Reconstituted milk made at 06:00 h</td>
<td>1</td>
<td>19:00</td>
<td>27</td>
<td>$1.4 \times 10^5$</td>
<td>&lt;10</td>
<td>&lt;3</td>
<td>&lt;3</td>
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<td></td>
<td>Reconstituted milk</td>
<td>2</td>
<td>09:30</td>
<td>–</td>
<td>$5.8 \times 10^4$</td>
<td>2.0 $\times 10^1$</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>Reconstituted milk for baby</td>
<td>2</td>
<td>– d</td>
<td>–</td>
<td>$1.2 \times 10^4$</td>
<td>&lt;10</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>Cocoa-dry milk-oats mixture</td>
<td>2</td>
<td>09:30</td>
<td>35</td>
<td>$7.0 \times 10^2$</td>
<td>&lt;10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Tripe (pre-cooked) purchased from neighbor</td>
<td>1</td>
<td>17:00</td>
<td>–</td>
<td>$1.8 \times 10^3$</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Tripe with sauce</td>
<td>1</td>
<td>18:15</td>
<td>80</td>
<td>$1.4 \times 10^4$</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Leftover meat sauce</td>
<td>1</td>
<td>19:00</td>
<td>27</td>
<td>$1.2 \times 10^4$</td>
<td>7.0 $\times 10^1$</td>
<td>&lt;3 / g</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>Salted, dry, raw fish</td>
<td>2</td>
<td>11:30</td>
<td>–</td>
<td>$1.2 \times 10^3$</td>
<td>–</td>
<td>1.0 $\times 10^1$</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Salted, dry, cooked fish</td>
<td>2</td>
<td>12:30</td>
<td>97</td>
<td>$7.0 \times 10^3$</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Cooked rice</td>
<td>2</td>
<td>12:30</td>
<td>75</td>
<td>$1.8 \times 10^4$</td>
<td>6.2 $\times 10^3$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Yuca</td>
<td>1</td>
<td>18:15</td>
<td>47</td>
<td>$5.0 \times 10^1$</td>
<td>&lt;10</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>Tap water</td>
<td>1</td>
<td>18:15</td>
<td>–</td>
<td>&lt;10</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Washing water from rice</td>
<td>2</td>
<td>11:30</td>
<td>–</td>
<td>$5.7 \times 10^7$</td>
<td>1.0 $\times 10^1$</td>
<td>–</td>
<td>1.1 $\times 10^6$</td>
</tr>
<tr>
<td>2</td>
<td>Milk from baby bottle</td>
<td>1</td>
<td>17:40</td>
<td>29</td>
<td>$1.6 \times 10^7$</td>
<td>&lt;10</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>Beans and rice leftover</td>
<td>1</td>
<td>18:35</td>
<td>28</td>
<td>$1.7 \times 10^5$</td>
<td>&lt;10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Potatoes leftover</td>
<td>1</td>
<td>18:00</td>
<td>30</td>
<td>$7.0 \times 10^3$</td>
<td>5.0 $\times 10^1$</td>
<td>2.0 $\times 10^1$</td>
<td>g</td>
</tr>
<tr>
<td>Sample Description</td>
<td>Time</td>
<td>Temperature</td>
<td>Aerobic Mesophilic Colony Count (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>--------------------------------------------</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beans after cooking</td>
<td>12:30</td>
<td>59</td>
<td>$1.2 \times 10^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice after cooking</td>
<td>12:30</td>
<td>93</td>
<td>$&lt;10$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spaghetti after cooking</td>
<td>12:30</td>
<td>49</td>
<td>$2.4 \times 10^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water in plastic jug</td>
<td>10:00</td>
<td></td>
<td>$&lt;10$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Tea in baby bottle</td>
<td>12:30</td>
<td>48</td>
<td>$5.8 \times 10^7$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>12:30</td>
<td>48</td>
<td>$4.5 \times 10^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice purchased</td>
<td></td>
<td></td>
<td>$8.5 \times 10^3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water from clay vessel</td>
<td>1</td>
<td></td>
<td>$3.4 \times 10^3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Egg tortilla leftover cooked at noon</td>
<td>17:00</td>
<td>25</td>
<td>$5.4 \times 10^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beans</td>
<td>14:30</td>
<td>27</td>
<td>$1.0 \times 10^{11}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled milk, leftover</td>
<td>17:00</td>
<td>28</td>
<td>$6.4 \times 10^8$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk boiled</td>
<td>10:00</td>
<td></td>
<td>$2.2 \times 10^8$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk boiled, stored in oil tin</td>
<td>10:00</td>
<td></td>
<td>$1.0 \times 10^{10}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice cream bar</td>
<td>17:00</td>
<td></td>
<td>$7.3 \times 10^8$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice cream bar</td>
<td>14:30</td>
<td></td>
<td>$6.4 \times 10^6$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice and beans</td>
<td>17:00</td>
<td>28</td>
<td>$7.8 \times 10^6$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plantin-milk-water mixture for baby</td>
<td>09:15</td>
<td>28</td>
<td>$1.8 \times 10^9$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well water before chlorination,</td>
<td></td>
<td></td>
<td>$&lt;10$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>La Delgada</td>
<td>2</td>
<td></td>
<td>$6.1 \times 10^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idem</td>
<td>2</td>
<td></td>
<td>$1.2 \times 10^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idem</td>
<td>2</td>
<td></td>
<td>$1.0 \times 10^3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All samples negative for *Salmonella and* Shigella.

*Not determined.*
well but had loose or watery stools. *Campylobacter* was isolated from two persons; one of whom had purported to have diarrhea.

Neither *Salmonella* nor *Shigella* was isolated from water vessels or taps in or at any of the homes. They were also not isolated from any food sample.

Usually, all foods in which temperatures were measured attained temperature exceeding 74°C during cooking; they usually were 90°C or above and frequently reached the boiling point (Table I). The only exception was spaghetti in which the temperature only reached 65°C, but remained between that temperature and 54°C for at least 43 min.

Although foods were frequently eaten promptly after cooking, some were held for 6 h or more before consumption (Table I). These usually had high AMCCs; nearly half had counts that exceeded $10^6$/g and many exceeded $10^8$/g (Table II). *Bacillus cereus* was recovered from 4 of 10 samples. One sample of 'moro' (rice and beans) had a *B. cereus* count of $6.0 \times 10^6$/g; a sample of rice and one of beans exceeded $10^3$/g. *Staphylococcus aureus* was isolated from 7 of 14 samples, once from milk in a quantity that exceeded $10^3$/g. Fecal coliforms were isolated from 8 of 14 samples of food, milk, ice-cream and tea in a baby bottle had quantities that exceeded $10^6$/ml.

Discussion

The sources of *Salmonella typhi* that may have infected persons in Buenos Aires and in La Delgada were not identified. Another etiologic agent may have been responsible. Hence, some of the clinically diagnosed cases of typhoid fever may in fact be other diseases, and consequently distort morbidity statistics. The cases in the present study may have ceased shedding *S. typhi* or were shedding them intermittently, or they had not indeed been infected with this agent at all. If it had been the responsible agent, it is surprising to not have recovered it from the environment, particularly, from the latrines. Only *S. agona* was found. This serotype is one of the ten most common isolates in many countries.

In Buenos Aires, sewage-effluent from toilet drains, latrine pits and drop privies was accessible to children while at play and to vectors, and it sometimes flooded some streets and seeped into low-laying houses during heavy rains. By no means however, did all persons in the community have direct contact with these effluents.

In both communities, water was chlorinated at its source. Even though cross connection and back syphonage potentials existed in Buenos Aires and in La Delgada water was stored in clay vessels, subject to contamination from hands and utensils. *Salmonella* and *Shigella* were not found and fecal coliforms were either negative or their counts were low. There was no indication that water was the source of the agents that caused bacterial diarrhea or the alleged typhoid fever.

Fly breeding was observed in some of the latrine pits, and the latrine platforms and risers were not fly-tight. Flies had ready access to all houses because windows and doors were not fly-tight. Cooked foods, however, were usually protected from flies by lids or inverted bowls or saucers placed over containers and dishes of food.
Although studies have reported association between fly populations and diarrheal disease rates, there is no satisfactory evidence that fly control is effective in reducing these rates, as stated in a document of the WHO (1985).

Foods were well cooked. They were seldom touched by hands after cooking; they were dispensed with utensils and put into poorly cleaned pans, bowls and dishes.

Cooked foods were commonly kept at room or outside temperatures for several hours while awaiting return of an absent family member, while transporting them to or keeping them in the fields for lunch or for serving the following day. This hazardous situation has been pointed out by: Bryan (1978), Bryan and Bartleson (1985), Bryan et al. (1981), Bryan et al. (1986), Capparelli and Mata (1975), Gilbert (1979), Rowland et al. (1978), and Van Steenberger et al. (1983). In this regard, bacterial spores (e.g., *B. cereus* and *C. perfringens*) which survive cooking could germinate and propagate to large numbers or elaborate toxins (in the case of *B. cereus*) during the prolonged-holding periods as demonstrated by Nester and Woodburn (1982).

In reference to propagation during holding, nearly half of the foods had AMCCs that exceeded $10^6$/g. *Bacillus cereus* was found in beans, rice, a mixture of them ('moro'), and a mixture of cocoa, oats, and dry milk. In the case of 'moro', large numbers were found. Under the right set of time-temperature and moisture conditions, emetic or diarrheagenic toxins could be elaborated as the cooked foods are held at room or outside temperatures for several hours as shown by Blakey and Priest (1980), Bryan et al. (1981), Gilbert (1979) and Nester and Woodburn (1982). Hence, rice and beans (which are commonly eaten in the Dominican Republic) may be important vehicles in the transmission of diarrheal diseases. Further laboratory and epidemiologic investigations are needed to prove or refute this hypothesis.

Counts exceeding $10^7$/g of *S. aureus* were found in a sample of milk which had been kept at room temperature. Since the milk had been heated, the contamination, no doubt, occurred afterwards, perhaps when pouring or dipping portions out or from pans, cans or jugs used to store the milk. Holding heated milk which probably contains relatively few competitive microorganisms at room temperature for long intervals is conducive to multiplication of staphylococci and possible elaboration of enterotoxins as described by Bryan (1976). This milk was used to make ice-milk that was sold at the local store.

Milk and formulae containing milk that was kept at room temperature were often found to contain fecal coliforms (Table II). Contamination after heating is the most likely event leading to this situation.

A critical control point is an operation (practice or procedure) at or by which a preventive or control measure can be exercised that will eliminate, prevent or minimize a hazard (WHO/ICMSF, 1982). Based on the observations made during the hazard analyses, critical control points for preparation of tripe stew, fish stew and vegetables are cooking and holding after cooking (these would be also applicable for meat, fish and poultry dishes). Thorough cooking is essential to kill enteric pathogens, and proper holding afterwards is essential to inhibit the germination of spores and the multiplication of resulting vegetative cells and bacterial contami-
nants that reached the foods after cooking. Depending upon circumstances, handling after cooking may also be a critical control point.

Based on the hazard analyses, the critical control point for preparation of spaghetti, rice, beans and 'moro' is holding after cooking. The hazard is that bacterial spores survive cooking and germinate, and the resulting vegetative cells multiply during holding. Acidification of the spaghetti sauce by tomato and vinegar is another possible control measure, depending on the amount of high-acid ingredients, mixing and contact time.

Depending on practices in households, reheating leftovers, cleaning of receptacles in which cooked foods are kept and utensils used to dispense them may also be critical control points.

If foods are liquid or contain large amounts of fluid, monitoring of temperatures during cooking or reheating can be done by ensuring that the foods boil and then simmer. Monitoring of solid foods is more difficult, but the high temperatures of cooking oil or fat should result in inactivation of vegetative forms of bacteria and some spores. Monitoring of holding practices is by time; holding at room or outside temperatures should not exceed 5 h. Monitoring cleanliness of storage-containers and utensil surfaces is done by ensuring that they are thoroughly washed.

From observations made in and around the homes, foods that were not eaten promptly after cooking posed a greater risk than any other environmental factors. The major hazard was holding cooked foods at room or outside temperatures for several hours.

Although certain hazards were observed as demonstrated by laboratory results and time-temperature measurements during the evaluations in the households visited, other hazards, no doubt, occur from time to time and when different foods are prepared. Additional ones, as well as good practices, must occur in other households. Hence, additional data needs to be collected before the situations can be considered typical of population groups in the Dominican Republic. Nevertheless, the control measures described are applicable at critical control points wherever the specific foods evaluated are prepared in a similar manner.

Preventive actions are based on the homemakers knowledge of hazards and practical control measures. This information must come from educational activities (which are focused on food-specific preparation and storage practices), from health education curricula in schools, and from training in homes in which informed homemakers reside. Such training awaits confirmation of these results by conducting hazard analyses in additional households coupled with socio-cultural data. The education must give caution about the identified hazards and provide economically-feasible and practical preventive measures and monitoring procedures, and stimulate the public to become motivated to take appropriate action to safeguard family members.

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References


Hazards and Critical Control Points of Vending Operations at a Railway Station and a Bus Station in Pakistan

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ABSTRACT

Hazard analyses (which included watching operations, measuring temperatures of foods throughout preparation and display, and sampling and testing for microorganisms of concern) were conducted of vending operations at a railway and a bus station in a large city in Pakistan. Commonly prepared foods which were surveyed included: rice, pulses, chick peas, ground meat and potato mixtures, meat stew, and okra. Temperatures were measured and samples were collected from a variety of other foods. Large numbers (107) of Clostridium perfringens were isolated from samples of pulses, ground meat dishes, and chick peas collected during display, 8 to 10 h after cooking. Aerobic colony counts were also high in these and other foods that were held for several hours, unless hot, at temperatures >55°C throughout the holding periods or periodic reheating practiced (which was done by a few vendors). Cooking was usually thorough, but spores survived which germinated during the display period. High temperature holding or periodic reheating maintained safe foods, and hence, are critical control points for these operations. Education about these matters ought to be directed at health and transportation authorities, vendors, and the public.

Foods are commonly vended at transportation centers such as railway or bus stations where there is constant and heavy movement of people. At these locations, people often wait for their initial transport or next connection and have short delays during loading and rest stops. During these periods, they may be either hungry or thirsty, and vendors are present to sell them foods and drinks.

Although there is no readily obtainable epidemiological data about the risks of foodborne diseases resulting from these foods in Pakistan, sparse information about the risk of street-vended foods in other developing countries has been published (28). Laboratory evidence shows that risk of spreading agents of foodborne disease via street-vended foods can be high or that such foods frequently have high microbial counts (1,6,9,10,21-24,26-29). Furthermore, foods that are vended in the stations include items that have been frequently identified as vehicles in outbreaks of foodborne diseases in countries where foodborne disease surveillance data are available (4).

Hazard analyses were conducted of food-vending operations in a railway station and at a bus station in a large city in Pakistan to identify critical control points at which simple, economical, and effective preventive measures can be applied.

MATERIALS AND METHODS

Description of vending operations

Foodservice facilities at the stations were permanent stands, stalls, and carts. A permanent stand at each was evaluated in detail and temperature measurements were made and samples taken from other operations.

Railway station. The railway station was a roofed structure that had a concrete platform which led to tracks. Two sets of stairs at each end of the station led to elevated walkways which crossed the tracks to another platform with a roof cover. On one end of the main station about a tenth of the mile away, there was a third platform with a roof cover. Municipal water was available from taps at the station; toilets were present.

The cooking area of the primary vendor (R1) was a hollow square formed by three wooden counters and a wall. Foods were cooked by gas and then held over charcoal much of the time during display in large pans with lids at the counter which faced the tracks. Several other similar vendor stands were located in a row the length of the main platform. There were also several other vendors (R2-R9) who sold foods from wooden push carts on one or more of the platforms. The carts usually had a gas or charcoal heater for cooking and reheating. Cooked items were usually held without a heat source, but the foods were occasionally reheated at various times for varying intervals.

Some of the vendors prepared foods at the site; others prepared them at home and brought them to the station for sale. Most of the vendors sold similar kinds of foods, which included chick peas (Kabla chana), pulses, ground meat and potatoes, okra, rice, a squash, and meat stew made of small cuts of meat and vegetables.

Bus station. Buses parked in a large lot for loading and unloading. Numerous food-vending operations were located along sidewalks on two sides adjacent to the lot.

The primary vendor (B1) was housed in a building where tables and chairs were available for customers. Foods were cooked over gas burners in the back and then transferred to the front. There, chick peas and rice were held in large pans over charcoal.
A man sat with the foods all day to stir and serve them and to keep them warm by periodically putting a gas burner under the pans. The other foods (meat stews, pulses, and vegetables) were displayed in pans on a counter at the sidewalk. Raw skinned chickens were hung over the counter to attract customers and to show that chicken was for sale. Another man sat nearby to serve the cooked foods and to cook the chicken on request. These foods were occasionally reheated by a gas heater.

Other vendors (B2-12) prepared and displayed foods either in small stalls along a sidewalk or on the sidewalk. Pulses, ground meat and potato, rice, meat stew, and vegetable dishes (frequently okra) were commonly sold. Additionally, vendors (a) cooked cuts of meat mixed with other ingredients on a large disk griddle, (b) squeezed sugar cane stalks and sold the juice, or (c) prepared and sold iced beverages of different varieties.

**Hazard analyses**

Hazard analyses consisted of (a) observing food preparation and storage practices to identify sources and modes of contamination; (b) measuring temperatures in internal regions of foods during or after cooking, periodically while holding foods on display and after reheating to evaluate survival, destruction, and growth; and (c) collecting samples of foods before and after sequential stages of preparation and testing them microbiologically (7). Additionally, these procedures were done sporadically at other permanent and transient vending operations at the stations.

Food temperatures were measured by inserting thermocouples (either type K or T) with needle-type sensors of various lengths into foods so that the sensing point was near the geometric center, and when feasible, so that the shaft was nearly covered by the food under study. Air temperatures were taken with type-T thermocouples with welded ends. Before use in foods, thermocouples were washed, rinsed, wiped dry with a paper tissue, and immersed three times in 95% alcohol and flame after each immersion. The thermocouple leads were plugged into either a battery-powered Pronto-Plus (Thermo Electric, Saddle Brook, NJ) hand-held, digital potentiometer or a battery-powered Technoterm 9300 (Testoterm GmbH & Co., Lenzkirch, Germany) hand-held, digital potentiometer.

Samples of approximately 200 g of food were collected either after cooking or reheating or during holding, as appropriate to the situation being studied. These were collected aseptically with a metal spoon after it had been washed, rinsed, and immersed three times in 95% alcohol and flame after each immersion. Samples were put into sterile Stomacher bags which were fastened at the top with either rubber bands or plastic bag fasteners. Bagged samples of hot foods were cooled by immersing into water; they were then put into an insulated plastic box containing ice. Samples were held in ice until they reached the laboratory where they were put into a refrigerator until analyses were begun, usually the following morning.

**Laboratory procedures**

Samples for analysis were homogenized according to the International Standards Organization (ISO) technique (16). They were premixed and 25 g of the mixture was transferred into 225 ml of 0.1% peptone water in a sterile plastic bag and homogenized in a Stomacher. Liquid analytical samples were thoroughly shaken. Decimal dilutions were made with 1 ml of the initial homogenate with 9 ml of sterile dilution fluid consisting of 0.1% peptone water and 0.075% (w/vol) agar (11). This formulation allowed thorough mixing and pipetting and was useful for spreading when the drop plating method was used. With a pipette tip, 0.05 ml of the selected dilution was carefully spread within one section. This technique was used for the enumeration of the viable (mesophilic aerobic) count, coliforms, and *Staphylococcus aureus* on plate count agar (Merck 5463), violet red bile agar (Merck-Germany 1406) containing 10 g lactose per 1, and Baird-Parker agar (Oxoid/Unipath CM 617) supplemented with tellurite and egg yolk emulsion (Unipath SR 54), respectively. All media were prepared according to the manufacturers instructions. Coliform plates were overlayed with selective medium after inoculation. Duplicate plates were made for assay of each sample.

Enumeration of *Bacillus cereus* was done on polymixin pyruvate egg yolk mannitol bromothymol blue agar (Unipath CM 617) supplemented with 50,000 IU of polymixin per 1 and egg yolk emulsion (Unipath SR 47). One hundredth ml of the dilution was spread on the agar in a 13-cm diameter petri dish. Tryptose sulfite cycloserine agar (Merck-Germany 11972), supplemented with 0.5 g D-cycloserine per 1, was used for the enumeration of *Clostridium perfringens*. Pour plate technique with 1 ml per dilution was applied according to ISO procedure (18).

According to the Pakistani laboratory policy and European procedures, incubation of plate count and coliforms was done at 37°C and not at the typical American prescribed temperature of 35°C. Plates were read after 20 h. Baird-Parker agar plates were read after 48 h at the same temperature. Inoculated polymixin pyruvate egg yolk mannitol bromothymol blue agar plates were held for 24 h at ambient temperature (28-32°C) which allowed intensive growth of bacilli.

Typical colonies of *S. aureus* were presumptively confirmed with the Staph-Rapid-Test (Roche Diagnostika, 07330386). This slide test uses stabilized sheep erythrocytes loaded with rabbit plasma containing fibrinogen that detects the clumping factor and immunoglobulin G which reacts with protein A. Final confirmation was done with subcultured colonies using the coagulase test according to ISO procedure (17).

Suspicious colonies of *B. cereus* were preliminarily identified with a combined staining procedure for spores and lipid granula according to Ashby and Burdon (13). Confirmation was done with at least two strains of every positive sample by ISO procedures (19). *C. perfringens* colonies were confirmed according to ISO procedure (18).

The isolation method for *Salmonella* followed the four-stage procedure of ISO 6579 (20). Preenrichment of 25 g or ml of the sample in 225 ml buffered peptone water for 16 h at 37°C was followed by enrichment in magnesium chloride malachite green medium and in selenite cystine medium for 24 h at 42 and 37°C, respectively. Plating was done on brilliant green phenol red agar (Unipath CM329) and bismuth sulfite agar (Difco 0073). Confirmation was done by the *Salmonella* Reference Laboratory of the National Institute of Health, Pakistan.

The pH was measured with a hand-held digital pH meter (model 2300 Testoterm, Lenzkirch, Germany), adjusted to sample temperature.

**RESULTS**

*Railway station*. Preparation and holding of meat stew with indication of hazards and critical control points by Vendor R1 are illustrated in Fig. 1. Ground meat and potatoes were prepared in a similar fashion, but ingredients differed. Preparation steps and hazards and critical control points for chick peas are illustrated in Fig. 2. Time-temperature exposures of the foods prepared by this vendor are shown in Fig. 3. Cooking temperatures exceeded 73.9°C (165°F) for all foods tested. The cooked foods were often held for many hours before they were sold and consumed.
Many of the foods were reheated periodically to various temperatures. Temperatures (measured at various times over a 2-d interval) of foods on display by vendors (other than R1) at the station are shown in Fig 4. Over 50% of the foods were at temperatures at which bacterial growth occurs; sometimes temperatures were optimum for growth of pathogenic foodborne bacteria. In all cases, the time of holding can be extended beyond that shown in the graphs because foods were often continued to be sold after the last temperature measurement, or they would be on other occasions.

Figure 1. Preparation and holding of beef stew by vendor R1.

Figure 2. Preparation and holding of chick peas by vendors R1.

Figure 3. Time-temperature exposures of foods during cooking and holding at a vendor’s stand (R1) in a railway station in a large city.

Figure 4. Temperatures of foods during holding or after reheating at vendors’ stands and carts in a railway station in a large city.

Results of laboratory analyses of 25 samples of foods collected from vendors at the railway station are given in Table 1. It lists temperatures of the foods at the time of sampling; these were frequently between 28°C (82.4°F) and 46°C (114.8°F). Nine of 21 (43%) samples of foods that were tested for C. perfringens were positive; four of these samples contained over 10⁵, and two contained more than 10⁶. Four of 15 (27%) samples were positive for B. cereus. Plate counts were usually higher when temperatures of the foods were 35°C (95°F) or below.

Bus station. Preparation and holding of rice by Vendor B1 and associated hazards and critical control points are illustrated in Fig. 5. This information for chicken is illustrated in Fig. 6. Pulses were prepared in a similar fashion to that for rice, but onion, garlic, tomato, and spices were added. Chick peas were prepared similarly to that done by Vendor R1 (Fig. 2). Time-temperature exposures of rice
TABLE 1. Microbial counts of foods prepared by vendors at a railway station and a bus station in Pakistan.

<table>
<thead>
<tr>
<th>Description of food</th>
<th>Place/vendor</th>
<th>Time</th>
<th>Temp °C</th>
<th>Plate count</th>
<th>Coliform</th>
<th>B.c.</th>
<th>C.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fried rice left overnight</td>
<td>R1</td>
<td>06:45</td>
<td>29</td>
<td>22 x 10^6</td>
<td>2.0 x 10^2</td>
<td>&lt; 10</td>
<td></td>
</tr>
<tr>
<td>Raw ground meat(^{b})</td>
<td>R1</td>
<td>07:45</td>
<td>28</td>
<td>22 x 10^4</td>
<td>4.0 x 10^4</td>
<td>&lt; 10</td>
<td></td>
</tr>
<tr>
<td>Ground meat after cooking</td>
<td>R1</td>
<td>07:50</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Chick peas</td>
<td>R1</td>
<td>12:15</td>
<td>34</td>
<td>4.8 x 10^6</td>
<td>&lt; 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground meat and potatoes (cooked)</td>
<td>R1</td>
<td>14:00</td>
<td>44</td>
<td>6.3 x 10^6</td>
<td>&lt; 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground meat and potatoes (cooked)</td>
<td>R1</td>
<td>15:55</td>
<td>41</td>
<td>2.2 x 10^6</td>
<td>&lt; 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chick peas</td>
<td>R1</td>
<td>16:20</td>
<td>30</td>
<td>6.5 x 10^7</td>
<td>&lt; 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground meat and potatoes (cooked)</td>
<td>R1</td>
<td>18:25</td>
<td>48</td>
<td>4.8 x 10^6</td>
<td>&lt; 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>R1</td>
<td>19:10</td>
<td>29</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Okra</td>
<td>R1</td>
<td>20:20</td>
<td>38</td>
<td>1.0 x 10^10</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat stew</td>
<td>R1</td>
<td>21:45</td>
<td>&lt; 45</td>
<td>3.5 x 10^6</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground meat and potatoes (cooked)</td>
<td>R1</td>
<td>21:50</td>
<td>&lt; 45</td>
<td>Spr.</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground meat (cooked)</td>
<td>R2</td>
<td>16:00</td>
<td>30</td>
<td>&lt; 10^2</td>
<td>1.1 x 10^6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses (fermenting)</td>
<td>R2</td>
<td>16:00</td>
<td>35</td>
<td>1.8 x 10^6</td>
<td>&lt; 10^2</td>
<td>6.0 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Ground meat (cooked)</td>
<td>R2</td>
<td>19:20</td>
<td>43</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses</td>
<td>R2</td>
<td>19:20</td>
<td>49</td>
<td>6.0 x 10^7</td>
<td>&lt; 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground meat (fermenting)</td>
<td>R3</td>
<td>16:37</td>
<td>46</td>
<td>3.8 x 10^6</td>
<td>&lt; 10^2</td>
<td>2.0 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Chick peas</td>
<td>R3</td>
<td>16:40</td>
<td>46</td>
<td>6.0 x 10^6</td>
<td>&lt; 10^2</td>
<td>1.4 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Ground meat (cooked)</td>
<td>R4</td>
<td>16:47</td>
<td>41</td>
<td>4.8 x 10^6</td>
<td>&lt; 10^2</td>
<td>5.0 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Ground meat (cooked)</td>
<td>R5</td>
<td>19:50</td>
<td>29</td>
<td>2.1 x 10^6</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground meat after reheating</td>
<td>R5</td>
<td>20:30</td>
<td>94</td>
<td>7.2 x 10^6</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground meat and potatoes (cooked)</td>
<td>R6</td>
<td>19:55</td>
<td>28</td>
<td>4.0 x 10^6</td>
<td>&lt; 10^2</td>
<td>1.6 x 10^6</td>
<td>1.0 x 10^6</td>
</tr>
<tr>
<td>Chick peas</td>
<td>R7</td>
<td>16:00</td>
<td>40</td>
<td>2.6 x 10^6</td>
<td>&lt; 10^2</td>
<td>1.6 x 10^6</td>
<td>1.0 x 10^6</td>
</tr>
<tr>
<td>Pulses</td>
<td>R8</td>
<td>16:25</td>
<td>36</td>
<td>~ 10^3</td>
<td>~ 10</td>
<td>3.0 x 10^3</td>
<td></td>
</tr>
<tr>
<td>Pulse burger</td>
<td>R9</td>
<td>17:00</td>
<td>37</td>
<td>6.0 x 10^4</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Bus station**

<table>
<thead>
<tr>
<th>Description of food</th>
<th>Place/vendor</th>
<th>Time</th>
<th>Temp °C</th>
<th>Plate count</th>
<th>Coliform</th>
<th>B.c.</th>
<th>C.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulses left overnight</td>
<td>B1</td>
<td>06:15</td>
<td>31</td>
<td>~ 10^4</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Meat stew left overnight</td>
<td>B1</td>
<td>06:20</td>
<td>34</td>
<td>6.0 x 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Rice shortly after cooking</td>
<td>B1</td>
<td>06:30</td>
<td>71-91</td>
<td>5.5 x 10^7</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chick peas</td>
<td>B1</td>
<td>06:45</td>
<td>91</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses after reheating</td>
<td>B1</td>
<td>08:30</td>
<td>68</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Meat stew</td>
<td>B1</td>
<td>08:30</td>
<td>63</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses after cooking</td>
<td>B1</td>
<td>08:45</td>
<td>83</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat stew after cooking</td>
<td>B1</td>
<td>09:50</td>
<td>87</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat stew</td>
<td>B1</td>
<td>12:10</td>
<td>34</td>
<td>9.1 x 10^6</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Pulses</td>
<td>B1</td>
<td>14:35</td>
<td>38</td>
<td>9.1 x 10^6</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Chicken (cooked)</td>
<td>B1</td>
<td>17:13</td>
<td>51</td>
<td>2.3 x 10^3</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Meat stew beginning of reheating</td>
<td>B1</td>
<td>18:45</td>
<td>48</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Rice</td>
<td>B2</td>
<td>17:30</td>
<td>37</td>
<td>1.3 x 10^4</td>
<td>&lt; 10^2</td>
<td>7.9 x 10^3</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Ground meat and potatoes (cooked)</td>
<td>B3</td>
<td>18:40</td>
<td>35</td>
<td>1.3 x 10^4</td>
<td>&lt; 10^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses</td>
<td>B4</td>
<td>18:50</td>
<td>31</td>
<td>2.9 x 10^6</td>
<td>3.7 x 10^4</td>
<td>&lt; 10^2</td>
<td>1.1 x 10^6</td>
</tr>
<tr>
<td>Beef cooked on griddle left overnight</td>
<td>B5</td>
<td>07:40</td>
<td>33</td>
<td>2.1 x 10^4</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Rice on lid of pan left overnight</td>
<td>B6</td>
<td>07:45</td>
<td>38</td>
<td>3.6 x 10^6</td>
<td>3.4 x 10^6</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Chick peas left overnight</td>
<td>B7</td>
<td>07:58</td>
<td>39</td>
<td>4.0 x 10^7</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Beef in pan left overnight (cooked on griddle)</td>
<td>B7</td>
<td>07:58</td>
<td>29</td>
<td>2.2 x 10^5</td>
<td>1.3 x 10^6</td>
<td>&lt; 10^2</td>
<td></td>
</tr>
<tr>
<td>Chick peas left overnight</td>
<td>B8</td>
<td>08:04</td>
<td>32</td>
<td>2.0 x 10^6</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Sugar cane juice (pH 4.2)</td>
<td>B9</td>
<td>08:45</td>
<td>9</td>
<td>~ x 10^4</td>
<td>9.8 x 10^6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lasi (curd and water) (pH 7.1)</td>
<td>B10</td>
<td>09:15</td>
<td>12</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10^2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Sharbat sandal (pH 7.1)</td>
<td>B11</td>
<td>09:20</td>
<td>8</td>
<td>1.6 x 10^6</td>
<td>2.0 x 10^7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice water</td>
<td>B12</td>
<td>09:25</td>
<td>23</td>
<td>1.8 x 10^7</td>
<td>10^8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Legend in table: Plate count = mesophilic aerobic colony count or aerobic plate count; B.c. = Bacillus cereus; C.p. = Clostridium perfringens; Spr./S = spreader; + = positive but not quantified; blank space = test not performed.
- \(^{b}\) Salmonella isolated from this sample.
Results of laboratory analysis of 25 samples of foods collected from vendors at the bus station and the temperature of the foods at the time of sampling are given in Table 1. It shows that relatively low microbial counts were found in samples of foods taken from Vendor B1. This was not the case for foods taken from other vendors. Pulses, left-over beef, leftover rice, and sugar cane had plate counts that exceeded $10^6$ CFU/g. Five of 20 contained coliform bacteria. Coliform counts in ground meal and potatoes, rice left overnight and collected from the top of the lid of a pan, beef left overnight, and sugar cane juice. A count of $10^6$ C. perfringens was found in a sample of pulses, and a count of $10^6$ B. cereus was recovered from a sample of rice.

**DISCUSSION**

Critical control points\(^a\) varied with the food, preparation steps, and duration of holding and display, but in most cases they were holding and reheating and sometimes cooking.

**Raw ingredients and formulation**

*Salmonella* was isolated from a sample of raw ground meat. This is not surprising because raw meat and raw poultry products are frequently contaminated by salmonellae. Dry foods and ingredients (e.g., rice, pulses, and...


Risks of Salmonellosis and Staphylococcal Food Poisoning From Pakistani Milk-based Confectioneries

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ABSTRACT

Hazards of milk-based products were conducted in three confectionery manufacturing shops in a city in Pakistan. The incoming khoa (a heat-concentrated milk product having a water activity of approximately 0.97) was initially contaminated with staphylococci and contained enterotoxin. The confectionery was subsequently cooked to temperatures that would be lethal to staphylococci, but staphylococci were often found in high numbers in the finished products. Recontamination occurred during handling. Khoa-filled confectionery and confectionery made from renin-processed cheese were contaminated by salmonellae. The contaminants reached the products either during cooling or handling after cooking. Multiplication occurred in the warm environment of the shop and may continue in products having high water activity during transport and within retail outlets. Critical control points are source of ingredients (particularly khoa), formulation, cooking (except for preformed enterotoxins), cooling, and cold storage. Milk-based products of the types evaluated pose a high risk of causing foodborne illness.

Milkborne and cream-filled pastry associated outbreaks of staphylococcal intoxication in developed countries are well documented, but the majority of these occurred many years ago before refrigerated storage of these products was commonplace (2,3,16). In middle eastern countries and the Indo-Pakistani subcontinent, milk-based confectioneries are still vehicles of staphylococcal enterotoxin. Although foodborne disease surveillance data are incomplete and reviews of outbreaks are rarely documented in the medical literature, newspaper articles occasionally report such outbreaks when they occur at social events such as weddings with many participants. Food poisoning outbreaks due to ingestion of milk products and milk-containing sweets are also reported in India, but these are rarely well documented (21). In one such report in Dharwar City, Karnataka, India, a family of eight was hospitalized with severe symptoms of gastroenteritis after eating a khoa (concentrated milk) product (28).

The Food Microbiology Laboratory of the National Institute of Health, Pakistan, received a cream-filled confectionery, made of khoa, that an ill person thought was responsible for his illness. Large numbers (10^7) of Staphylococcus aureus and enterotoxin A were isolated from the confectionery. The source of the product, however, could not be traced, but a similar product was found in a large confectionery shop. A sample of it revealed the same quantity of S. aureus. Its source was traced to a manufacturer in a large city. To gather more information on these situations, hazard analysis critical control point evaluations were conducted in three small confectionery manufacturing establishments.

MATERIALS AND METHODS

Facilities evaluated

Two of the confectionery manufacturing establishments were located in a bazaar area and the other along a busy street in a large city in Pakistan. The facilities consisted of two or more rooms within which, raw ingredients were stored, products were prepared, cooked, cooled, and decorated, and finished product were stored until delivered to a sales room on the same premise or at other locations.

Products encountered

Khoa is milk (usually from buffaloes) that is boiled until it concentrates to a semisolid to solid consistency; preparation steps are illustrated in Fig. 1. It is used both as a filling (Fig. 2) and as an ingredient in confectionery products. Burfi is a khoa-based confectionery which is topped with nuts and silver flake; its preparation is illustrated in Fig. 3. Ghulab jamun is a chilled ball of khoa with coconut. Rus gula is a cheese-based confectionery; its preparation is shown in Fig. 4. Mithoo is a hard brownish-yellow colored sweet. Ladoo is a yellow course grained ball-shaped confectionery. Moli pak is a burfi-like colored square cube. Rus malai is a chilled khoa- and cheese-based ball-shaped confectionery which contains nuts; a flow diagram of preparation steps is illustrated in Fig. 5. The figures illustrate flow processes for the products.

Hazard analyses

The hazard analyses consisted of (a) observing food preparation and storage practices to identify sources and modes of contamination; (b) measuring temperatures in internal regions of foods throughout or after cooking and periodically during holding and cooling to evaluate survival, destruction, and growth; (c) collecting samples of foods before and after sequential stages of *A microbiological hazard is unacceptable contamination, survival or growth of foodborne pathogens (10).
RISKS OF SALMONELLA AND STAPHYLOCOCCAL FOOD POISONING

Legend
- Contamination hazard
+ Microbial growth likely
CCP Critical control point
  a Operation would be a critical control point
  if cooling and cold storage during delivery
  and display were practiced

Figure 1. Preparation of Khoa.

Figure 2. Preparation of khoa-filled confectionery in a small establishment.

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Figure 4. Preparation of cheese-based confectionery (rus gula) in a small establishment.

Figure 5. Preparation of rus malai, chilled khoa- and cheese-based confectioneries in a small establishment.

Laboratory procedures

Samples for analysis were homogenized according to the International Standards Organization (ISO) technique (/I). They were premixed and 25 g of the mixture were transferred into 225 ml of 0.1% peptone water in a sterile plastic bag and homogenized in a Stomacher. Decimal dilutions were made with 1 ml of the initial homogenate with 9 ml of sterile dilution fluid consisting of 0.1% peptone water and 0.075% (w/v) agar (6). This formulation allowed thorough mixing and pipetting and was useful for spreading when the drop plating method was used. With a pipette tip, 0.05 ml of the selected dilution was carefully spread within one section. This technique was used for the enumeration of the "total" (mesophilic aerobic) viable count, coliforms, and S. aureus on plate count agar (Merck 5463), violet red bile agar (Merck 1406) containing 10 g lactose per l. and Baird-Parker agar (Oxoid/Unipath CM 617) supplemented with tellurite and egg yolk emulsion (Unipath SR 34), respectively. All media were prepared according to the manufacturer’s instructions. Coliform plates were overlayed with selective medium after inoculation. Duplicate plates were made for assay of each sample.

According to the Pakistani laboratory policy and European procedures, incubation of plate count and coliforms was done at 37°C and not at the typical American prescribed temperature of 35°C. These plates were read after 24 h, and Baird-Parker agar plates were read after 48 h at 37°C.

Typical colonies of S. aureus were presumptively confirmed with the Staph-Rapid-Test (Roche Diagnostics, 07330386). This slide test uses stabilized sheep erythrocytes loaded with rabbit plasma containing fibrinogen that detects the clumping factor and immunoglobulin G which reacts with protein A. Final confirmation was done with subcultured colonies using the coagulase test according to ISO procedure (12). Two strains of every positive sample were tested for their ability to form toxins using the SET-REPLA test kit (Unipath, TD 900). This test is based on the principle of reverse passive latex agglutination.

The isolation method for Salmonella followed strictly the four-stage procedure of ISO (13). Pre-enrichment of 25 g or ml of the sample in 225 ml buffered peptone water for 16 h at 37°C was followed by enrichment in magnesium chloride malachite green medium and in selenite cystine medium for 24 h at 37°C, respectively. Plating was done on brilliant green phenol red (Unipath CM329) and bismuth sulfite (Difco 0073) agar. Confirmation was done by the Salmonella Reference Laboratory of the National Institute of Health, Pakistan.

Water activity was measured with a Novasina instrument (Novasina, Pfaffikon, Switzerland) having a temperature stabilized chamber for three samples. Adjustment and determination of water activity were done according to the manufacturer’s instructions. The mean measurement time was 4 h. The pH was measured with a hand-held digital pH meter (Model 2300 Testoterm, Lenzkirch, Germany), adjusted to sample temperature.

RESULTS

Actual or potential hazards and critical control points are indicated on Fig. 1-5. During heating of the khoa and confectioneries, temperatures exceeded 74°C (165°F) and were usually near boiling for approximately 20 min. Opportunities for contamination from handling of confectioneries and for microbial growth during storage of khoa were obvious at all establishments.

Laboratory results for confectionery collected during various stages of processing and of finished products are
listed in Table 1. Three samples of khoa contained more than 10^8 mesophilic aerobic CFU per g, more than 10^6 S. aureus per g. Counts of khoa-filled confectioneries exceeded 10^4 mesophilic aerobic CFU/g, and S. aureus counts ranged from 10^3 to 10^5/g. Staphylococcal enterotoxin type A was recovered from eight of 10 samples of khoa and khoa-filled confectioneries in Plant A and from khoa in Establishment A and from khoa in Establishment C. The enterotoxin-positive samples contained at least 8 x 10^9 S. aureus per g. A cheese-containing confectionery (rus gula) had mesophilic aerobic colony and coliform counts that ranged from <10^2 to 10^9/g. Salmonellae were isolated from a khoa product after filling, from cheese and from rus gula in Establishments A, and from a khoa-filled confectionery in Establishment B.

Water activity of two samples of khoa was 0.97; pH ranged between 6.5-6.8. Another sample had a water activity of 0.82. Water activity of a sample of mithoo was 0.59; that of ghulab jaman was 0.87; that of burfi was 0.82; that of ladoo was 0.82 and 0.81 (manufactured at two establishments); and that of moli pak was 0.87.

### DISCUSSION

Based on the laboratory data, risk of acquiring staphylococcal food poisoning from khoa-containing confectioneries is high. Sporadic reports confirm that the problem exists. Cream-filled products have been notorious vehicles of staphylococcal enterotoxins and, hence, responsible for many outbreaks of staphylococcal food poisonings (1,2,8,16). Milk-based products have also been identified as vehicles of salmonellae and responsible for outbreaks of salmonellosis (1).

**Milk**

Numerous investigations have shown the presence of pathogens in milk (9). Studies in India have shown very high counts of S. aureus in raw milk used for khoa preparation (20). Furthermore, S. aureus (10^6 inoculum) grew within 6 h in sterilized skim milk and in raw milk having a low microbial count, and reach populations of 10^9 in 24 h (22). All but one of 14 strains isolated from khoa produced at least one enterotoxin in sterilized milk and eight of 14 strains produced detectable enterotoxin in raw milk.

### TABLE 1. Microbial counts of foods prepared in small manufacturing plants and shops.*

<table>
<thead>
<tr>
<th>Plant/Shop</th>
<th>Food</th>
<th>Status/ Process</th>
<th>Temp (°C)</th>
<th>Plate count</th>
<th>Coliform</th>
<th>S. aureus Type enterotoxin</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Khoa</td>
<td>Finished</td>
<td>37</td>
<td>3.6 x 10^9</td>
<td>2.4 x 10^9</td>
<td>2.8 x 10^9</td>
<td>A</td>
</tr>
<tr>
<td>A</td>
<td>Khoa</td>
<td>Sugar and khoa</td>
<td>37</td>
<td>4.2 x 10^9</td>
<td>1.0 x 10^7</td>
<td>1.4 x 10^9</td>
<td>A</td>
</tr>
<tr>
<td>A</td>
<td>Khoa-filled confectionery</td>
<td>After cutting and filling</td>
<td>37</td>
<td>1.1 x 10^9</td>
<td>1.4 x 10^6</td>
<td>1.2 x 10^9</td>
<td>A</td>
</tr>
<tr>
<td>A</td>
<td>Khoa-filled confectionery</td>
<td>Finished</td>
<td>37</td>
<td></td>
<td></td>
<td>8.0 x 10^5</td>
<td>A</td>
</tr>
<tr>
<td>A</td>
<td>Cheese</td>
<td>Prepared in shop</td>
<td>37</td>
<td>2.8 x 10^7</td>
<td>1.0 x 10^6</td>
<td>&lt;10^2</td>
<td>+</td>
</tr>
<tr>
<td>A</td>
<td>Cheese</td>
<td>After forming</td>
<td>37</td>
<td>1.6 x 10^8</td>
<td>1.4 x 10^6</td>
<td>&lt;10^2</td>
<td>+</td>
</tr>
<tr>
<td>A</td>
<td>Rus gula</td>
<td>Finished</td>
<td>37</td>
<td>6.0 x 10^5</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>+</td>
</tr>
<tr>
<td>A</td>
<td>Rus gula</td>
<td>After cooling</td>
<td>37</td>
<td>1.6 x 10^6</td>
<td>1.4 x 10^4</td>
<td>&lt;10^2</td>
<td>+</td>
</tr>
<tr>
<td>A</td>
<td>Rus gula</td>
<td>After cooking and cooling</td>
<td>58</td>
<td>1.5 x 10^5</td>
<td>1.4 x 10^4</td>
<td>&lt;10^2</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>Khoa</td>
<td>Finished</td>
<td>30-40</td>
<td>1.9 x 10^7</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Khoa-filled confectionery</td>
<td>Finished</td>
<td>30-40</td>
<td>1.8 x 10^7</td>
<td>1.8 x 10^7</td>
<td>4.0 x 10^4</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Burfi</td>
<td>Finished</td>
<td>30-40</td>
<td>6.6 x 10^9</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Ghulab jaman</td>
<td>Finished</td>
<td>30-40</td>
<td>2.2 x 10^9</td>
<td>1.0 x 10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Rus gula</td>
<td>Finished</td>
<td>30-40</td>
<td>&lt;10^5</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Rus gula</td>
<td>Finished</td>
<td>30-40</td>
<td>6.8 x 10^8</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Ladoo</td>
<td>Finished</td>
<td>30-40</td>
<td>4.1 x 10^6</td>
<td>1.3 x 10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Yellow sugar-flour ball</td>
<td>Before heating</td>
<td>30-40</td>
<td>1.7 x 10^5</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Yellow sugar-flour ball</td>
<td>Before cooling</td>
<td>30-40</td>
<td>2.0 x 10^9</td>
<td>2.2 x 10^9</td>
<td>1.6 x 10^9</td>
<td>A</td>
</tr>
<tr>
<td>C</td>
<td>Khoa</td>
<td>Finished</td>
<td>30-40</td>
<td>4.0 x 10^8</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>Burfi</td>
<td>Finished</td>
<td>30-40</td>
<td>7.4 x 10^9</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>Ghulab jaman</td>
<td>Finished</td>
<td>30-40</td>
<td>3.1 x 10^8</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>Ladoo</td>
<td>Finished</td>
<td>30-40</td>
<td>9.0 x 10^8</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>Moli Pak</td>
<td>Finished</td>
<td>30-40</td>
<td>9.0 x 10^8</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
<td>-</td>
</tr>
</tbody>
</table>

*Legend: + = positive, - = negative, blank space = no test performed.

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Khoa

*Khoa* is usually manufactured in rural areas and transported to cities. It is purported that *khoa* is boiled for approximately 15-20 min. The batch observed during this investigation was boiled for several minutes, but the duration was not recorded. Boiling should kill salmonellae, staphylococci, shigellae, and viruses that may have been acquired either from the raw milk or while the products were handled. Staphylococcal enterotoxins would, however, most likely survive (2,17,18,26).

Despite the heating, large numbers of staphylococci were isolated from *khoa* (during this study and those of others). Varadaraj and Nambudripad (26) found both enterotoxins and deoxyribonuclease in *khoa* and suggested the possibility of heat injury and recovery (24). *Khoa* is prepared in open pans, and finished products are likely to be handled while being put into containers which in many cases have neither been effectively washed nor sanitized. Hand contact would be expected. Hence, recontamination by staphylococci and coliform bacteria could occur after heating.

Storage of *khoa* where produced, during its delivery, and while in the confectionery-manufacturing plant is done without cooling or cold-storage facilities. Hence, staphylococcal growth (27) and enterotoxin production can readily occur in *khoa* having a water activity of 0.97 (and probably down to 0.92). During the warmer months in Pakistan, the temperature is favorable for staphylococci to grow rapidly and produce enterotoxins. The high staphylococcal and coliform counts indicate growth during holding.

According to Varadaraj and Nambudripad (25), milk is concentrated during *khoa* preparation to a semisolid/solid mass resulting in a product of 26-28% moisture. Staphylococcal enterotoxins were produced by strains of *S. aureus* in *khoa* with moisture levels between 26-48% within 2 d while stored at 25-35°C (23). In a second study, *S. aureus* inoculated at a level of 10⁰ CFU/g into *khoa* with moisture of 26-28, 38-42, and 45-48% reached 10⁵-10⁶ in 24 h. Counts of 10⁶-10⁷ were attained in 48 h at the higher two moisture ranges when held at room temperature (23). No significant growth was observed when the products were held under refrigeration (4-5°C).

The few samples of *khoa* taken from the establishments surveyed during this investigation had *S. aureus* counts as high as 10⁸. Similar counts of staphylococci (10⁴-10⁵) were found in 16 of 33 samples of *khoa* collected in India (14). Sharma et al. (19) reported average staphylococcal counts of 5 x 10³/g from 200 samples of *khoa* collected in Udaipur, India. Ghodekar et al. (7) isolated staphylococci in average numbers of 7 x 10, 6.7 x 10, and 2 x 10⁴/g from samples of *khoa* that they classified as good, fair, and poor, respectively. The average number of staphylococci in 100 market *khoa* samples collected in Bangalore and Mysore, India, was 4.0 x 10⁵/g; 21% of the isolates produced enterotoxins (21).

According to Varadaraj and Nambudripad (25), casein is coagulated during heating of milk when *khoa* is prepared, and it takes up moisture resulting in microenvironments of high water activity. Within these, staphylococci were observed to be present in clusters containing large numbers of cells. The clusters gradually increased in size as storage progressed from 24 to 72 h. The loose texture of *khoa* allowed disintegration of the staphylococcal clusters so that cells can be rapidly disseminated upon stirring and mixing with other ingredients.

**Khoa-based products**

*Khoa* is used as an ingredient in many confectionery products and may be contaminated with pathogens and contain staphylococcal enterotoxin. Furthermore, microorganisms may reach *khoa*-based products from other ingredients. For example, bacterial spores become incorporated into the product from wheat flour, nuts and sugar, and improperly cleaned equipment. Enteric pathogens (e.g., *Salmonella*) may gain entrance into the product from milk, contaminated cooling water, possibly other ingredients, and carriers.

Within confectionery establishments, the widespread use and handling of *khoa* spreads staphylococci and other organisms (e.g., salmonellae and coliform bacteria) to ready-to-eat products. Conditions conducive to cross-contamination were obvious. Furthermore, persons who would have resident staphylococci on their hands mix, roll and knead *khoa*, and then test cooked products for doneness, cut cooked products, cut nuts, and decorate and fill confectioneries by hand. Holding conditions, later, are conducive to bacterial multiplication and enterotoxin production. Salmonellae and large numbers of coliforms and staphylococci were isolated from these items. Since initial contamination from *khoa* was so high, the relative importance of the additional contamination from handling is difficult to evaluate. In the way that the product is handled, however, the risk of contamination from hands of sweet-shop workers is high.

Subsequently, as *khoa*-filled confectioneries are held in the place of manufacture, while being transported, and while on display in shops without refrigeration, bacterial multiplication and toxin production in a product of relatively high water activity are quite likely. This is particularly so in the warmer months when temperatures are near optimum for growth of staphylococci. The water activity values of many *khoa*-filled products permit multiplication of staphylococci and enterotoxin production.

**Cheese-based products**

Cheeses have also been implicated as vehicles of staphylococcal enterotoxin and salmonellae (3,9,15). The initial source of salmonellae in confectionery products could have come from raw milk, *khoa* prepared from it, cooling water, or a carrier during mixing and forming balls. In one establishment (A), workers handled cheese confectioneries when they were formed into balls. Cross-contamination could explain the presence of salmonellae.

During heating of cheese balls in a sugar syrup, temperatures of 100°C were attained for a prolonged duration. Salmonellae should have been killed by this time-temperature exposure unless the high sugar content provided protection. Heating in a syrup would lower water activity of the product. Nevertheless, salmonellae and large numbers (up to 10⁶) of coliform bacteria were found in the cooled products.
Identified critical control points

Critical control points for confectionery manufacturing are raw ingredients, formulation, cooking (except when enterotoxins are present), and cooling and cold storage. Furthermore, avoiding hand contact of products during preparation of items that are not to be subsequently heated and the use of cleaned and sanitized containers and trays will minimize opportunities for contamination.

Obtaining raw ingredients (e.g., khoa) from a safe source (i.e., one that protects the product from postheating contamination) is a critical control point. Since the khoa is often processed in villages remote from the place of manufacturing confectioneries, coordination by health agencies at national, provincial, and local levels is needed. For the situation in India, Varadaraj and Nambudripad (25) recommended institution of stricter hygienic and sanitation practices to prevent or at least minimize contamination during preparation and during manufacturing of confectionery made from khoa. Because of the relatively high water activity of khoa, receipt of a cold product would be a form of monitoring, but such facilities are not common in Pakistan. Hence, the existing products pose a high risk.

Formulation can be a critical point if it is done in a way that produces a product with a water activity of 0.92, ideally 0.85, or below. Modifying formulations [to use sugars that are more effective in reducing water activity and to add high concentrations of sugars (high sugar to water ratio)] to achieve these values by manufactures of cream-filled pastry have been important reasons for the decline of staphylococcal food poisoning in developed countries. Without devices to measure water activity, however, routine monitoring and verification are not feasible. The water-to-sugar ratio will have to be used as a guide.

Boiling of milk during khoa production and heating of confectioneries during preparation are critical control points. Staphylococcal enterotoxins, however, survive and contamination can occur during further preparation.

The decline in incidence of foodborne diseases from cream-filled products in developed countries has been influenced by food workers not handling these products during filling and by refrigeration during holding, transporting, and displaying those that have water-activity values above 0.85 (7). Keeping products at temperatures of 7°C or below during holding in the place of manufacturing, transporting, displaying for sale, and while in homes is a critical control point (4). This is essential to prevent growth and enterotoxin production in khoa and in its products of high (greater than 0.92) water activity. This practice, however, requires major outlays of capital which is difficult to arrange in developing countries. Without these actions, however, milk-based confectioneries will remain high risk products for causing staphylococcal food poisoning.

Although the hazard analyses were incomplete, many hazards were observed or measured, and some were confirmed by laboratory results. Additional work needs to be done to confirm and enlarge these findings. Nevertheless, the data should alert health authorities in developing countries that foods processed and prepared in small manufacturing plants and shops can be hazardous, and that such foods pose health risks of varying degrees to persons who consume these products. It is recommended that the hazard analysis critical control point approach be used to study foods prepared in cottage industries and small-volume food processors (5,29). Practical, economical measures are needed to prevent or control the hazards, and simple (but effective) means of monitoring critical control points are essential to ensure food safety. The only other options are to either (a) take action to remove hazardous products from the market or (b) acknowledge the high risk that milk-based confectioneries are likely to be contaminated, and that their consumption may result in staphylococcal food poisoning and salmonellosis and so inform the public.

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Hazards and Critical Control Points of Street-Vending Operations in a Mountain Resort Town in Pakistan

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ABSTRACT

Hazard analyses (which included watching operations, measuring temperatures of foods throughout preparation and display, and sampling and testing for microorganisms of concern) were conducted of some typical street-vending operations in a mountain resort town in Pakistan. Salmonellae were isolated from ground meat, chicken flesh (from all operations surveyed), cutting boards, egg shells (eggs used in pulse patties), and buffalo milk (used for milk shakes). Greater than 107 cfu/ml of coliform bacteria were isolated from raw milk, ice-cream mixes and products, and pulse-patty mix. Time-temperature exposure during cooking was adequate to kill salmonellae, but there were potential for recontamination from cutting boards, knives, and hands of the vendor. Buffalo milk was held in a freezer and not boiled by the vendor as is usual in Pakistani homes to retard spoilage. Hence, because pathogens were not killed, milk shakes were a health risk. Pulse patties were not always thoroughly cooked, so pathogens could have survived. Holding stacks of them on a griddle for several hours would have allowed germination and growth of bacterial spores and growth of resulting cells. Health agency personnel in developing countries, vendors, and consumers of these foods need to be informed of the hazards and appropriate preventive measures.

Laboratory evidence, however, shows that high microbial counts are common and foodborne pathogens are sometimes present in street-vended foods (1,6,9,10,20-22,24,26-29). Certain foods (e.g., chicken, rice) that are commonly sold by street vendors are frequently identified as vehicles during investigations of outbreaks of foodborne disease in countries that have surveillance activities (4,8). Ice cream has been implicated as a vehicle of foodborne illnesses (3,13,23).

Because of these situations, hazard analyses were conducted of some typical street-vending operations in a mountain resort town. Risks posed on tourists were assessed and critical control points at which simple, economical, and effective preventive measures could be applied were identified.

MATERIALS AND METHODS

Description of street-vending operations

One vending operation (Vendor D) prepared and cooked chicken and rice, meat stew, and vegetable dishes. The kitchen consisted of three rooms located below the main street level. A long room was used for cooking, which was done on gas stoves, and holding cooked foods. A second room was used for salad preparation, washing utensils and pans, and storing mostly raw foods in a small deep freezer. Another room was used for cutting raw chickens and by the staff for resting. Cooked foods were displayed in covered pans on a counter at the sidewalk. Cooked foods, other than the chickens, were periodically reheated over a gas burner. Skinned raw chickens were hung above the counter to attract customers. These chickens were usually cooked on demand; otherwise, after cooking, they were displayed on top of the lids of pans. More precooked chickens, however, were sold than freshly cooked ones so they usually were held for a long duration.

Two other vendors (Vendors B and C) sold cooked chickens. In these operations, approximately a dozen skinned chickens were displayed by hanging them over the front counters. Chickens were cut on boards and cooked, usually on demand, over gas burners. One of these was located on a side street; others of the same kind were nearby. The other stand was located a few steps below the sidewalk of the main street.

Another vendor (Vendor E) was located outside, a few steps above the sidewalk of the main walking street near the entrance.
to a few shops. The stand consisted of four counters that formed a hollow square. Pulse patties (sometimes referred to as burgers because of their shape and method of cooking) were prepared. Pulses were cooked on a gas burner and then taken elsewhere for grinding. Afterwards, the ground pulses were mixed with water, vegetables, spices, and eggs, and the patties were formed by hand. The patties were cooked on a large iron griddle over a gas flame. After cooking, they were stacked three to six high at the edge of the griddle and stayed there until sold. Sales were infrequent and many were left over from the previous day and left at the end of the days of the survey. These were kept overnight in a compartment of the stand.

Up the street at a corner, another vendor (Vendor F) prepared the same item. This vendor did not have a stand, only a griddle, a couple boxes, and a chair. A sample of pulse patties was collected, but a hazard analysis was not done because the vendor departed with cooking apparatus when the survey team left the stand.

The fifth vendor (Vendor G) for which a hazard analysis was done prepared ice cream. The shop had two rooms, one for preparation and storage of products and ingredients and the other for customers to sit. An ice-cream freezing machine was located at the front of the preparation room along a sidewalk. Most of the customers purchased the ice cream from the sidewalk and ate it as they strolled through the town. Several other ice-cream vendors which had similar operations or sold ice cream made elsewhere were located on the same street. Another vendor (Vendor A) sold foods just outside a covered market. Only a few food temperatures were measured and a few samples collected there because this vendor was out of the jurisdiction of the city health authority.

Hazard analyses

Hazard analyses were conducted at three operations (referred to as vendors D, E, F) that vended foods along or adjacent to the main street of the mountain resort town. Other operations were visited where operations were observed, food temperatures measured, and samples collected. The hazard analyses consisted of (a) observing food preparation and storage practices to identify sources and modes of contamination; (b) measuring temperatures of internal regions of foods during or after cooking and periodically during holding and display to evaluate survival, destruction, and growth; and (c) collecting samples of foods at various stages of preparation and testing them for microbial concerns.

Food temperatures were measured by inserting thermocouples (either type K or T) with needle-type sensors of various lengths into foods so that the sensing point was near the geometric center, and when feasible, so that the shaft was nearly covered by the food being studied. Air temperatures were taken with type-T thermocouples with welded ends. Before use in foods, thermocouples were washed in soapy water, rinsed, wiped dry with a paper tissue, and immersed three times in 95% alcohol and flame after each immersion. The thermocouple leads were plugged into either a battery-powered Pronto-Plus (Thermo Electric, Saddle Brook, NJ) hand-held, digital potentiometer or a battery-powered Technoltem 9300 (Testosystem Gesellschaft & Co., Lenzkirch, Germany) hand-held digital potentiometer.

Samples of approximately 200 g were collected either after cooking or preparation or during holding to evaluate a potential hazard. These were collected aseptically with a metal spoon after it had been washed, rinsed, and immersed three times in 95% alcohol and flame after each immersion. Samples were put into sterile Stomacher bags which were fastened at the top with either rubber bands or plastic bag fasteners. Cutting boards and sets of approximately 10 skinned chickens were swabbed with sterile ear swabs which had been moistened with sterile peptone water. After swabbing, the used end of the swab was cut with scissors which had been dipped into 95% alcohol and flame after each of three dippings. The cut end was directed to fall into a tube of Salmoneilia enrichment broth. Samples were held in ice during transit to the laboratory where they were put into a refrigerator until analyses were begun, usually the following morning.

Laboratory procedures

Samples for analysis were homogenized according to the International Standards Organization (ISO) technique (15). They were premixed and 25 g of the initial mixture were transferred into 225 ml of 0.1% peptone water in a sterile plastic bag and homogenized in a Stomacher. Liquid analytical samples were thoroughly shaken. Decimal dilutions were made with 1 ml of the initial homogenate with 9 ml of sterile dilution fluid consisting of 0.1% peptone water and 0.075% (wt/vol) agar (11). This formulation allowed thorough mixing and pipetting and was useful for spreading when the drop plating method was used. With a pipette tip, 0.05 ml of the selected dilution was carefully spread within one section. This technique was used for the enumeration of the viable (mesophilic aerobic) count, coliforms, and Staphylococcus aureus on plate count agar (Merck 5463), violet red bile agar (Merck-Germany 1406) containing 10 g lactose per 1, and Baird-Parker agar (Oxoid/Unipath CM 617) supplemented with tellurite and egg yolk emulsion (Unipath SR 54), respectively. All media were prepared according to the manufacturer's instructions. Coliform plates were overlayed with selective medium after inoculation. Duplicate plates were made for assay of each sample.

Enumeration of Bacillus cereus was done on polymixin pyruvate egg yolk mannitol bromothymol blue agar (Unipath CM 617) supplemented with 50,000 IU of polymixin per 1 and egg yolk emulsion (Unipath SR 47). One hundred ml of the dilution was spread on the agar in a 13-cm diameter petri dish.

Tryptose sulfite cycloserine agar (Merck-Germany 11972), supplemented with 0.5 g D-cycloserine per 1, was used for the enumeration of Clostridium perfringens. Pour plate technique with 1 ml per dilution was applied according to ISO (17).

According to the Pakistani laboratory policy and European procedures, incubation of plate count and coliforms was done at 37°C and not at the typical American prescribed temperature of 35°C. Plates were read after 20 h. Baird-Parker agar plates were read after 48 h at the same temperature. Inoculated pyruvate egg yolk mannitol bromothymol blue agar plates were held for 24 h at ambient temperature (28-32°C) which allowed intensive growth of bacilli.

Typical colonies of S. aureus were presumptively confirmed with the Staph-Rapid-Test (Roche Diagnostika, 07330836). This slide test uses stabilized sheep erythrocytes loaded with rabbit plasma containing fibrinogen that detects the clumping factor and immunoglobulin which reacts with protein A. Final confirmation was done with subcultured colonies using the coagulase test according to ISO procedure (16).

Suspicious colonies of B. cereus were preliminarily identified with a combined staining procedure for spores and lipid granula according to Ashby and Burdon (12). Confirmation was done with at least two strains of every positive sample by ISO procedures (18). C. perfringens colonies were confirmed according to ISO procedure (17).

The isolation method for Salmonella followed the four-stage procedure of ISO 6579 (19). Preenrichment of 25 g or ml of the sample in 225 ml buffered peptone water for 16 h at 37°C was followed by enrichment in magnesium chloride malachite green medium and in selenium cystine medium for 24 h at 42°C and

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*Microbial hazards refer to contamination, survival, and growth of foodborne pathogens (14).*

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37°C, respectively. Plating was done on brilliant green phenol red agar (Unipath CM320) and bismuth sulfide agar (Difco 0073). Confirmation was done by the Salmonella Reference Laboratory of the National Institute for Health, Pakistan.

RESULTS

Preparation and holding of chickens at three locations are illustrated in Fig. 1: hazards and critical control points are noted. Time-temperature exposures of chickens during cooking and holding at a restaurant where the foods were displayed in and on pans on a counter along the sidewalk are shown in Fig. 2. Salmonellae were isolated from all of three groups of raw skinned chickens, two of three swabs of cutting board surfaces, and a sample of ground raw meat. Salmonella worthington was isolated from a swab sample of chickens; this was the only culture serotyped.

![Diagram of food preparation and holding process](image)

**Figure 1. Preparation and vending of chicken.**

Pulse-patty preparation, associated hazards, and critical control points are illustrated in Fig. 3. Time-temperature exposures of pulses and pulse patties are shown in Fig. 4. Temperatures of several patties were measured consecutively; the connecting lines represent the highest and lowest temperatures measured at any particular time but not necessarily the same patty in subsequent measurements. Dots inside this area represent temperatures of other individual patties. Unsold patties were held overnight at ambient temperatures and put out for display (with or without reheating) the next day.

The foods contained either >10⁶ of CFU or spreaders which make accurate counts difficult (Table 1). Coliform bacteria were isolated from three of five samples. C. perfringens were isolated from two of seven samples of pulse patties. Salmonella was isolated from a group of four egg shells that were to be discarded after breaking.

Steps of ice-cream and milk-shakes preparation are illustrated in Fig. 5. Raw buffalo milk which was used in milk shakes contained 10⁶ CFU/mL. 10⁴ coliform bacteria, 10⁵ S. aureus, and Salmonella (Table 1). Coliform counts were between 10⁶-7/g in the ice-cream products, and plate counts ranged from 10⁴-5 CFU/g. The ice mix was held at room temperature for longer than 4 h.

In all cases, the time of holding can be extended beyond that shown in the graphs. Foods were often continued to be sold after the last temperature measurement.

DISCUSSION

Hazards were associated with most steps of the vendors operations. Those of most significance from a public health perspective are described, critical control points* determined, and control actions suggested.

**Ingredients and cross-contamination**

In this study, salmonellae were isolated from most raw foods of animal origin (e.g., poultry flesh, egg shells, raw ground meat, buffalo milk) that were sampled. These bacteria are commonly found on raw foods of animal origin throughout the world (13). Either inadequate cooking or

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* A critical control point is an operation (practice, procedure, process, or location) at which a preventive or control measure can be exercised that can eliminate, prevent, or minimize hazards (7,14).
Figure 3. Preparation and holding of pulse patty by Vendor E.

Figure 4. Time-temperature exposures of pulses and pulse patties during cooking and holding by a vendor in a resort town.
Cereals, vegetables, and spices are apt to contain bacterial spores. Spores and even enteric bacteria may also enter products from dry milk. Water which is used for preparation and washing utensils is another potential source of enteric bacteria, but this was not confirmed during the hazard analyses.

Cooking

Cooking is a critical control point for chicken and pulse burgers. Chickens were cooked to temperatures (usually greater than 90°C/194°F) sufficiently high to kill human viruses and vegetative forms of mesophilic bacteria. Pulse patties, which contained eggs, were handled extensively during mixing and forming. Furthermore, they were not always cooked to temperatures that would be lethal to pathogens. Those measured attained temperatures of approximately 60°C (140°F) for about 7 min during cooking and the initial stages of holding at the side of the griddle.

It is quite likely that some pathogens would survive in patties given such heat treatment.

It is common practice to boil milk shortly after it is received in homes in Pakistan. This action kills vegetative forms of foodborne pathogens as well as delays spoilage. Such heating, however, was not done in the ice-cream store. This was obvious from a plate count of 10^6 CFU/ml and isolation of *Salmonella* and the lack of facilities for heating. Spoilage was prevented by storing raw buffalo milk in a deep freezer. This procedure, however, did not eliminate salmonellae from the milk. Either using pasteurized milk or boiling milk is essential for ensuring the safety of fresh milk used in ice-cream (milk) mixes and isolation of *Salmonella* and the lack of facilities for heating.

Handling and cleaning

The presence of staphylococci in the ice-cream mixes suggest contamination during preparation. Milk shakes were

### TABLE 1. Microbial counts of foods prepared by street vendors in a mountain resort town in Pakistan

<table>
<thead>
<tr>
<th>Description of food</th>
<th>Place/Time</th>
<th>Temp</th>
<th>Plate count</th>
<th>S. a.</th>
<th>B. c.</th>
<th>C. p.</th>
<th>Sal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice left overnight</td>
<td>A 09:25</td>
<td>25</td>
<td>Spr.</td>
<td>Spr.</td>
<td>&lt;10</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pulse patty left over</td>
<td>A 09:30</td>
<td>25</td>
<td>9.3 x 10^7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw ground meat mix</td>
<td>A 10:50</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken flesh (swab of group of 10)</td>
<td>B 11:30</td>
<td>Amb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutting board (2 x 4 inch board) after prep</td>
<td>B 11:35</td>
<td>Amb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken flesh (swab of group of 10)</td>
<td>C 11:45</td>
<td>Amb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutting board (round log)</td>
<td>C 11:50</td>
<td>Amb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken flesh (swab of group of 6 from freezer and group of 10 hanging at counter display)</td>
<td>D 12:00</td>
<td>Amb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutting board (round log)</td>
<td>D 13:10</td>
<td>Amb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken (in chicken and rice dish)</td>
<td>D 08:45</td>
<td>43</td>
<td>Spr.</td>
<td></td>
<td>&lt;10</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chicken (piece of wing, cooked and held at ambient temperature for many hours)</td>
<td>D 21:15</td>
<td>3.0 x 10^9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse patty left overnight</td>
<td>E 08:30</td>
<td>25</td>
<td>Spr.</td>
<td>&lt;10^2</td>
<td>5.0 x 10^3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg shells (group of four)</td>
<td>E 09:50</td>
<td>Amb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse patty after mixing and forming, before cooking</td>
<td>E 10:05</td>
<td>32</td>
<td>1.1 x 10^9, 2.2 x 10^9</td>
<td>&lt;10^2</td>
<td>5.0 x 10^3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse patty after cooking</td>
<td>E 10:15</td>
<td>60</td>
<td>Spr.</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Pulse patty</td>
<td>E 18:50</td>
<td>29</td>
<td>Spr.</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Pulse patty</td>
<td>E 21:30</td>
<td>29</td>
<td>5.6 x 10^9, 6.0 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Pulse patty</td>
<td>F 17:20</td>
<td>29-39</td>
<td>4.9 x 10^9, 6.0 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Dry milk</td>
<td>G 10:35</td>
<td>29</td>
<td>4.2 x 10^9, 4.3 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Raw milk in freezer</td>
<td>G 10:36</td>
<td>1</td>
<td>2.6 x 10^9, 6.0 x 10^9, 6.0 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen ice cream from machine</td>
<td>G 10:37</td>
<td>-6</td>
<td>7.6 x 10^9, 4.2 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Chocolate ice-cream mix, previously made</td>
<td>G 10:38</td>
<td>-7</td>
<td>2.6 x 10^9, 1.0 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanilla ice-cream mix, freshly made</td>
<td>G 12:15</td>
<td>23</td>
<td>9.0 x 10^9, 5.6 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Chocolate ice-cream mix, freshly made</td>
<td>G 16:30</td>
<td>24</td>
<td>7.7 x 10^9, 1.6 x 10^9, 2.0 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Raw buffalo milk used for milk shakes</td>
<td>G 16:31</td>
<td>24</td>
<td>1.4 x 10^9, 1.4 x 10^9, 1.4 x 10^9, 1.4 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Vanilla ice cream mix freshy made</td>
<td>G 16:32</td>
<td>23</td>
<td>1.4 x 10^9, 9.2 x 10^9, 1.4 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Banana milk shake</td>
<td>G 21:45</td>
<td>7</td>
<td>1.4 x 10^9, 2.0 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Vanilla ice cream from freezer</td>
<td>G 21:46</td>
<td>Frozen</td>
<td>7.7 x 10^9, 1.6 x 10^9</td>
<td>&lt;10^2</td>
<td></td>
<td>&lt;10^2</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

*Legend in table: Plate count = mesophilic aerobic colony count or aerobic plate count; S. a. = *S. aureus*; B. c. = *B. cereus*; C. p. = *C. perfringens*; Sal = *Salmonella*; Amb = ambient air temperature; Spr. = spreader; + = positive; - = negative; blank space = test not performed.*
further subjected to contamination during peeling and cutting fruits which were added prior to blending. The pulse-patty mixture was subjected to contamination from the vendor’s hands during rolling and shaping.

Poorly cleaned (and not disinfected) pans and utensils are additional sources of contamination. Pans and utensils were usually just rinsed and wiped with a damp multipurpose cloth for a few seconds. In the ice-cream shop, a soapy solution was sometimes used for cleaning by one of the workers but not by others. The same wiping cloth, however, was used by all. Rinsed pans were not inverted so microorganisms could have multiplied in the water and food residues in the improperly cleaned containers.

Holding

Epidemiological data from industrialized countries show that the primary factor that contributes to outbreaks of foodborne diseases is holding cooked foods at ambient (room or outside) temperature for several hours (2,5,8,25). This situation also ought to be a significant factor contributing to outbreaks of foodborne diseases elsewhere, and hence, holding of cooked foods is a critical control point. During the summer time, even in the mountainous region of Pakistan, climatic temperature is conducive for growth of common foodborne pathogens, including *C. perfringens*, *Salmonella*, *S. aureus*, and *B. cereus*.

Cooked dishes and pulse patties were held for many (sometimes exceeding 12) hours at outside ambient temperature while on display by the vendors. High counts of mesophilic aerobic microorganisms indicate bacterial growth. Similar situations have been observed with street-vended foods elsewhere (6,9). Although high counts of *B. cereus* or *C. perfringens* were not found in the samples tested, the potential for their presence, germination, and growth of resulting cells exists. Safety of street-vended foods must focus on reduction of time of holding on display and holding foods above temperatures at which pathogenic bacteria multiply. Criteria for control are to either hold foods for a short duration (i.e., less than 4 h) or keep them at temperatures at or higher than 54.4°C (130°F).

Ice-cream mixes were held at room temperatures for more than 4 h before freezing. Risk of the ice cream becoming a vehicle of foodborne illness increases with every hour of holding of the mix. The high mesophilic aerobic colony and coliform counts suggest either (a) extensive contamination from ingredients or during preparation of the mixes or (b) microbial growth during holding after preparation or (c) both.

Cooling

Freezing is an important control measure to prevent growth of bacteria in ice-cream mixes. Hence, it should be done as soon as practicable after preparation of a batch of mix. Otherwise, the mix should be stored in the deep freezer (or a refrigerator, if available) after preparation until it is put into the freezing dispenser.

Cooling of cooked foods was not practiced by the vendors surveyed. This practice requires either refrigeration or ice. If refrigeration facilities were available, leftover pulse patties should be kept cold overnight. Nevertheless, hazardous situations may have previously occurred while the patties were held at the edge of the griddle.

Reheating

Pulses patties which are stacked on the griddle should be reheated thoroughly prior to sale. Preformed *B. cereus* emetic toxin or staphylococcal enterotoxins, however, would not be inactivated by this practice.

Needed public health actions

Prospective consumers of street-vended foods should be alerted to the hazards associated with holding cooked foods at outside (warm) ambient temperature for long durations. Furthermore, they should be cautioned to request that the cooked foods they select be either hot or be reheated.

The hazard analyses undoubtedly point out high risks of eating certain street-vended foods. They are particularly valuable in countries that have either no or only rudimentary foodborne disease surveillance activities. Results ought to be used to set priorities for food safety programs and education of public health officials and street vendors. Hazard analysis critical control point evaluations indicate where attention for preventive measures need to be focused.

ACKNOWLEDGMENTS

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Hazards and Critical Control Points of Street-Vended Chat, a Regionally Popular Food in Pakistan

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ABSTRACT

A hazard analysis (which included watching operations, measuring temperatures of foods throughout preparation and display, and sampling and testing for microorganisms of concern) was conducted of a street vendor's operation. Chat is a popular dish in certain areas of Pakistan. It consists of sliced cooked potatoes, fried graham and pulse dough, and chick peas or red beans garnished with lasi (a fermented milk) and a fruit syrup. Staphylococci reached the cooked potatoes during peeling, cutting, and other handling. These bacteria increased up to 10⁶ while the contaminated foods were held for several hours. Counts up to 10⁶ Bacillus cereus were isolated from the cooked doughs after a 6-h or longer holding period. Large numbers (usually >10⁵) of coliform bacteria and aerobic mesophilic colonies (10⁶⁺) were isolated from all foods after handling and holding for several hours. Ingestion of these foods must be considered high risk unless handling of cooked items can be kept to a minimum and the time of holding reduced to less than 6 h. Critical control points are handling after cooking and holding on display. Health agency personnel in developing countries, vendors, and consumers of these foods need to be informed of the hazards and appropriate preventive measures.

Foods are commonly vended on streets and sidewalks in Pakistan. One category of these that is regionally popular is known as chat. It consists of potatoes, chick peas (Kabla chana) or red beans (or both), a graham flour-based fried food known as boondi, and a pulse flour fried food known as bhalla. A portion of each is put on a dish and topped with fermented milk (lasi) and a sweet sauce.

Little epidemiological data are available to associate these foods with illnesses, but similar items (e.g., potato salad, pinto beans) have been identified as vehicles elsewhere (2,3). Street-vended foods have shown epidemiological links with illness (25), and laboratory results have shown high counts and the presence of foodborne pathogens on street-vended foods (15,8,9,17-19,21,23-26). To assess microbiological hazards (i.e., contamination, survival, and growth) and risks associated with chat, a hazard analysis was conducted of preparation at a small facility and subsequent display of chat items at a vending stand. This operation was typical of other chat operations.

MATERIALS AND METHODS

Description of food preparation and vending operations

Foods were prepared about a half mile from where they were vended. This was done in a dingy room of a building which had a concrete floor. Potatoes and chick peas were cooked by gas heat, and boondi and bhalla were fried in oil. After cooking, potatoes were peeled and sliced, and the other items were transferred to pans and bowls where they were positioned and shaped by hand. A raw vegetable salad was also prepared. Ingredients and preparation steps for the major foods are depicted in Fig. 1-6. In addition to these items, an apricot sauce was prepared by rehydrating dried apricots, adding chili, and heating. The foods were covered by a cloth and conveyed to the vending site on a wooden cart.

The vendor's stand was constructed of wood with glass at the upper half at front and sides. It was painted and lettered in bright colors. At the vending site, the foods were garnished with peppers by hand. Lasi was purchased from another vendor and mixed with ice at the stand. Bowls and pans were displayed in the stand until they were sold or returned to the place of preparation for overnight storage. Other nearby vendors of the same product had stands of similar design.

Hazard analysis

The hazard analysis consisted of (a) observing food preparation and display practices to identify sources and modes of contamination, (b) measuring temperatures of internal and sometimes near surface regions of foods during or after cooking and while the foods were displayed for sale, and (c) collecting samples of foods during various stages of preparation and during display and testing them for microorganisms of concern.

Food temperatures were measured by inserting thermocouples (either type-K or -T) with needle-type sensors of various lengths into foods so that the sensing point was near the geometric center, and when feasible, so that the shaft was nearly covered by the food under study. Air temperatures were taken with type-T thermocouples with welded ends. Before use in foods, thermocouples were washed in soapy water, rinsed, wiped dry with a paper cloth and conveyed to the vending site on a wooden cart.

Other nearby vendors of the same product had stands of similar design.

‡ A microbiological hazard refers to unacceptable contamination, survival, or growth of foodborne pathogens (12).
Samples of approximately 200 g of foods were collected either after cooking or during holding, to evaluate contamination, survival, and growth of microorganisms and to assess risks associated with the operations. These were collected aseptically with a metal spoon after it had been washed, rinsed, and immersed three times in 95% alcohol and flamed after each immersion. Samples were put into sterile Stomacher bags which were fastened at the top with either rubber bands or plastic bag fasteners. Samples were put into a container of ice and held until they reached the laboratory where they were put into a refrigerator until analyses were begun, usually on the following morning.

Laboratory procedures

Samples for analysis were homogenized according to the International Standards Organization (ISO) technique (13). They were premixed and 25 g of the mixture was transferred into 225 ml of 0.1% peptone water in a sterile plastic bag and homogenized in a Stomacher. Decimal dilutions were made with 1 ml of the initial homogenate with 9 ml of sterile dilution fluid consisting of 0.1% peptone water and 0.075% (wt/vol) agar (10). This formulation allowed thorough mixing and pipetting and was useful for spreading when the drop plating method was used. With a pipette tip, 0.05 ml of the selected dilution was carefully spread within one section. This technique was used for the enumeration of the "total" (mesophilic aerobic) viable count, coliforms, and *Staphylococcus aureus* on plate count agar (Merck 5463), violet red bile agar (Merck-Germany 1406) containing 10 g lactose per l and Baird-Parker agar (Oxoid/Unipath CM 617) supplemented with tellurite and egg yolk emulsion (Unipath SR 54), respectively. All media were prepared according to the manufacturer's instructions. Coliform plates were overlayed with selective medium after inoculation. Duplicate plates were made for assay of each sample.

Enumeration of *Bacillus cereus* was done on polymixin pyruvate egg yolk mannitol bromthymol blue agar (Unipath CM 617) supplemented with 50,000 IU of polymixin per l and egg yolk emulsion (Unipath SR 47). One hundredth ml of the dilution was spread on a 13-cm diameter petri dish.

Tryptose sulfite cycloserine agar (Merck-Germany 11972), supplemented with 0.5 g D-cycloserine per l, was used for the enumeration of *Clostridium perfringens*. Pour plate technique with 1 ml per dilution was applied according to ISO procedure (15).

According to the Pakistani laboratory policy and European procedures, incubation of plate count and coliforms was done at 37°C and not at the typical American prescribed temperature of 35°C. These plates were read after 24 h, Baird-Parker agar plates were read after 48 h at the same temperature. Polymixin pyruvate egg yolk mannitol bromthymol blue agar plates were incubated for 24 h at ambient temperature (28-32°C) which resulted in intensive growth of bacilli.
Typical colonies of *S. aureus* were presumptively confirmed with the Staph-Rapid-Test (Roche Diagnostica, 07330386). This slide test uses stabilized sheep erythrocytes loaded with rabbit plasma containing fibrinogen that detects the activity of the clumping factor and immunoglobulin G which reacts with protein A. Final confirmation was done with subcultured colonies using the coagulase test according to ISO procedure (14). Two strains of every positive sample were tested for their ability to form toxins using the SET-REPLA test kit (Unipath, TD 900). The test is based on the principle of reverse passive latex agglutination.

Suspicious colonies of *B. cereus* were preliminarily confirmed with a combined staining procedure for spores and lipid granula according to Holbrook and Anderson (17). Confirmation of *B. cereus* was done with at least two strains of every positive sample by ISO procedures (16). *C. perfringens* colonies were confirmed according to ISO procedures (15).

**RESULTS**

Flow diagrams of preparation and holding of chat items with notations of hazards and critical control points are illustrated in Fig. 1-6. Time-temperature exposures of chat items during cooking, preparation, transporting, display, and holding throughout much of a day's operation are shown in Fig. 7. Foods remained within a temperature range that would promote rapid multiplication of bacteria for many hours. Observations supplemented by a few temperature measurements of foods on display at other nearby vendors indicated that the holding pattern was similar to that of the vendor surveyed.

Results of samples of foods taken during preparation and display are shown in Table 1. All but one sample had mesophilic aerobic colony counts that exceeded one million CFU/g; eight had counts greater than 10². Coliforms were present in all of 18 samples, all but one exceeded 10⁴; eight samples of them exceeded 10⁶. *B. cereus* was isolated from
HACCP OF STREET VENDED CHAT

Legend
- Hazard of contamination likely
+ Hazard of bacterial growth likely/bacterial growth unlikely, depending on pH of curd and time of holding

Figure 5. Preparation and holding of laji.

ONIONS*
LETTUCE*
MINT*
RADISH*
PEEL *
CHOP *
CHOP *
CUT *

(CCP)
MIX *
TRANSFER TO TRAY
PUT ON CART
CONVEY TO VENDING SITE

CCP
HOLD (+)
SELL

Legend
- Hazard of contamination likely
+ Hazard of bacterial growth likely

CCP Critical control point

Figure 6. Preparation and holding of salad.

five of 16 samples. An isolate from bhalla after soaking was cell-culture and Oxid-test positive. Hence, it appeared to be a producer of diarrheal-type enterotoxin. C. perfringens was not isolated from 12 samples. Upwards to 5.6 x 10^3 S. aureus per g was isolated from eight of 14 samples. Staphylococcal enterotoxin type A was produced by iso-

A critical control point is an operation (practice, procedure, process, or location) at which hazards can be eliminated, prevented or minimized (6.12).

DISCUSSION

The data clearly show the value of conducting hazard analysis critical control point evaluations to detect foodborne disease hazards and to focus attention on situations where control action is needed. This procedure is particularly valuable in countries that have either no or only rudimentary foodborne disease surveillance activities.

Ingredients

Potatoes are grown underground; cereal and legume products are subject to soil contamination during growing, harvesting, and storage. Hence, spores of B. cereus will be frequently present in graham and pulse flours and on chick peas and potatoes.

Cooking

The foods were cooked to temperatures sufficiently high to kill vegetative forms of bacteria, but some bacterial spores would survive. Hence, cooking is not a critical control point for spore-laden foods. An operation (or operations) after cooking must be employed as a critical control point.

Handling foods and hygienic practices

The cooked potatoes were peeled, cut, and stacked on trays by hand. Even chick peas were stacked and shaped in bowls and later garnished with peppers and tomatoes by hand. Salad ingredients were cut and mixed by hand. Consequently, high counts of coliforms and staphylococci were found. Although there is not a direct association between coliforms and enteric pathogens, Salmonella typhi, Shigella, viruses of hepatitis A, and Norwalk-like viruses

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Holding and displaying

for over 6 h presents a high risk; the risk increases substantially with every additional hour of holding. Daytime temperatures while on display for many hours; sometimes

are hazards of varying risks depending on exposure and hygiene practices of the persons who handle the foods.

Hand washing by the vendors was not observed, but this would have minor impact on the staphylococci because they reside in human skin (2,20). Facilities for hand washing were not readily available. Utensils and vessels were rinsed in water and rubbed by hand and a damp multiple-purpose cloth.

Avoiding hand contact of cooked foods is an important measure to minimize chances of contamination. Slicing, mixing, and stacking cooked chat items are critical control points if practical procedures, such as using clean utensils, rather than bare hands, would be used. Ensuring that such practices are followed by vendors, however, would be a difficult task. Alerting vendors of the hazards and teaching them safe food handling techniques would aid in this endeavor. If, however, hand contact of cooked potatoes and other items either cannot or will not be avoided, effective time-temperature controls during holding for display must follow.

**Holding and displaying**

The cooked chat items were held at outside ambient temperatures while on display for many hours; sometimes more than 12 h. Holding foods at warm outside temperature for over 6 h presents a high risk; the risk increases substantially with every additional hour of holding. Daytime temperatures (greater than 40°C at mid-day hours) were conducive for promoting microbial growth. Foods unsold on the day of preparation were held overnight at ambient room temperatures and put out for display the next day. As a result of the holding, high counts of mesophilic aerobic microorganisms were found. This situation with street-vended foods has been observed elsewhere (5,8,9).

The large numbers of staphylococci indicate that these bacteria grew in the foods following contamination. The tens of thousands of B. cereus cells indicate that their spores germinated and resulting vegetative cells multiplied while the foods were held on display.

The major factor that contributes to outbreaks of foodborne diseases in developed countries is holding cooked foods at ambient (room or outside) temperature for several hours (4,7,22). This situation must contribute to foodborne outbreaks in Pakistan (and other developing countries) where the practice is commonplace. Hence, holding foods while being displayed by street vendors calls for a critical control point.

If the duration of holding is short, pathogenic bacteria will have little time to multiply to large numbers. Such a practice could be implemented by preparing a limited amount of food for service at lunch time and either chilling or discarding leftovers. Another batch would then be prepared after lunch for evening sales. Chat items should not be held at warm outside air temperatures for over 4 h.
definitely not for 6 h, or longer. This procedure is contrary, however, to the usual practices by vendors.

Cold storage of the prepared foods is another option. Refrigerator units will be out of the financial resources of many vendors. Ice is an option, but it will melt rapidly during hot weather and will need to be periodically replaced. This practice unfortunately will not be commonly used because of cost and the inconvenience of getting replacement ice.

Holding must be periodically monitored by the vendors, and this action verified by health authorities. To achieve this, vendors must become aware of the hazards that are associated with their operations and become motivated to control them and have timepieces and thermometers for monitoring. These measures will only happen with any reasonable degree of certainty if health agency personnel, street vendors, and the public are informed about the hazards and convinced of associated health risks.

**Risks of acquiring foodborne illnesses from chat and prevention**

Based upon this study and observations of other chat-vending operations, the ingestion of these foods must be considered a high risk of acquiring foodborne illness. The greatest concern is that of staphylococcal food poisoning resulting from ingesting the potatoes. All of the factors that usually contribute to outbreaks of staphylococcal food poisoning—colonized persons handling cooked, moist proteinaceous foods, holding at warm outside temperatures, and lapse of several hours between preparing and eating—are operative (4). The pulses, chick peas, and graham flour products pose risks of illness caused by *B. cereus* and *C. perfringens*. The excessive handling of the cooked products during mixing, cutting, stacking, and garnishing also poses risks of contamination by enteric bacteria, viruses, and parasites.

**ACKNOWLEDGMENTS**

This study was done as a part of a joint program of the Food Safety Unit, World Health Organization; National Institute of Health, Government of Pakistan; German Technical Development Agency (GTZ); and the Industry Council for Development (ICD). Thanks are given to the following persons for their technical assistance in this project: Mohammad Taffail, Abdul Ghufoor, Nazir Hussain Shah, Gul Mohammad, Food Microbiology Laboratory, NIH, Islamabad, Pakistan.

**REFERENCES**

Hazards and Critical Control Points of Food Preparation and Storage in Homes in a Village and a Town in Pakistan

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ABSTRACT

Hazard analyses were conducted in 13 homes in each of a village and a town in Pakistan. Pulses, lentils, chick peas, potatoes, rice, and combinations of them, curd, and weaning preparations were commonly prepared in both locations, and meat dishes were prepared in the town. Cooked foods were left, usually at room ambient temperature, overnight in over 50% of the homes. Samples of foods cooked in the morning and eaten at noon usually had mesophilic aerobic colony counts less than 10° CFU/g, but those left overnight usually ranged between 10°-9 CFU/g. Coliform bacteria were isolated from 77% of samples; many of the counts exceeded 10°/g. Greater than 10°/g Staphylococci aureus were isolated from curd and buffalo milk which had been previously heated. Clostridium perfringens were isolated from 18% of samples; once from pulses left overnight in quantities exceeding 10°/g. Only three samples contained Bacillus cereus. Salmonella was not recovered from any of 28 samples. Hazards were primarily associated with holding the foods after preparation. Critical control points are cooking, manipulation of foods after cooking, holding cooked foods, and reheating.

In industrialized countries, from 20-40% of the reported outbreaks of foodborne diseases have been traced to mishandling of foods in homes (4, 6, 30, 32). It is unknown whether these statistics are applicable to situations in developing countries, but foodborne diseases ought to be even more prevalent in developing countries because mishandling of foods and poor storage practices are commonplace. Despite the lack of epidemiological data, foodborne disease hazards can be identified and attention drawn to important food safety measures through hazard analysis critical control point evaluations (e.g., 12, 20). To assess risks in Pakistan, these evaluations were made of food preparation and storage practices in homes in a rural village and a mountain resort town.

MATERIALS AND METHODS

Description and selection of households

Hazard analyses of varying degrees of completeness were conducted in 13 homes in a rural village and in the same number in a mountain town. The village population was over 4,000; its economy centered on farming. It had a small center with a mosque and a few shops (e.g., barber, part-time slaughterhouse, tea and cold drink shops, material shop, small markets, and food vendors). Houses in the village were constructed of mud brick which were covered by stucco; floors were of concrete. Walls frequently surrounded the property forming a courtyard. Cooking was done either in a combined parlor-kitchen, a separate small building outside the main living quarters, or outside in the courtyard. Cooking fuels were gas, sticks, or dried dung. Water supply was from either community or individual wells. Sewage from houses drained to the streets. Clothes and buffalo washing were done in a small lake at the outskirts of the village.

The town was located in a rolling mountainous region. Most of the homes that were included in the survey were downhill from the business area. The homes usually had a parlor, a kitchen, and one or more bedrooms. Cooking was usually done by gas, and because of the high altitude, pressure cookers were sometimes used. All but one house was connected to a municipal water supply, but in some of the houses, running water was only available for an hour in the early morning. Sewage ran into open drains onto streets and then into ditches which drained into streams.

In the village, homes that were studied were initially selected on the basis of those reporting (during a previous nutrition survey) the feeding of weaning foods to babies. Later, however, the selection criterion was changed to households that were preparing foods of a type (e.g., meat, rice) that elsewhere have been identified as vehicles of foodborne diseases. Mothers from five households reported children with diarrhea. In the mountain town, all of the households selected had either babies or children under five years old who at that time had diarrhea, and mothers had brought them to a local health clinic for treatment. The surveys were carried out over a 6-d period in the village and a 3-d period in the town. Each day, hazard analyses were conducted in two or more households. Samples of leftover foods were collected wherever available.

Hazard analyses

The hazard analyses consisted of (a) observing food preparation and storage practices to identify sources and modes of contamination; (b) measuring temperatures in internal regions of foods throughout or just immediately after cooking and periodically during holding of the cooked foods to evaluate survival, destruction, and growth; and (c) collecting samples of foods before and after sequential stages of preparation and of leftovers and testing them microbiologically. The pathogens selected for testing were judged on the basis of the foods under study and

* A microbiological hazard is unacceptable contamination, survival, or growth of foodborne pathogens (12, 20).

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situations that may have allowed contamination of the foods or survival or growth of microbes in them.

Food temperatures were measured by inserting thermocouples (either type K or T) with needle-type sensors of appropriate lengths so that the sensing point near the geometric centers and with most of the shaft surrounded by the food under study. Air temperatures were measured with type T thermocouples with welded ends. Before use in foods, thermocouples were washed in soapy water, rinsed, and immersed three times in 95% alcohol and flamed after each immersion. The thermocouple leads were plugged into either a battery-powered Pronto-Plus (Thermo Electric, Saddle Brook, NJ) or a battery-powered Testoterm (Testoterm GmbH & Co., Lenzkirch, Germany) hand-held, digital readout potentiometer.

Sample units were collected from some of the foods either after cooking, after holding for specified times, or after overnight holding. These were collected aseptically with a metal spoon after it had been washed, rinsed, and immersed three times in 95% alcohol and flamed after each immersion. The samples were put into sterile Stomacher bags which were fastened at the top with either rubber bands or plastic bag fasteners. Bagged samples of hot foods were cooled with water and then put into an insulated box that contained ice. The samples were held on ice until they reached the laboratory where they were put into a refrigerator until analyses were begun, usually on the following morning.

Laboratory procedures:

Samples for analysis were homogenized according to the International Standards Organization (ISO) technique (21). They were premixed and 25 g of the mixture was transferred into 225 ml of 0.1% peptone water in a sterile plastic bag and homogenized in a Stomacher. Liquid analytical samples were thoroughly shaken. Decimal dilutions were made with 1 ml of the initial homogenate with 9 ml of sterile dilution fluid consisting of 0.1% peptone water and 0.075% (wt/vol) agar (16). This formulation allowed thorough mixing and pipetting and was useful for spreading when the drop plating method was used. With a pipette tip, 0.05 ml of the selected dilution was carefully spread within one section. This technique was used for the enumeration of viable (mesophilic aerobic) count, coliforms, and Staphylococcus aureus on plate count agar (Merck 5463), violet red bile agar (Merck-Germany 1406) containing 10 g lactose per 1, and Baird-Parker agar (Oxoid/Unipath CM 617) supplemented with tellurite and egg yolk emulsion (Unipath SR 54), respectively. All media were prepared according to the manufacturer's instructions. Coliform plates were overlayed with selective medium after inoculation. Duplicate plates were made for assay of each sample.

Enumeration of Bacillus cereus was done on polymixin pyruvate egg yolk mannitol bromothymol blue agar (Unipath CM 617) supplemented with 50,000 IU of polymixin per 1 and egg yolk emulsion (Unipath SR 47). One hundred ml of the dilution was spread on a 13-cm diameter petri dish.

Tryptose sultfite cycloserine agar (Merck-Germany 11972), supplemented with 0.5 g D-cycloserine per 1, was used for the enumeration of Clostridium perfringens. Pour plate technique with 1 ml per dilution was applied according to ISO procedure (23). According to the Pakistani laboratory policy and European procedures, incubation of plate count and coliforms was done at 37°C and not at the typical American prescribed temperature of 35°C. These plates were read after 20 h. Baird-Parker agar plates were read after 48 h at the same temperature. Inoculated polymixin pyruvate egg yolk mannitol bromothymol blue agar plates were held for 24 h at ambient temperature (28-32°C) which promoted intensive growth of bacilli.

Typical colonies of S. aureus were presumptively confirmed with the Staph-Rapid-Test (Roche Diagnostika, 07330386). This slide test uses stabilized sheep erythrocytes loaded with rabbit plasma containing fibrinogen that detects the activity of the clumping factor and immunoglobulin G which reacts with protein A. Final confirmation was done with subcultured colonies using the coagulase test according to ISO procedure (22). Suspicious colonies of B. cereus were preliminarily identified with a combined staining procedure for spores and lipid granula according to Holbrook and Anderson (19). Confirmation was done with at least two strains of every positive sample by ISO procedures (24). C. perfringens colonies were confirmed according to ISO procedure (25).

The isolation method for Salmonella followed the four-stage procedure of ISO (25). Preenrichment of 25 g or ml of the sample in 225 ml buffered peptone water for 16 h at 37°C was followed by enrichment in magnesium chloride malachite green medium and in selenite cystine medium for 24 h at 42 and 37°C, respectively. Plating was done on brilliant green phenol red (Unipath CM329) and bismuth sulfite (Difco 0073) agar. Confirmation was done by the Salmonella Reference Laboratory of the National Institute of Health, Pakistan.

The pH was measured with a hand-held digital pH meter (Model 2300 Testoterm, Lenzkirch, Germany), adjusted to sample temperature.

RESULTS

Food preparation activities in both communities were similar. They consisted of preparing raw ingredients, cooking, and holding foods at ambient temperatures or occasionally in refrigerators until consumption. Sometimes foods were reheated just prior to eating. Potatoes, other vegetables, pulses, chick peas, and meat stews were the dishes commonly prepared during the visits. Several spices of similar variety were used in each of the preparations. Weaning foods included special cereal preparations and buffalo milk, a roti (bread) and sugar preparation (choori), halwa (a mixture of wheat, ghee, and sugar), potato-based dishes, and household foods.

Flow diagrams of selected food preparation are illustrated in Fig. 1-4; hazards and critical control points are noted. Dal (pulses), seeds of peas, beans, or lentils, is a popular food in Pakistan. Onion, garlic, red chili, coriander, salt, water, and sometimes ghee were mixed with the pulses, cooked, and held until served (not illustrated in figures). Lentils, chick peas (Kabia chana), and rice were prepared in a similar manner. Two staple foods are often combined with flavoring ingredients. As an example, preparation of knichri (lentils and rice) is illustrated in Fig. 1. Combinations of potatoes and rice, chick peas and rice, chick peas and potatoes, potatoes and eggs, and mixed vegetables were prepared in similar ways. Preparation of beef stew in a home in the mountain town is shown in Fig. 2. Pressure cookers were helpful because of the high altitude. (Similar procedures were used in the village when meat was available, but pressure cookers were not used.) Babies were sometimes fed a commercial cereal product or porridge mixed with milk (Fig. 3). Halwa consists of wheat, ghee, water, sugar, and cinnamon seeds. Choori is roti (a bread) and sugar. There was considerable handling during their preparation, without subsequent cooking. Both foods were either fed or given to children. Preparation of

\* A critical control point is a practice or a procedure at which a control measure can eliminate, prevent, or minimize a hazard (12).
Figure 1. Preparation and holding of knichri in a village home.

Figure 2. Preparation and holding of beef stew in a town home.

curd in a village home is illustrated in Fig. 4. Curd was frequently mixed with spices to make a sauce in which roti was dipped.

Time-temperature exposures of foods during preparation and storage are listed in Table 1. Most of the foods attained temperatures that exceeded 73.9°C (165°F). These and some other foods reached temperatures that exceeded 54.4°C (130°F) for 45 min or longer. Three foods (custard: potato, tomato, onion, egg mixture: chicken necks), however, were undercooked.

Cooking of chick pea dishes and subsequent holding of leftovers in two homes is compared in Fig. 5. In one home, the chick pea dish was put into a refrigerator after serving: in the other, the chick pea dish was kept at ambient temperature in the kitchen, which was common practice in the homes visited. The refrigerated chick pea dish was only in a temperature range (49-21°C) within which bacteria
TABLE 1. Temperature exposures of foods during cooking in village and town homes.

<table>
<thead>
<tr>
<th>Product</th>
<th>Highest temperature °C attained</th>
<th>Min above 130°F/54.4°C</th>
<th>Min above 165°F/73.9°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chick peas/potatoes</td>
<td>98.8</td>
<td>100</td>
<td>52</td>
</tr>
<tr>
<td>Chick peas/rice</td>
<td>96.5</td>
<td>103</td>
<td>58</td>
</tr>
<tr>
<td>Custard (processed)</td>
<td>51.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Halwa</td>
<td>87.0</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Onion/ghee</td>
<td>96.5</td>
<td>77</td>
<td>45</td>
</tr>
<tr>
<td>Potatoes/rice</td>
<td>96.5</td>
<td>75</td>
<td>48</td>
</tr>
<tr>
<td>Potatoes/tomatoes/onion/egg</td>
<td>58.5°E</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Dal (pulses)</td>
<td>97.5</td>
<td>73</td>
<td>53</td>
</tr>
<tr>
<td>Sweet rice</td>
<td>96.0</td>
<td>103</td>
<td>65</td>
</tr>
<tr>
<td>Tomato/onion</td>
<td>96.0</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Town</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef/potatoes</td>
<td>83.4</td>
<td>~165</td>
<td>~115</td>
</tr>
<tr>
<td>Chicken necks</td>
<td>58.8</td>
<td>&lt;1</td>
<td>0</td>
</tr>
<tr>
<td>Meat stew</td>
<td>89.5</td>
<td>198</td>
<td>130</td>
</tr>
<tr>
<td>Potatoes/onions</td>
<td>76.5</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Potatoes/rice</td>
<td>66.7</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Tomato/onion/egg</td>
<td>77.2</td>
<td>18</td>
<td>3</td>
</tr>
</tbody>
</table>

* Probe inside potato, broth boiled.

multiply rapidly for approximately 2 h. The dish kept at room temperature was in this range for over 20 h and near the optimal temperature for B. cereus most of that time. Foods left overnight were found in over 50% of the homes. In the town, foods (six on 2 d and nine on another) were left overnight in each of the households surveyed.

Results of laboratory analyses of samples of foods collected in households in the rural village is given in Table 2, and this information for households in the town is presented in Table 3. Foods cooked in the morning and eaten at noon or shortly thereafter usually had mesophilic aerobic colony counts of less than 10⁶ CFU/g. Cooked foods that were either left overnight or at ambient temperatures for several hours usually had mesophilic aerobic colony counts between 10⁵ over 10⁶ CFU/g.

Coliform bacteria were isolated from 16 (76%) of 21 samples. Counts >10⁷/g were found in curd with water, leftover mutton broth, buffalo milk (three samples), and water collected from several vessels in one house in which the source was turned on only an hour a day. Staphylococcc were isolated from two (12.5%) of 16 samples; greater than 10⁶ were isolated from samples of curd in water and buffalo milk. B. cereus was isolated from only three (6%) of 49 samples, in quantities in the range of 10⁴-10⁵/g. C. perfringens was isolated from eight (21%) of 38 samples, but usually in quantities of less than 5.0 x 10⁴/g. An exception was a sample of pulses which had a count of 1.0 x 10⁷/g. Salmonellae were not found in either 15 samples of foods from homes in the rural village or 13 samples of foods from homes in the town.

**DISCUSSION**

Results of hazard analyses in homes in a rural community and a mountain town in Pakistan did not vary greatly from the results of the studies conducted in the Dominican Republic, Peru, or Thailand (9-11,26-28). Hazards included (a) raw foods of animal origin; (b) vegetables, sugar, and spices which contain bacterial spores; (c) handling (e.g., cutting, peeling, scrapping, grinding, mixing); (d) heating too low of temperatures or for too short of time to kill vegetative forms of pathogens; and (e) holding foods for a sufficiently long interval within a temperature range that permits bacterial multiplication.

In the rural community, many of the wells were open and subject to contamination from above, from vessels used

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![Figure 5. Time-temperature exposures of chick pea dishes at two homes; leftovers in one home were refrigerated; leftovers in the other were kept in a kitchen at ambient temperature.](image-url)

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to collect the water, and from the person collecting the water, but not from any obvious sewage pollution. In the town, water was subject to the potential of back siphonage, but not from any obvious sewage pollution. The large numbers suggest either bacterial survival and subsequent growth despite the low pH of the curd or contamination from storage vessels. Although plate and coliform counts were higher than World Health Organization recommendations for drinking water (34,35), they were not as high as those recovered from many of the food samples. This situation has also been observed by Black et al. (2).

Large numbers of mesophilic aerobic microorganisms and coliforms were commonly found in buffalo milk. The milk was often either given to babies or young children or mixed with a cereal preparation and then fed to babies. In Pakistan and many other countries where pasteurized milk is either expensive or not readily available, raw milk is usually boiled shortly after it is received in a home. If any

is left, it is usually boiled again in the evening and again in the morning. Nevertheless, high microbial counts were found in samples of buffalo milk obtained from homes. The large numbers suggest either bacterial survival and subsequent growth despite the low pH of the curd or bacterial growth in the milk before fermentation and sur-

\[\text{Counts per g/ml}\]

<table>
<thead>
<tr>
<th>Description of food</th>
<th>Household</th>
<th>Time</th>
<th>Temp (°C)</th>
<th>Plate count</th>
<th>Coliform</th>
<th>S. a.</th>
<th>B. c.</th>
<th>C. p.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kulfa (vegetable) in curd leftover</strong> (pH 4.8)</td>
<td>1</td>
<td>11:35</td>
<td>31</td>
<td>5.0 x 10⁷</td>
<td>&lt;10⁸</td>
<td>&lt;10³</td>
<td>&lt;10⁸</td>
<td></td>
</tr>
<tr>
<td>Curd with water (pH 3.7)</td>
<td>1</td>
<td>11:39</td>
<td>27</td>
<td>4.6 x 10⁷</td>
<td>1.8 x 10⁶</td>
<td>2.4 x 10⁹</td>
<td>&lt;10⁶</td>
<td>2.0 x 10⁶</td>
</tr>
<tr>
<td>Ras (wheat flour and water mixed and kneaded, held for longer than 1 h)</td>
<td>1</td>
<td>13:10</td>
<td>31</td>
<td>1.2 x 10⁷</td>
<td>&lt;10³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef broth, left overnight</td>
<td>2</td>
<td>10:00</td>
<td>35</td>
<td>5.0 x 10⁴</td>
<td>&lt;10⁶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses leftover from 05:00</td>
<td>2</td>
<td>10:05</td>
<td>Spr.</td>
<td>&lt;10⁶</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses left overnight (malodorous)</td>
<td>2</td>
<td>11:20</td>
<td>35</td>
<td>8.6 x 10⁵</td>
<td>&lt;10⁶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td>2</td>
<td>11:30</td>
<td>38-50</td>
<td>3.0 x 10⁶</td>
<td>&lt;10⁹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Custard made from commercial powder, after cooking (pH 6.7)</td>
<td>2</td>
<td>12:05</td>
<td>51</td>
<td>&lt;10³</td>
<td>&lt;10³</td>
<td>&lt;10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water from pot</td>
<td>2</td>
<td>12:10</td>
<td>Amb</td>
<td>8.0 x 10⁴</td>
<td>6.2 x 10⁷</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled milk with anise (for baby)</td>
<td>3</td>
<td>11:25</td>
<td>35</td>
<td>1.8 x 10⁶</td>
<td>&lt;10³</td>
<td>&lt;10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled milk with cereal</td>
<td>3</td>
<td>11:28</td>
<td>35</td>
<td>2.0 x 10⁶</td>
<td>&lt;10⁶</td>
<td>&lt;10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken curry leftover, refrigerated</td>
<td>3</td>
<td>12:05</td>
<td>9</td>
<td>3.8 x 10⁶</td>
<td>&lt;10³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baby cereal powder</td>
<td>3/4</td>
<td>11:35</td>
<td>Amb</td>
<td>2.0 x 10⁶</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutton broth, left overnight</td>
<td>4</td>
<td>10:40</td>
<td>33</td>
<td>9.0 x 10⁵</td>
<td>4.0 x 10⁸</td>
<td>&lt;10³</td>
<td>&lt;10³</td>
<td></td>
</tr>
<tr>
<td>Boiled milk</td>
<td>4</td>
<td>13:40</td>
<td>34</td>
<td>1.1 x 10⁷</td>
<td>1.4 x 10⁷</td>
<td>&lt;10³</td>
<td>&lt;10³</td>
<td></td>
</tr>
<tr>
<td>Pulses after soaking</td>
<td>5</td>
<td>10:35</td>
<td>25</td>
<td>1.5 x 10⁴</td>
<td>&lt;10³</td>
<td>&lt;10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses at time of eating</td>
<td>5</td>
<td>12:00</td>
<td>32</td>
<td>3.0 x 10⁶</td>
<td>&lt;10³</td>
<td>&lt;10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choori (roti &amp; sugar)</td>
<td>6</td>
<td>11:37</td>
<td>35</td>
<td>2.8 x 10⁶</td>
<td>&lt;10³</td>
<td>1.2 x 10⁷</td>
<td>&lt;10³</td>
<td></td>
</tr>
<tr>
<td>Well water from well</td>
<td>6</td>
<td>12:00</td>
<td>Amb</td>
<td>5.0 x 10⁴</td>
<td>&lt;10³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses cooked in morning and kept in refrigerator</td>
<td>6</td>
<td>12:15</td>
<td>10</td>
<td>3.8 x 10⁶</td>
<td>1.0 x 10⁷</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice and lentils (knichri) after cooking</td>
<td>7</td>
<td>13:25</td>
<td>49</td>
<td>1.8 x 10⁷</td>
<td>&lt;10³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet rice</td>
<td>8</td>
<td>13:10</td>
<td>32</td>
<td>2.2 x 10⁷</td>
<td>&lt;10³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice and potatoes (potato pullao)</td>
<td>9</td>
<td>13:25</td>
<td>62</td>
<td>6.0 x 10⁷</td>
<td>&lt;10³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chick peas and rice, refrigerated</td>
<td>10</td>
<td>16:43</td>
<td>13</td>
<td>1.4 x 10⁸</td>
<td>&lt;10³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes leftover just started to be reheated</td>
<td>10</td>
<td>11:55</td>
<td>37</td>
<td>1.8 x 10⁸</td>
<td>&lt;10³</td>
<td>&lt;10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pumpkin leftover</td>
<td>11</td>
<td>10:00</td>
<td>32</td>
<td>7.8 x 10⁴</td>
<td>&lt;10³</td>
<td>&lt;10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses leftover</td>
<td>11</td>
<td>10:45</td>
<td>33</td>
<td>5.8 x 10⁴</td>
<td>8.0 x 10⁷</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chick peas and potatoes</td>
<td>11</td>
<td>16:30</td>
<td>32</td>
<td>5.4 x 10⁴</td>
<td>&lt;10³</td>
<td>&lt;10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes left overnight</td>
<td>12</td>
<td>10:30</td>
<td>Spr.</td>
<td>2.0 x 10⁷</td>
<td>Spr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chick peas leftover (fermenting)</td>
<td>13</td>
<td>11:15</td>
<td>31</td>
<td>1.4 x 10⁸</td>
<td>3.0 x 10⁷</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes left overnight</td>
<td>13</td>
<td>10:15</td>
<td>31</td>
<td>1.8 x 10⁷</td>
<td>Spr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water from community well</td>
<td>11:15</td>
<td>22</td>
<td>4.4 x 10⁷</td>
<td>2.0 x 10⁷</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water at boy's school</td>
<td>10:00</td>
<td>27</td>
<td>6.2 x 10⁵</td>
<td>6.0 x 10⁵</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\*Legend in table: plate count = mesophilic aerobic colony count or aerobic plate count; S. a. = Staphylococcus aureus; B. c. = Bacillus cereus; C. p. = Clostridium perfringens; Amb = ambient air temperature; Spr./S = spreader; blank space = test not performed.

\(\text{Counts per g/ml}\)
TABLE 3. Microbial counts of foods prepared in urban homes with babies having diarrhea*.

<table>
<thead>
<tr>
<th>Description of food</th>
<th>Household</th>
<th>Time</th>
<th>Temp (°C)</th>
<th>Plate count</th>
<th>Coliform</th>
<th>S. a.</th>
<th>B. c.</th>
<th>C. p.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables (tenda and mercs) left overnight (pH 4.8)</td>
<td>1</td>
<td>09:30</td>
<td>25</td>
<td>Spr.</td>
<td>7.6 x 10⁴</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Dal (graham pulses) left overnight</td>
<td>1</td>
<td>09:35</td>
<td>47</td>
<td>2.2 x 10⁶</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffalo milk (boiled 22:00) and cereal preparation</td>
<td>1</td>
<td>09:30</td>
<td>25</td>
<td>8.0 x 10⁴</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereal preparation &amp; buffalo milk for baby from bottle</td>
<td>1</td>
<td>11:30</td>
<td>38</td>
<td>2.2 x 10⁷</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffalo milk</td>
<td>1</td>
<td>14:30</td>
<td>32</td>
<td>1.6 x 10⁴</td>
<td>1.0 x 10⁷</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Buffalo milk</td>
<td>1</td>
<td>19:25</td>
<td>25</td>
<td>6.8 x 10⁴</td>
<td>2.0 x 10⁷</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Rice and potatoes for baby left overnight</td>
<td>1</td>
<td>19:25</td>
<td>24</td>
<td>5.6 x 10⁷</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef stew broth leftover from lunch to be reheated and served at supper</td>
<td>1</td>
<td>19:30</td>
<td>32</td>
<td>2.4 x 10⁷</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooked ground meat leftover</td>
<td>2</td>
<td>09:20</td>
<td>26</td>
<td>4.4 x 10⁴</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Okra left overnight (pH 5.2)</td>
<td>2</td>
<td>09:30</td>
<td>22</td>
<td>Spr.</td>
<td>&lt;10²</td>
<td>1.0 x 10⁷</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Pulses</td>
<td>2</td>
<td>11:55</td>
<td>21</td>
<td>Spr.</td>
<td>&lt;10²</td>
<td>1.0 x 10⁷</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Buffalo milk</td>
<td>2</td>
<td>19:00</td>
<td>22</td>
<td>1.8 x 10⁷</td>
<td>8.0 x 10⁴</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Younus (vegetable and mutton) left overnight</td>
<td>3</td>
<td>09:30</td>
<td>22</td>
<td>4.6 x 10⁷</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffalo milk with water, boiled at 09:00</td>
<td>3</td>
<td>10:50</td>
<td>28</td>
<td>Spr.</td>
<td>8.0 x 10⁶</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Pulses left overnight</td>
<td>4</td>
<td>09:30</td>
<td>22</td>
<td>8.2 x 10³</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lentils left overnight</td>
<td>4</td>
<td>09:45</td>
<td>21</td>
<td>7.0 x 10⁸</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutton and potatoes left overnight</td>
<td>5</td>
<td>11:05</td>
<td>23</td>
<td>Spr.</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver left overnight</td>
<td>6</td>
<td>11:25</td>
<td>26</td>
<td>6.4 x 10⁶</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beans left overnight</td>
<td>6</td>
<td>09:30</td>
<td>23</td>
<td>4.4 x 10⁷</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water from two tanks and a thermos (municipal water turned on an hour daily)</td>
<td>7</td>
<td>20:20</td>
<td>24</td>
<td>1.0 x 10⁶</td>
<td>1.2 x 10⁶</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Potato and ginger (prepared 2 days before)</td>
<td>7</td>
<td>20:25</td>
<td>24</td>
<td>6.8 x 10⁸</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable and green chilli left overnight (pH 3.9)</td>
<td>7</td>
<td>09:30</td>
<td>25</td>
<td>6.4 x 10⁸</td>
<td>2.4 x 10⁴</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Arbi (starchy root) left overnight</td>
<td>8</td>
<td>09:30</td>
<td>23</td>
<td>1.1 x 10⁶</td>
<td>4.0 x 10⁴</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Pulses</td>
<td>8</td>
<td>11:15</td>
<td>23</td>
<td>6.1 x 10⁶</td>
<td>1 x 10⁴</td>
<td>1.0 x 10⁷</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Rice left overnight</td>
<td>9</td>
<td>09:30</td>
<td>23</td>
<td>5.2 x 10⁷</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td>9</td>
<td>11:25</td>
<td>22</td>
<td>1.3 x 10⁷</td>
<td>1.6 x 10⁷</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Chick peas and lamb's feet</td>
<td>9</td>
<td>20:00</td>
<td>29</td>
<td>2.0 x 10⁷</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chick peas and lamb's feet, freshly cooked</td>
<td>10</td>
<td>12:35</td>
<td>52</td>
<td>Spr.</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulses left overnight</td>
<td>10</td>
<td>09:30</td>
<td>22</td>
<td>4.8 x 10⁴</td>
<td>2.0 x 10⁴</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice with chicken</td>
<td>10</td>
<td>12:30</td>
<td>23</td>
<td>5.2 x 10⁷</td>
<td>&lt;10²</td>
<td>4.0 x 10⁴</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Boiled rice left overnight</td>
<td>11</td>
<td>12:55</td>
<td>24</td>
<td>1.4 x 10⁸</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef and vegetables</td>
<td>12</td>
<td>20:00</td>
<td>30</td>
<td>8.6 x 10⁸</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef left overnight</td>
<td>12</td>
<td>09:30</td>
<td>23</td>
<td>Spr.</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef and potatoes</td>
<td>13</td>
<td>18:45</td>
<td>15</td>
<td>Spr.</td>
<td>&lt;10²</td>
<td>&lt;10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Legend in table: Plate count = mesophilic aerobic colony count or aerobic plate count; S. a. = Staphylococcus aureus; B. c. = Bacillus cereus; C. p. = Clostridium perfringens; Spr./S = spreader; blank space = test not performed.

vival. Fermentation, so as to sufficiently lower the pH of curd, would be considered a critical control point, but the potential for the presence of staphyloenterotoxin exists. Varadaraj and Ranganathan, for example, showed that S. aureus grew and produced thermostable deoxyribonuclease in curd and other fermented milks and in association with lactic cultures (33).

Weaning foods and foods given to children often had high mesophilic aerobic colony and coliform counts. Foodborne pathogens sought during the laboratory examination of samples were found. Nevertheless, the results indicate a high probability of microbial growth. Foods would be sporadically contaminated by pathogens. Rice, beans, and cereal products which are sometimes fed to children in Pakistan have been shown to be contaminated by pathogens or have high bacterial counts elsewhere (1,3,5,7,11,13,15,17,29,31).

Foods were usually cooked to high temperatures that would inactivate vegetative forms of pathogens. Exceptions were custard, a potato dish, and chicken necks. The custard was a rehydrated processed food; the initial microbial count was quite low. The potato temperature was that of the

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center of a cut piece of potato in a liquid that boiled. Shortly after cooking, the chicken necks were given to children. The necks did not attain a temperature that would guarantee destruction of Salmonellae and other enteric pathogens; thus, they posed a risk.

Cooking is a critical control point for foods that contain milk, meat, poultry, and eggs which are frequently contaminated by enteric pathogens. Cooking, however, is not a critical control point for cereals, pulses, lentils, beans, rice, or foods containing spices because some spores that are frequently present would not be killed, although vegetative forms would be killed. For moist foods, a temperature of 73.9°C (165°F) will inactivate large numbers of parasites, viruses, and vegetative bacteria in less than a minute. Temperatures greater than 54.4°C (130°F) can also produce lethal effects if the exposure is of sufficient duration, often hours.

Foods that were not eaten promptly after cooking were commonly held for 12 or more hours (e.g., overnight) before consumption. This practice has been observed in homes in other developing countries (2.9-11.26-28) during the holding interval, bacteria (including pathogens) multiplied and reached large quantities.

Most of the samples of foods collected a few to several hours after cooking contained large numbers of CFU/g. If pathogens contribute to a significant proportion of this total, risk of gastrointestinal following consumption would be high. For example, both B. cereus and C. perfringens can multiply rapidly in pinto beans, and at 37°C (98.6°F) reach numbers sufficiently high to cause foodborne illness in 4 to 6 h and at 23°C (73.4°F) reach these quantities in 12 h (29). The optimal temperature for the growth of B. cereus in rice is between 30°C (86°F) and 37°C (98.6°F), but growth can occur at decreasing rates down to 15°C (59°F) and up to 43°C (109.4°F) (18). These investigators further observed that this organism grew to large numbers in rice held at room temperature. Foods (e.g., chick peas, pulses, meat and poultry stews) frequently encountered in the households in Pakistan were within the temperature range of 15-43°C for several hours, often overnight. A sample of pulses, for example, contained ten million C. perfringens per g. Ingestion of this batch of pulses would pose a high risk of infection. Pulses were found leftover on six occasions. This reemphasizes the high risk.

Holding foods (except those that have a low pH or low water activity) after cooking is a critical control point. Allowing potentially hazardous foods to remain at either room or outside temperature for several hours is the most frequently occurring factor that contributes to the causation of foodborne illnesses (4.6,14,30,32). The safest procedure is to eat foods promptly after cooking. If foods either must be prepared ahead of serving or are left over and are to be eaten at a subsequent meal or when someone returns home several hours after meal time, the foods should be stored in shallow containers and cooled rapidly in refrigerators. This solution, however, is only applicable if cooling facilities are available and within the economic resources of the preparer or recipient of the foods. In the town, only 15% of the homes surveyed had refrigerators; in the village, 25% of the homes surveyed had refrigerators. Cooked leftover foods were kept in some of them, and in these cases, foods could cool rapidly enough to discourage the development of large numbers of bacteria.

Most of the cooked foods were only contacted by spoons during dispensing. Foods were usually covered while they were being held, which keeps dust and flies out, but the humidity around the foods remains high and aids in maintaining high water activity in them. The handling of halwa ingredients during preparation or of the choori dough and then feeding the foods to children were obvious hazards. Touching cooked foods is a commonly identified factor that leads to outbreaks of staphylococcal food poisoning, shigellosis, septic sore throat, hepatitis A, and Norwalk gastroenteritis (6). If the contaminants are bacteria and the foods are to be held subsequently, the problem is intensified because of bacterial growth. Handling activities could be considered critical control points, but ones that would be difficult to control.

Reheating of leftover foods and milk is another critical control point. It is often the last line of defense. As with cooking, temperature exposures need to be sufficiently high and of sufficient duration to inactivate large numbers (in problem situations frequently larger than in the raw foods) of infectious microorganisms or heat-labile toxins. Heat-stable toxins, however, will not be inactivated. Hence, food safety rests with preventing formation of toxins by either eating foods before they are formed or by rapidly cooling them or holding them at temperatures below or above which the toxins can be formed.

As hazards are identified by hazard analyses or epideimiological or other scientific studies, preventive measures which are practical under prevailing customs and circumstances must be chosen from known measures or they must be devised. This information needs to be conveyed to public health personnel so that they can focus attention and set program priorities on these matters. Food safety activities must concentrate on informing homemakers about specific hazards of the foods they usually prepare and practical preventive measures that can be applied at critical control points. Data within this paper indicate where emphasis ought to be put. Continued hazard analyses in other homes and for other food items ought to indicate additional hazards and preventive measures.

ACKNOWLEDGMENTS

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REFERENCES


Spin-off from space travel

Food associated diarrhoeal diseases are preventable and need not occur. The concept of the hazard analysis critical control point (HACCP) is the most viable means to this end yet devised

by Silvia Michanie and Frank L. Bryan

It was in order to provide absolutely safe foods for astronauts while travelling in space in the 1960s that the hazard analysis critical control point (HACCP) concept evolved in the United States. During the following decade, the HACCP approach was adopted by the US Food and Drug Administration in cooperation with the food-processing industry as a means of providing safe low-acid canned foods.

Since then, many food processors and some food-service operators have found that the HACCP approach does not only provide a high degree of assurance of food safety and quality; it is also economically advantageous. It is rational since it is based on historical data about causes of illness and spoilage; it is comprehensive in that it relates to ingredients and subsequent use of products as well as to the process; it is continuous in that problems are detected when they occur and action is taken then to correct them; and it is systematic in that it is a comprehensive plan covering step-by-step procedures. And it offers greater assurance than either testing final products or making periodic inspections.

By hazard we mean the unacceptable contamination, growth or survival of micro-organisms that might cause illness or spoilage, and/or the unacceptable production (or persistence in foods) of toxins of microbial metabolism. Severity is the magnitude of the hazard or the degree of consequences that can result when a hazard exists. Risk is an estimate of the probability that a hazard will occur.

A critical control point is an operation (practice, procedure, process or location) or a step of an operation at which a preventive or control measure can be exercised. This measure will eliminate, prevent or minimise any hazard that has occurred prior to this operation.

The criteria that ensure control at critical control points are specified limits or characteristics. They may be physical (time, perhaps, or temperature), chemical (concentration of salt or acetic acid), or biological (sensorial or microbiological).

Monitoring means checking that a processing or handling procedure at each critical control point meets the established criteria. It involves systematic observation, measurement and recording of the significant factors needed to prevent or control hazards. The monitoring procedures chosen must enable action to be taken to rectify an out-of-control situation or to bring the product back within acceptable limits either before start-up or during processing or preparation. So one great advantage of the HACCP system is immediate response to hazardous situations.

During hazard analyses, sources and modes of contamination are sought. Measurements establish whether disease-causing microbes survive cooking or other processes, and whether they multiply during intervals between preparation and ingestion. Certain foods may or may not lend themselves to supporting the growth of microbes. Samples may be collected and analysed for the presence and quantity of disease-causing and/or spoilage microbes. Each step of the food flow is considered and illustrated on a diagram, with the hazards and critical control points highlighted.

Answers have to be found to a number of questions. For instance, what are the raw ingredients? What is the pH (degree of acidity) of the final product? What is the time-temperature exposure of the product during processing of preparation? Will the product be eaten immediately after preparation, or will it be stored hot, cold or at ambient temperature?

This looks rather sophisticated for use in homes, yet these analyses have been conducted in homes in a capital city, an island city, a suburban shanty town, a mountain pueblo and a rice-farming village. The variety of these settings provides a stringent test of the validity of the approach, and it has passed the test.

In addition to contamination that may be specific to certain kinds of raw food, such factors as traditions, education, economic resources, sanitary facilities, personal hygiene and environmental sanitation may further contribute to hazards during preparation, when food is kept after being cooked, or while leftovers are handled. The
Hazard analysis involves finding out whether disease-causing microbes survive cooking or other processes. A hospital kitchen in Santiago, Chile.

Photo WHO/Almasy

greatest hazard, however, is holding cooked foods at ambient temperature for six hours or longer between preparation and eating. This allows time for microbial contaminants to multiply to quantities large enough to cause enteric illness or to generate toxins. Reheating of the leftover foods is often not enough to inactivate microbes that have grown during long holding periods.

All these observations reveal the hazards and indicate the critical control points of culture-associated food preparation procedures. The next step of the HACCP approach is for persons who are knowledgeable of food microbiology and hygiene to select or devise preventive and control measures and criteria. These have to be both economically feasible and culturally acceptable, and must also be communicated to the public either through organized community action or national educational campaigns.

As the diarrhoeal diseases are studied in all their aspects more intently than in the past, it is becoming clear that contaminated and mishandled foods are the major vehicles of transmission. So the educational follow-up of HACCP evaluations may be the most valuable function of the HACCP concept. This is particularly so in countries that lack foodborne disease surveillance activities. The information gathered can be used to inform the health and social authorities, train public health personnel and educate the adult public and schoolchildren.

Food associated diarrhoeal diseases are preventable and need not occur. The HACCP concept is the most viable means to this end yet devised.
Le progrès venu de l'espace

Il ne devrait plus y avoir de maladies diarréiques liées à l'alimentation, car elles sont évitables; à ce jour, la méthode des points de contrôle critiques dans l'analyse des risques est le moyen le plus prometteur d'y parvenir.

Silvia Michanie et Frank L. Bryan

L'approche dite HACCP, sigle anglais significant « méthode des points de contrôle critiques dans l'analyse des risques », a été mise au point aux États-Unis, dans les années 60, dans le but de munir les astronautes d'aliments absolument sûrs pendant leur séjour dans l'espace. La décennie suivante, la Food and Drug Administration américaine adopta, avec le concours de l'industrie alimentaire, l'approche du HACCP qui permet de produire des conserves alimentaires sûres avec moins d'acides.

Depuis, de nombreuses entreprises dans l'industrie alimentaire et quelques-unes dans la restauration se sont rendu compte que non seulement l'approche du HACCP leur assure un haut degré de sûreté et qualité alimentaires, mais qu'elle permet en outre de réaliser des économies. Elle est rationnelle car elle est basée sur les données existantes concernant les causes de maladie et de détérioration; elle est complète puisqu'elle concerne à la fois les ingrédients, la fabrication et l'usage qui sera fait des produits; elle est continue car elle permet de découvrir et corriger les problèmes au moment où ils se déclarent; elle est systématique puisqu'il s'agit d'un plan global composé de démarches graduées. En outre, elle est plus fiable que les analyses sur les produits finis ou les contrôles périodiques.

Par un risque, nous entendons toute contamination, croissance ou survie inacceptables de micro-organismes qui pourraient provoquer des maladies ou des détériorations, ou encore la production ou persistance inacceptables dans les aliments de toxines du métabolisme microbien.

La gravité est l'importance du risque ou des conséquences qui peuvent résulter de l'existence éventuelle d'un risque. Quant au risque, c'est l'estimation de la probabilité que celui-ci se produira.

Un point de contrôle critique est la phase d'une opération (pratique, procédure, procédé ou lieu) d'une étape d'une opération où une mesure de prévention ou de contrôle peut être prise pour éliminer ou minimiser tout risque qui s'est créé avant cette opération et prévenir des risques pouvant se produire après.

Les critères des contrôles aux points critiques ont la forme de limites ou caractéristiques précises. Ils peuvent être de nature physique (temps, peut-être, ou température), chimiques (la concentration de sel ou d'acide acétique ou biologiques (sensoriels ou microbiologiques).

La surveillance continue signifie que l'on vérifie à chaque point de contrôle critique qu'un procédé de fabrication ou de manipulation correspond bien aux critères établis. Elle passe par l'observation, le relevé des données et l'enregistrement systématique des principaux éléments nécessaires à la prévention ou l'élimination des risques. Les procédures de surveillance doivent permettre d'effectuer les régiages nécessaires ou de ramener les caractéristiques du produit dans des limites acceptables avant le démarrage ou au cours de la fabrication ou préparation. Ainsi, l'un des avantages du système du HACCP est sa capacité de réagir immédiatement aux situations à risques.

Les analyses des risques visent à déceler les sources et moyens de contamination. À l'aide des données relevées, il est déterminé si des microbes pathogènes surviennent à la cuisson ou à d'autres procédés et s'ils se multiplient entre la préparation et la consommation du produit. Certains aliments peuvent être plus susceptibles de favoriser le développement de microbes que d'autres. Des échantillons peuvent être pris pour analyser la présence et la quantité des microbes pathogènes ou susceptibles d'avoir avancé dans le produit. Chaque stade du passage des aliments est étudié et illustré par un schéma qui souligne les risques et les points de contrôle critiques.

Il faut trouver des réponses à de nombreuses questions. Quels sont, par exemple, les ingrédients bruts? Quel est le pH (degré d'acidité) du produit fini? Quel est le taux d'exposition température du produit pendant la fabrication ou préparation? Le produit sera-t-il consommé dès sa préparation ou sera-t-il conservé au chaud, au froid ou encore à température ambiante?

Tout cela paraît un peu trop raffiné pour la maison, et pourtant ces analyses ont été faites dans des foyers dans une capitale, dans une ville insulaire, dans un bidonville, dans une communauté de montagne et dans un village de cultivateurs de riz. La diversité de ces lieux représente une mise à l'épreuve rigoureuse de la méthode, et elle l'a réussie.

Outre l'éventuelle contamination spécifique à certains types d'aliments à l'état brut, des facteurs tels que les traditions, l'éducation, les ressources financières, les équipements sanitaires, l'hygiène personnelle et l'assainissement de l'environnement peuvent augmenter les risques, pendant la préparation et lorsque la nourriture est conservée après sa préparation ou que l'on reprend les restes. Le risque le plus grand résulterait, cependant, de la conservation d'aliments cuisinés à température ambiante pendant six


L'étude de la résistance de microbes pathogènes à la cuisson et à d'autres traitements fait partie de l'analyse des risques. La cuisine d'un hôpital à Santiago du Chili.

 Photographie OMS/P. Almay

heures ou plus avant leur consommation. C'est alors que des microbes contaminant ont le temps de se multiplier au point d'être assez nombreux pour provoquer une maladie intestinale ou générer des toxines. Souvent, il ne suffit pas de réchauffer les restes pour neutraliser les microbes qui se développent pendant une longue période de conservation.

Toutefois, ces observations révèlent les risques et montrent les points de contrôle critiques dans la manière de cuisiner, inhérents à la culture. L'approche du HACCP prévoit ensuite la sélection ou conception de mesures de contrôle et critères par des spécialistes en microbiologie alimentaire et hygiène. Ces mesures, qui doivent être à la fois économiquement possibles et culturellement acceptables, doivent être portées à la connaissance de la population soit par des actions communautaires concertées soit par des campagnes nationales d'éducation.

À l'heure où les différents aspects des maladies diarrhéiques sont étudiés plus à fond que dans le passé, il devient évident que les aliments contaminés et mal manipulés sont leur principal agent de transmission. Aussi, les suites à donner aux constatations du HACCP sur le plan de l'éducation pourraient être la partie la plus précieuse de cette méthode, surtout dans les pays qui n'ont pas de structures de surveillance pour les maladies transmises par les aliments. Les renseignements recueillis peuvent être utilisés pour informer les autorités sanitaires et sociales, former les personnels de santé publique et éduquer la population adulte et les écoliers.

Il ne devrait plus y avoir de maladies diarrhéiques liées à l'alimentation, car elles sont évitables ; à ce jour, l'approche du HACCP est le moyen le plus prometteur d'y parvenir.
Las enfermedades diarreicas de origen alimentario se pueden prevenir y no deberían aparecer. Con este fin, el medio más viable que ha encontrado hasta ahora para lograr un objetivo es el concepto de punto crítico de control en el análisis de riesgos.

por Silvia Michanie y Frank L. Bryan

Un punto crítico del control es una operación (práctica, procedimiento, proceso o ubicación) o una fase de una operación en la que puede aplicarse una medida preventiva o de control. Esta medida eliminará, prevendrá o minimizará cualquier riesgo que se haya producido antes de aplicarla.

Los criterios que garantizan el control en los puntos críticos de control operaciones o durante el procesamiento o la preparación. Así pues, una garantía del sistema PCCAR es que proporciona una respuesta inmediata a las situaciones de riesgo.

En los análisis de riesgos se trata de encontrar fuentes y modos de contaminación, mediante mediciones se establece si los microbios patógenos sobreviven a la cocción a otros procesos y si se multiplican en los intervalos que median entre preparación e ingesta. Ciertos alimentos pueden o no prestar a la multiplicación de los microbios. Cabe la posibilidad de obtener muestras y analizarlas para determinar si se encuentra la presencia y la cantidad de microbios causantes de enfermedades y/o deterioro de alimentos. Cada etapa del "itinerario" de un alimento se considera y se representa gráficamente en un diagrama, donde se destacan los riesgos y los puntos críticos de control.

Se han de dar respuestas a ciertas cuestiones. Por ejemplo, ¿cuáles son los ingredientes brutos? ¿Qué pH (grado de acidez) tiene el producto acabado? ¿Cuál es la exposición tiempo-temperatura del producto durante el procesamiento o la preparación? ¿Va a cometerse el producto inmediatamente después de preparado o se va a almacenar caliente, frío o a la temperatura ambiente?

Aunque estos análisis parecen demasiado complicados para practicarlos en las casas, ya se han empleado en domicilio en una capital, en un centro urbano de una isla, en una barrida suburbana miserable, en una aldea de la montaña y en un poblado arrocero. El método ha superado esta prueba brillantemente en sitios tan diversos, lo que demuestra su validez.

Además de la contaminación que puede ser específica de ciertos tipos de alimentos crudos, hay ciertos factores como las tradiciones, la educación, los recursos económicos, las instalaciones sanitarias, la higiene perso-
El análisis de riesgos obliga a investigar si los alimentos cocinados o sometidos a otros procesos siguen conteniendo microbios patógenos. Obsérvese esta cocina de un hospital de Santiago (Chile).

FOTO OMS / APH NV

nal y el saneamiento del medio, que pueden dar lugar a riesgos durante la preparación de los alimentos. Cuando la comida preparada se deja en reserva o cuando se aprovechan los restos. Sin embargo, el mayor riesgo procede de mantener a la temperatura ambiente los alimentos cocinados durante seis horas o más entre la preparación y la comida. Los contaminantes microbianos tienen así tiempo de multiplicarse hasta una cantidad suficientemente alta para provocar enfermedades entericas o producir toxinas. El recalentamiento de las sobras no basta a menudo para inactivar los microbios que proliferan durante los largos periodos de conservación.

Todas estas observaciones revelan los riesgos e indican los puntos críticos de control de los procedimientos de preparación de alimentos utilizados en distintos medios culturales. La etapa siguiente del concepto de PCCAR es el establecimiento de la selección de medidas y criterios de prevención y control por personas expertas en microbiología e higiene de los alimentos. Estos criterios y medidas han de ser económicamente viables y culturalmente aceptables, al par que habrán de comunicarse al público mediante campanas nacionales de educación o acciones comunitarias organizadas.

Como hoy se estudian las enfermedades diarreicas en todos sus aspectos con más atención que en el pasado, cada vez está más claro que los principales vehículos de transmisión son los alimentos contaminados e incorrectamente manipulados. De ahí que el seguimiento educacional de las evaluaciones del PCCAR sea probablemente la función más valiosa del concepto, sobre todo en los países donde no se realizan actividades de vigilancia de las enfermedades alimentarias. La información reunida puede utilizarse para informar a las autoridades sanitarias y sociales, así como para formar personal de salud y para educar al público adulto o juvenil.

Las enfermedades diarreicas de origen alimentario se pueden prevenir y no deben producirse. Con este fin, el concepto del PCCAR representa el medio más viable que se ha encontrado hasta ahora.
APLICACION DEL SISTEMA DE PELIGROS POTENCIALES E IDENTIFICACION Y CONTROL DE LOS PUNTOS CRITICOS PARA MEJORAR LA CALIDAD E INOCUIDAD DE LOS ALIMENTOS

Silvia Michaelis y Fernando Quevedo

ANALIZAR
LOS PELIGROS POTENCIALES

DETERMINAR
LOS PCC

ESPECIFICAR
LOS CRITERIOS

MONITORIZAR
LOS PUNTOS CRITICOS

VERIFICAR
APLICACIÓN DEL SISTEMA DE PELIGROS POTENCIALES E IDENTIFICACIÓN Y CONTROL DE LOS PUNTOS CRÍTICOS PARA MEJORAR LA CALIDAD E INOCUIDAD DE LOS ALIMENTOS*

Silvia Muchias1 y Fernando Quevedo2

En el marco de la Quinta Reunión del Comité Coordinador Regional del Codex Alimentarius para América Latina y el Caribe (CCLAC), se realizó del 9 al 10 de febrero de 1987, el III Taller sobre Normalización de Alimentos y Salud en América Latina y el Caribe, en La Habana, Cuba, con el auspicio de la Organización Panamericana de la Salud, Organización Mundial de la Salud y el Comité Estatal de Normalización de la República de Cuba. Este Taller tuvo por objeto responder a la necesidad que experimentan los países de América Latina y el Caribe de incrementar las acciones encaminadas a la protección de la salud de sus poblaciones, así como al desarrollo y establecimiento de prácticas equitativas en el comercio de alimentos.

Participaron en este Taller funcionarios de la Argentina, Brasil, Costa Rica, Cuba, Ecuador, Guyana, Haití, México, Nicaragua, República Dominicana y observadores de la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO), el Consejo de Normas de la Comunidad del Caribe (CARICOM), el Instituto Centroamericano de Investigación y Tecnología Industrial (ICAII) y representantes de Australia y España.

En el marco del Taller se presentaron las siguientes ponencias:

a) Problemas presentados en la salud de los consumidores por los residuos de medicamentos veterinarios en los alimentos,

b) Importancia y utilización de alimentos a base de proteínas vegetales obtenidas de plantas oriundas de los países de la región y c) la que a continuación se presenta.

Las enfermedades vinculadas con los alimentos constituyen uno de los problemas de salud más extendidos en el mundo actual. Por ejemplo, las enteritis y otras enfermedades diarreicas se encuentran entre las cinco primeras causas de mortalidad en la mayoría de los países de América Latina y el Caribe. Al mismo tiempo, hay un creciente reconocimiento de que los alimentos contaminados son causa de una alta proporción de casos de diarreas.

Desafortunadamente, hasta el momento, muy pocos países de la región han desarrollado sistemas de vigilancia epidemiológica de las enfermedades transmitidas por alimentos (ETA), que permitan conocer oportunamente y adecuadamente la ocurrencia de brotes de estas enfermedades. Además, en los países con elevado desarrollo tecnológico, las estrategias utilizadas hasta el momento no han conseguido aún prevenir la presentación de las ETA. Tradicionalmente se han empleado tres medios para controlar los peligros de origen microbiano en los alimentos: la educación y el adiestramiento, la inspección de los establecimientos de procesamiento o preparación de alimentos y de las operaciones que realizan, y las pruebas y análisis microbiológicos. Los programas de algunos países utilizan la combinación de estos tres instrumentos.

La educación y el adiestramiento para el control de los peligros de origen microbiano están principalmente dirigidos hacia el desarrollo y difusión del conocimiento de las causas de la contaminación microbiana, incluyendo la supervivencia y/o el crecimiento de los microorganismos contaminantes y están orientados a técnicos, manipuladores y, en algunos países, a los consumidores.

Sin embargo, es mucho más efectivo que los conceptos sobre higiene personal, saneamiento de la comunidad y higiene de los alimentos se impartan sobre todo durante los ciclos de educación primaria y secundaria.

A nivel de elaboración de alimentos, la extensión del adiestramiento depende de la complejidad técnica de los procesos utilizados y del grado de responsabilidad de los operarios, según la función asignada. El personal adiestrado debería ser capaz de seleccionar y utilizar las medidas de control esenciales para obtener alimentos inocuos.

La inspección de alimentos, como método más antiguo utilizado para el control, no ha sido suficientemente eficaz en el alcance de los objetivos que las leyes y reglamentos propugnan. El control del cumplimiento de las Buenas Prácticas de Elaboración de Alimentos, contenidas o no en reglamentos de inspección, es un medio realizado sobre la base de la inspección de las instalaciones edilicias, de los equipos y de la observación de las prácticas higiénicas del personal. Es de destacar que, por la general no existe en los reglamentos o manuales de inspección, una indicación de la importancia relativa de los diversos requisitos y esto puede dar lugar a interpretaciones subjetivas, llevadas a criterio del inspector. La dificultad en discriminar entre los requisitos relevantes y otros de menor importancia, resulta a menudo en la sobreestimación de la inspección de operaciones o pasos que

* Basado en la presentación realizada en el marco del III Taller OPS/OMS sobre Normalización de Alimentos y Salud para América Latina y el Caribe. La Habana, Cuba 9 al 10 de febrero de 1987.
no lo requieran, produciendo generalmente un aumento de los costos sin reducir significativamente los peligros de origen microbiano que el proceso y/o el alimento pueden entrañar.

Por otro lado, el análisis microbiotológico de los alimentos, como medio para vigilar el peligro microbiológico que estos vehiculizan, surgió en el presente siglo. Se toman muestras de ingredientes, de materiales obtenidos de puntos seleccionados del proceso y del producto final. Tal muestreo y los análisis ayudan a determinar el cumplimiento de las Buenas Prácticas de Elaboración, Manipulación y Distribución. En algunos casos, se analizan los alimentos para buscar la posible presencia de microorganismos patógenos y/o sus toxinas. Sin embargo, a manudo los análisis se realizan para detectar microorganismos indicadores de la posible presencia de organismos patógenos y/o microorganismos alteradores.

Los criterios microbiológicos —estándares, especificaciones y pautas— que establecen como aceptable las especificaciones y la presencia de microorganismos patógenos, establecen como aceptable la supervivencia y/o multiplicación de las semillas. Por ejemplo, se aplica un sistema de评分 de la probabilidad de que se produzca una contaminación de alguna especie microbiana que a su juicio ocasionarían riesgos para el productor. La determinación de la probabilidad de que ocurren esos riesgos para el productor es de suma importancia.

Un nuevo enfoque

Mientras que las estrategias de control mencionadas se utilizan ampliamente, se están probando otras alternativas que buscan controlar la calidad de los alimentos y minimizar los riesgos asociados con el consumo de alimentos. Un ejemplo de esto es el uso del análisis microbiológico en la industria de alimentos. Por ejemplo, la mayor parte de las semillas que se compran para el procesamiento de alimentos son de alta calidad y se utilizan para la producción de alimentos a nivel industrial. Sin embargo, es importante tener en cuenta que el uso del análisis microbiológico no garantiza la inocuidad de los alimentos, ya que pueden tolerarse ciertos niveles de contaminación microbiana.

El sistema está constituido por los siguientes componentes o pasos secuenciales (Fig. 1):

Identificación de los peligros potenciales y evaluación de la gravedad. Estos se identifican durante la cría, cosecha, procesamiento/manufactura, distribución, comercialización, preparación y uso de una materia prima o de un producto alimenticio.

En este contexto peligro significa el desarrollo, la supervivencia o contaminación con microorganismos no aceptables desde el punto de vista de la inocuidad o alteración de los alimentos y/o la producción o permanencia, igualmente inaceptable, de productos del metabolismo microbiano (por ej., toxinas, histaminas, enzimas) en el alimento. Gravedad es la magnitud del peligro o las consecuencias que ese peligro ocasiona.

Determinación de los Puntos Críticos de Control necesarios para controlar el o los peligros que se han identificado. Un punto crítico de control es un sitio, proceso o etapa del proceso en el que se puede intervenir para mejorar la inocuidad del producto final, evitando o minimizando el riesgo asociado. Un punto crítico de control es aquel en el que se puede intervenir para mejorar la inocuidad del producto final, evitando o minimizando el riesgo asociado.

Un nuevo enfoque en sistema, es decir integral, racional y contínuo, de prevención y organización, con miras a lograr la inocuidad de los alimentos, mejorar la calidad y disfrutar las parrillas ocasionalmente por su alteración. Este enfoque se aplica en todos los pasos de la cadena alimentaria desde la producción (por ej., la cría de ganado, la siembra de las semillas) pasando por la elaboración o proceso, la comercialización y finalmente al nivel del usuario final, por ej., el uso que hace del alimento al ama de casa o los servicios de comidas para colectividades.
1.0 Análisis de los peligros potenciales

El análisis de los peligros potenciales consiste en evaluar todos los procedimientos alimentarios a la producción, distribución y uso de las materias primas o alimentos con el objeto de:

a. Identificar las materias primas potencialmente peligrosas y los alimentos que pueden contener sustancias tóxicas, microorganismos patógenos, o un número elevado de microorganismos alteradores, y/o que pueden permitir la multiplicación de microorganismos;

b. Identificar, por medio de análisis en cada paso de la cadena alimentaria, las fuentes potenciales y los puntos específicos de contaminación;

c. Determinar la posibilidad de los microorganismos de sobrevivir o multiplicarse durante la producción, el procesamiento, la distribución y el almacenamiento previo al consumo; y

d. Evaluar los riesgos y la gravedad de los peligros identificados.

1.1 Identificación de los peligros

La información epidemiológica es la que brinda la mejor evidencia de que existe un peligro respecto a un producto dado. Si puede demostrarse la relación entre la aparición de un brote de ETA y el consumo de un alimento, no hay duda de que existe un peligro que requiere control. Se debe establecer el origen del peligro, como así también las medidas correctivas.

Además de la evidencia epidemiológica de un peligro microbiano, se requiere información sobre aspectos relacionados con la producción, el procesamiento, el almacenamiento, la distribución y el uso de un alimento en particular. Sin embargo, a nivel de producción se puede disponer de información precisa sobre la composición del alimento, con frecuencia es difícil para el productor relacionar esos datos con los efectos del almacenamiento, la distribución y la utilización del alimento, debido a que éste desconoce los detalles de todos los posibles abusos o maltratos que en este caso expuesto el alimento. Como consecuencia de esta falta de información es necesario efectuar numerosos controles con el objeto de salvaguardar el producto. Antes de someter un producto a un análisis de peligros, se debería consultar a especialistas en alimentos que posean amplios conocimientos del proceso del producto y de su utilización. El microbiólogo deberá, como mínimo, hacerse las siguientes preguntas en relación con los posibles peligros:

a. ¿Cuál es la composición del alimento?

b. ¿Las materias primas son satisfactorias desde el punto de vista microbiológico?

c. ¿Cuál es el pH?

d. ¿Cuál es la actividad acuosa (aw)?

e. ¿Se utilizan conservadores?

f. El pH, la aw y la presencia de conservadores van a prevenir el desarrollo microbiano?

2.0 Puntos críticos de control

¿Qué es un punto crítico? En esencia es una etapa del proceso o una manipulación en la que uno o más peligros potenciales, identificados como resultado del análisis de peligros, puede ser prevenido o reducido mediante el ejercicio del control en esa etapa de la operación. En algunos puntos un solo operación en un punto crítico de control puede ser utilizada para reducir un o más peligros microbianos; por ejemplo, la pasteurización de la leche. Sin embargo, en la mayoría de las operaciones se debe aplicar una combinación de procedimientos en uno o más puntos críticos para eliminar los peligros. En algunas operaciones no es posible eliminar todos los peligros con el control de los puntos críticos, sólo es posible reducir la magnitud del peligro. Por ejemplo, es importante considerar la manipulación posterior del alimento durante el sacrificio, la evisceración y la posterior manipulación para reducir la contaminación cruzada y la multiplicación microbiana de los peligros.

¿Qué constituye una contaminación inaceptable, una supervivencia o desarrollo de microorganismos patógenos o de alteración de calidad? Al considerar estos puntos, se debe realizar una evaluación de la gravedad del peligro. Por ejemplo, la búsqueda de microorganismos anaerobios...
Evaluación del peligro

El análisis del o los peligros, para tener significación, debe ser cuantitativo; es necesario evaluar el riesgo y su magnitud o gravedad.

Consecuentemente, nos debemos preguntar: ¿El peligro estará siempre presente, ocurrirá una vez por año, una vez por año? ¿Estará amenazada la calidad del producto? ¿Se producirá una alteración del producto o las consecuencias en términos de alteración o de provocar una ETA son triviales? Las respuestas a estas preguntas indicarán el grado de recursos económicos que se deberán utilizar en el control del peligro. Por ejemplo, la producción de toxinas por Clostridium botulinum es un peligro severo en carnes curadas enlatadas (estabilizadas) que reciben un proceso térmico inferior al requerido para la desinfección de ese agente. Se puede demostrar que Clostridium botulinum puede desarrollarse cuando se inocula en estos productos. Sin embargo, se han producido toneladas de carnes enlatadas curadas sin evidencia de peligro botulínico. Por lo tanto, si bien el peligro es grave, el riesgo es extremadamente pequeño.

Los peligros asociados con la producción del o con el uso de un alimento en particular deben evaluarse mediante este sistema, en términos de su gravedad y de la probabilidad de su ocurrencia en relación con la Salud Pública o un interés comercial. Si los puntos críticos no existen y el riesgo y la gravedad de los peligros se consideran elevados, el producto no debe bajar de ello. La elección de este sistema se debe a su capacidad para aplicar régimen de control de los peligros de manera efectiva. El análisis del peligro en el proceso de producir o manipular el producto, es útil en esta etapa. El control de los peligros microbianos es esencial para evitar que se produzcan productos contaminados con microorganismos patógenos, lo que puede resultar en enfermedades alimentarias o intoxicaciones alimentarias.

3.0 Selección de los criterios para el control

Es muy importante identificar el o los criterios que deberán usarse para controlar los peligros en los puntos críticos. Por ejemplo, la combinación de tiempo-temperatura que se requiere para productos enlatados, el pH o la acidez en productos fermentados, el control de humedad en la conservación de productos deshidratados, la temperatura durante la distribución de productos refrigerados, la actividad acuosa en alimentos de humedad intermedia. Todos estos criterios deben incluirse como especificaciones en los manuales de preparación de los productos. Deberán, además, incluirse las tolerancias a la especificación, cuando existan. Para un solo punto crítico puede ser posible aplicar varias medidas de control, y para varios puntos críticos puede ser necesaria una combinación de controles. La elección de las opciones de control dependerá de la utilidad, del costo y de la capacidad para aplicar el control seleccionado a una operación particular.

4.0 Monitorización

Como se señaló anteriormente, la monitorización es la comprobación de que el proceso se está realizando de manera efectiva. La monitorización de los puntos críticos debe realizarse en un momento o en un momento determinado del proceso. La monitorización se realiza por medio de la observación visual, la evaluación sensorial, la medición de parámetros físicos, los controles químicos y los análisis microbiológicos. La observación visual se utiliza principalmente para detectar cualquier desviación y estar en condiciones de controlar la información a tiempo para que se tomen las medidas correctivas, antes de que se haga necesario el rescate del producto.

Los principales monitores empleados son la observación visual, la evaluación sensorial, la medición de parámetros físicos, los controles químicos y los análisis microbiológicos. Los monitores químicos son importantes para la determinación de cloro libre en el agua utilizada en el enlatado de la leche, son medidas de control útiles. Igualmente los controles de temperatura, pH, contenido salino, y humedad pueden realizarse con rapidez y son de utilidad en el monitoreo de un proceso, cuando estos factores son los medios para controlar un punto crítico particular.

Por otro lado, los análisis microbiológicos tienen una aplicación limitada en la monitorización de los puntos críticos; el tiempo que requieren estos análisis no permite tomar medidas correctivas rápidas. Pueden ser de utilidad en el control de la calidad de algunas materias primas; no obstante, en la mayoría de los casos, el análisis microbiológico sirve como prueba complementaria de las observaciones o evaluaciones sensoriales y de los análisis físico-químicos rápidos. Por ejemplo, en algunos países, el mayor énfasis ha sido puesto en la aplicación de este sistema para controlar el peligro craneo en la preparación de conservas enlatadas de alimentos poco ácidos. En estos productos el peligro grave es, por ejemplo, la proliferación de Clostridium botulinum y la presencia de toxinas. El peligro severo en carnes curadas enlatadas se evitará al aplicar varios métodos de control. Los controles microbianos se realizarán en el control de superficies y en el control de los equipos. Varios grupos de investigadores trabajan en el desarrollo de métodos microbiológicos rápidos para controlar el inconveniente derivado del tiempo que insumen estos análisis.

Además de la necesidad de obtener resultados rápidos, es conveniente utilizar métodos simples, que puedan ser adecuados para la aplicación práctica. Generalmente, la monitorización se realiza con la intervención de personas con escasos conocimientos técnicos. Las microprocesadoras que se emplean para monitorizar un punto crítico están programadas para dar una respuesta antes de que se presente una situación de riesgo o de control, y se emplean cada vez más en estos países. Si bien las grandes industrias procesadoras de alimentos tienen personal técnico adiestrado en controles químicos, físicos y microbiológicos, el personal responsable del control y de la línea de producción generalmente no posee esos conocimientos técnicos. Por este motivo, los métodos de monitorización utilizados en la línea de producción deben ser accesibles al personal no técnico. Se hará entonces necesario el adiestramiento del personal en métodos simples y fáciles de aplicar.

Una vez establecidos los métodos de monitorización y los criterios que indican si la operación está o no bajo control, será necesario especificar la frecuencia de la monitorización y los métodos de muestreo que deberán emplearse. La fra-
10 mas simples posibles. usa de listas de control, en las que
de la cadena alimen­

taria se establecerá con relación al pe­

tiempo y la temperatura, al pH, el conten­
dido de sal, etc.— es posible revisar los re­
sultados del monitoreo con respecto a un
punto crítico. Todos esos registros deben
estar disponibles para que los revise y

evalúe el personal de control de calidad.

La falta o carencia de registro imposibilita­
rá conocer cuando un proceso está o no
bajo control.

Hemos analizado la monitoreización en
lo que respecta al procesamiento, pero
este sistema se aplica a todas las etapas
de la cadena alimentaria, desde la pro­
ducción de la materia prima pasando por
todas las etapas hasta llegar al uso que
hace del alimento el consumidor, ya sea
en el hogar o en los servicios de comidas
para colectividades. Los puntos críticos
existen en cada etapa de la cadena y
deben estar monitoreados; es necesario
registrar los resultados de la monitorea­
ización.

5.0 Verificación

La verificación es la utilización de con­
troles adicionales para comprobar que el
sistema funciona correctamente; es decir
que es operativo. La verificación puede
usarse cuando este sistema de control se
aplica por primera vez o en un proceso
nuevo, o como parte de la revisión conti­
nuva de un programa establecido con an­
terioridad.

Este nuevo enfoque, en función de las
ventajas que presenta, debiera ser utiliza­
do en la reorientación y reorganización
de los Programas de Protección de Alimen­
tos de los países de la región.

Estamos convencidos de que la apli­
cación de este sistema a la preparación
de alimentos en los hogares, en los servi­
cios de comedas para colectividades, en
la industria artesanal, y en la pequeña, me­
diana y gran industria redundará en una
diminución de la morbi-mortalidad oca­
sionada por las enfermedades transmiti­
das por los alimentos y reducirá las pérdi­
das económicas, como así también, ma­
jorará el aspecto nutricional y la calidad hi­
giénica de los alimentos.

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FIG. 1 ETAPAS DEL SISTEMA DE ANALISIS DE PELIGROS
Y CONTROL DE PUNTOS CRITICOS
The role of HACCP and predictive microbiology in the efforts of WHO to improve food safety

Dr Fernando Quevedo

SUMMARY

The last three decades have witnessed an increase in the number and frequency of foodborne diseases (FBD's). Numerous 'new' etiological agents have made their appearance. Chronic complications and sequelae demonstrate that foodborne diseases have 'come of age'. Certain food products, unsuspected before, are acting now as vehicles for transmission of these diseases. The traditional approaches used by national administrations for the control of the quality and safety of foods are not giving the expected results for the prevention of FBD's. Moreover, the increased pressure to feed large populations in ever-expanding cities makes the task of control more difficult for the national authorities, considering the scarcity of financial and human resources assigned to them. And in many countries there is no firm national commitment towards ensuring a safe food supply.

The traditional approaches already mentioned have progressed in a different manner. Many national and international efforts have been made to update the food legislation and regulations. The work accomplished by the FAO/WHO Codex Alimentarius Commission has been exceptional. The educational approach is presently better understood and is growing. The epidemiology of foodborne diseases is seeking its appropriate place in all these efforts. The progress accomplished in modern epidemiology is being applied for FBD studies and investigations. The inspection approach has been maybe the most passive, and the most active has been the analytical sector, in which the chemical laboratories have been the more dynamic.

Food microbiology has evolved from very simple tasks such as 'total counts' and 'coliform' determinations to the search for pathogens and their metabolites and the microbial ecology of foods. Realizing that the examination of the final products were not contributing effectively enough to the prevention of foodborne diseases and to the improvement of the quality of foods, food microbiologists created and developed the HACCP system as a valuable contribution to Total Quality Management. Food microbiologists also started to develop predictive microbial modelling to contribute to a better design of HACCP and to facilitate the work of food technologists.

The World Health Organization has participated actively in all these processes. Several projects on training, research and development of the different aspects of food safety have been promoted by its Headquarters and Regional Offices. HACCP has been the subject of various WHO activities since 1974, and is receiving increasing attention. Predictive microbial modelling will receive WHO support, not only when applied to the food industry, but also if it is studied in relation to food prepared at the home level, by cottage industries, and the ever-growing street food sector. As a general strategy to improve Food Quality and Safety, WHO is advocating a shared approach, with the participation of the three sectors involved: governments, food industries and consumers.

* Presented at the 2nd International Conference on Predictive Microbiology and HACCP, Laval, France, 10-11 June 1992

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THE ROLE OF HACCP AND PREDICTIVE MICROBIOLOGY
IN THE EFFORTS OF WHO TO IMPROVE FOOD SAFETY

Dr Fernando Quevedo

Introduction.- Advances in science, technology and public health achieved during this 'century of hygiene' led to the impression that the majority of transmissible diseases, including foodborne diseases (FBD's), would be prevented, at least in the most 'developed' countries. The adequate control of the cholera epidemics reoccurring in certain such countries supports this belief. The dramatic reduction in botulism was another 'proof'. But, for various reasons which will be discussed later, in the last three decades FBD's which had apparently been under control began again to be a real problem, as they increased in number of cases and frequency, and 'new' or 'emergents' pathogens have also appeared.

This increase in the number of known FBD etiologic agents is evident. If we compare some of the 'classic' books on foodborne diseases we can appreciate how their numbers have been progressively increasing. In Dack's book (1943) and in the second edition of Tanner and Tanner (1953), only a few foodborne disease bacterial agents were mentioned; years later in 1969 in his book Riemann added some agents. Later, in 1979, Riemann & Bryan's book discussed in detail, seven bacterial agents, in addition to viruses, parasites and mycotoxins. One specific chapter discussed "Infections and Intoxications caused by other bacteria", but 

Yersinia enterocolitica, Listeria monocytogenes, Campylobacter jejuni, and others, were at that time considered as "bacteria not conclusively proved to be food-borne". However, in 1972 a very comprehensive list of food-borne diseases agents, including a classification and summary, had been published by Bryan, the second edition was published in 1982, and a reprint in 1984, this useful publication is also considered a 'classic', but it needs periodic updating because 'new' agents continue to 'appear'. Various possible explanations for these 'emerging' diseases have been published, Cox has identified seven basic reasons, that are, according to him, interrelated and very rarely mutually excluded:

1.- Changes in eating habits; 2.- Changes in perception and awareness of what constitutes hazards, risks, and hygiene; 3.- Demographic changes; 4.- Changes in primary food production; 6.- Changes in handling and preparation practices; 7.- Changes in the behaviour of microorganisms.

Other examples of 'emergents' are: Vibrio vulnificus, Campylobacter jejuni, Cryptosporidium parvum, Plesiomonas shigelloides, domoic acid, Escherichia coli O157:H7, etc.

In addition to the growing number of outbreaks and cases, and the increase in the number of agents, it is also interesting to remember that in recent years 'uncommon' food-vehicles have produced outbreaks of botulism, salmonellosis, viral infections, etc (Tables 1, 2, and 3). "Records" of the 'biggest' and 'deadliest' outbreaks have also occurred. Tables 4, 5 and 6 give some examples of these records.

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It has to be emphasized that the FBD's have 'come of age'. This means that not only do they produced the 'typical' symptoms of diarrhoea, vomiting, malaise, etc. but they can also lead to joint diseases, autoimmune thyroid diseases, neural/neuromuscular disorders, renal diseases, diseases of the heart and vascular system, and other sequelae\(^3\), obliging the health authorities in a growing number of countries to take these foodborne illnesses more seriously.

A Joint FAO/WHO Expert Committee on Food Safety recently stated that "illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity\(^5\). Every year WHO receives information from its Collaborating Centers and from national authorities and scientific institutions all over the world which confirms that foodborne diseases are increasing in number and frequency, and in spite of the efforts that are being made, the problem will continue to grow if other strategies are not applied. Some examples of the increase in FBD's are given in the figures attached.

In countries without good systems for food control and protection it is understandable why this is happening, but in some of the industrialized countries with good infrastructure and programmes the situation is similar. What has failed? What continues to fail?

The logical conclusion is that the traditional approaches for both food control and for the prevention of FBD's, approaches based on food inspection, laboratory analysis, food legislation, education, etc., have not been successful. Before commenting on the evolution or accomplishments of these approaches, it is important to remember that food safety has "to be seen against the backdrop of a series of interrelated and complex problems which block the possibilities of many national governments to provide protection against unsafe and fraudulent foods"\(^6\). It is necessary to recognize that during these last decades new global challenges to health and the environment have arisen, including the profound changes in the growth and distribution of population all over the world\(^7\). Related to these changes are rapid urbanization and the creation of 'megacities', one increasing the risks of food contamination and the other creating more demanding needs to feed immense populations. This problem, and the increasing dependence of all countries on large quantities of imported food\(^8\), makes very difficult the task of the authorities to control the quality and safety of foods. Also, the available technical capabilities, physical infrastructure and equipment and the financial constrains faced by the governments of numerous countries (and local governments in the case of cities), for these control activities, compared with the infrastructure and the technological advances accessible to the private sector, requires serious a consideration of the situation and the need to adopt new strategies.

One of the traditional approaches that has received much attention in the past, and will continue to receive in the future, is food legislation and regulation. Here, countries might be categorized by groups: one formed by countries that have excellent and up-to-date legislation, and appropriate mechanisms and infrastructure for its enforcement; another group could be composed of countries with very good legislation, but without the financial or technical possibilities or without political support for its enforcement; and the third group that of countries with an obsolete legislation, and no well-organized means of control. In the field of standards and codes of practice the work accomplished by the Joint FAO/WHO Codex Alimentarius Commission, created in 1962, is remarkable.
The epidemiological surveillance of foodborne diseases which aims at getting a better understanding of the problem and thus establish a basis for political decisions and for the appropriate funding of food safety activities, dont have a parallel development in the countries. Some industrialized countries have developed good systems and good mechanisms of information. The WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe, is under the responsibility of the WHO Regional Office for Europe and the Robert von Ostertag Institute of Berlin, an FAO/WHO Collaborating Centre, is the managerial centre. The Programme aims at: - identifying the causes and epidemiology of foodborne diseases in the Region; - distributing the gathered and collated information to those concerned, and - to cooperate with national authorities in their efforts to strengthen the prevention and control of foodborne diseases. The WHO Regional Office for the Americas has stimulated the organization of an active Latin American Network of Epidemiological Surveillance of Foodborne Diseases, which has published good information. The modern epidemiology is using new investigative tools in the study of important outbreaks or epidemics of FBD's.

Food Inspection, one of the oldest and most employed means for food control has not progressed to the same extents as other approaches. Moreover, food inspectors in many countries are not provided with the minimum set of tools for their work. In many places, inspectors are not even given a simple thermometer, and they can only use their senses to inspect the food commodities and make decisions. The same has been happening with the traditional inspection of meat, poultry, and meat and poultry products. At the request of the US Department of Agriculture (USDA), the Food and Nutrition Board, Commission of Life Sciences of the US National Research Council appointed a Committee on the Scientific Basis of the Nation's Meat and Poultry Inspection Program, which produced an important report. It was recognised that traditional inspection methods have remained unchanged for nearly 70 years, and that slaughter inspectors rely almost completely on sight, smell, and touch to discern abnormalities in animals and carcasses. Recognizing the importance of the system, the Committee recommended that the principles of HACCP "...be applied more rapidly and comprehensively in plant operations" and that "To achieve both operational efficiency and protection of public health, critical control points must be identified, inspectors trained in the HACCP approach, and procedures regularly monitored". In relation to this, it has also been stated, in New Zealand, that "although a traditional approach to postmortem meat inspection dominates current programs, scientific evidence increasingly suggests that some practices are inappropriately focused. Allocation of inspection resources in modern meat production and processing systems should reflect a distribution according to risk, rather than a distribution according to the classical rules of meat inspection".

For some time now, health education in food safety has been advocated as one of the most useful ways to prevent FBD's. Many and imaginative methodologies have been used. WHO is promoting this approach together with community involvement, in order to have food safety integrated into primary health care delivery systems.

The analysis of the final product has been one of the most commonly used approaches by national and local authorities. If a sample gave results that were not within the allowed standards, the authorities took action, mainly punitive ones. The disadvantages of this approach are very well known. However the analytical services will continue to offer an extraordinary contribution to food quality and safety control.
The sector devoted to chemical analysis has been very active, and has made a great strides in the scientific and technical progress. Modern techniques with an extraordinary specificity and sensibility have been developed for the chemical analysis of foods. Of course there are still technical problems to solve, but the principal obstacle to using the modern analytical methodology in many places is the high cost entailed for equipment and of the execution of the analyses.

Food microbiologists have also been very active. In the traditional approach they were given the task of analysing the final products, specially performing 'total counts' and 'coliform tests', they also had to determine the presence of some pathogens or their toxins. More than sixty years ago, food microbiologists started to use mathematical modelling of microbial kinetics, specially for the calculation of the death kinetics of Clostridium botulinum spores in heat processes. At the same time Eijkman and later Butiaux, proposed some prevention approaches in lieu of 'control' of final products. Forty years ago, Ingram and Mossel promoted the study of the microbial ecology of foods, opening new avenues for research and for the interpretation of the behaviour of the microbes in foods. One of their works is still referred to nowadays. In the Sixties food microbiologists started to proposed a system that now is worldwide known as Hazard Analysis Critical Control Point (HACCP). In 1962, the International Commission on Microbiological Specifications for Foods (ICMSF) was created. This international group has contributed actively to the sistematization of many of these developments, and to the recognition of the importance of food microbiology for food quality and safety. Because of this importance, WHO organized two meetings of the Expert Committee on the Microbiological Aspects of Food Hygiene in 1967 and in 1976.

HACCP: some historical information.- The concept of "critical control points" was introduced 'in society' at the 1971 National (US) Conference on Food Protection, when Bauman presented two background papers for defining, systematizing and locating them. The Conference approved some recommendations. One of these was the widespread use of the concept of hazard analysis of each food and food system and the establishment of critical control points to ensure quality. It is well known that the system was engineered by the NASA, the Natyck Laboratories and The Pillsbury Company. In 1974, FDA applied the HACCP concept to the Low Acid Canned Foods. In subsequent years many articles and research papers appeared showing the results of the application of HACCP to different foods and processes. A comprehensive book on HACCP, edited by ICMSF, was published in 1988.

The Codex Alimentarius Commission has been showing increased interest in the application of HACCP, and recently the Committee on Food Hygiene appointed a commission, headed by Catherine Adams and Tony Baird Parker, to prepare a review of the concept. The working paper prepared (Conference Room Document No 8) that was presented at the 25th Session of the Committee on Food Hygiene of the FAO/WHO Codex Alimentarius Commission (October-November 1991), recalled that HACCP's successful application requires the full commitment and involvement of management and the workforce. It also requires a team approach; "this team should include appropriate experts such as production personnel, microbiologists, chemists and engineers". The document also highlighted the fact that the application of HACCP is compatible with the implementation of quality management systems, such as ISO 9000.
HACCP is a system which identifies specific hazard(s), (biological, chemical or physical). The document referred to above defines seven principles:

**Principle 1** Identify the potential hazard(s) associated with food production at all stages until consumption. Assess the likelihood of occurrence of hazard(s) and identify the preventative measures for their control. **Principle 2** Determine the points/procedures/operational steps that can be controlled to eliminate the hazard(s) or minimize its likelihood of occurrence (CCP). **Principle 3** Establish target level(s) and tolerances which must be met to ensure the CCP is under control. **Principle 4** Establish a monitoring system to ensure control of the CCP by schedule testing or observations. **Principle 5** Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control. **Principle 6** Establish procedures for verification which includes supplementary tests and procedures to confirm that HACCP is working effectively. **Principle 7** Establish documentation concerning all procedures and records appropriate to these principles and their application.

The document was well accepted by the participants at that meeting. Recently, in USA, Tompkin introduced slight modifications to that document and presented it for discussion at the national group dealing with microbiological criteria for foods.

**HACCP and the efforts of WHO in promoting Food Safety.** The World Health Organization considers HACCP a very useful methodology that could be instrumental in the prevention of foodborne diseases all over the world. Shortly after HACCP presentation at the US Food Protection Conference, WHO promoted the discussion of this concept at the Inter American Conference on Food-Borne Toxi-Infections held in Guatemala in 1974. Following the recommendations of this conference, teaching of the concept was included in the postgraduate courses on Food Microbiology & Hygiene organized by PAHO/WHO. In 1976, the WHO Expert Committee on Microbiological Aspects of Food Hygiene discussed HACCP and insisted upon the need for cost-benefit studies in relation to this approach.

In 1982, ICMSF and WHO met to discuss HACCP and its application in food hygiene. A similar activity was held at a later date regarding the application of HACCP for the prevention and control of foodborne salmonellosis.

In 1983, the Joint FAO/WHO Expert Committee on Food Safety concludes that "The hazard analysis critical control point (HACCP) approach (which is a sequential investigative control activity) is a desirable alternative to certain traditional control options (e.g. routine inspection or microbiological sampling and testing of raw meat). It is therefore recommended that local, regional, and national governments should initiate the training of their administrative, laboratory and field personnel to prepare them for this approach. Furthermore, a food-processing or food-preparation operation should be selected and hazard analysis made of appropriate aspects of the food chain and/or of individual establishments or homes. The food selected might be an infant weaning food prepared in a rural home, a food prepared in a home or small shop and vended on the street, a food prepared in a tourist hotel, or one manufactured in a food-processing plant. Critical control points of the operation should be identified, cost-effective control measures chosen or devised, and monitoring procedures initiated. Educational efforts concerned with prevention can be directed at persons at all levels of the food chain at which contamination, survival, or proliferation of etiological agents occurs, or at the public who might be at risk because of their food habits. "After some experience has been gained in the application of the HACCP system as a key approach in the improvement of food safety, FAO and WHO should review their contributions to
the success of programmes, take note of the problems that have arisen, and give assistance in the preparation of procedural guides for homes, mass-catering establishments, and processing plants". FAO and WHO can also assist by arranging for the recruitment of experts to direct the HACCP evaluation or to conduct training in its application, and/or by advising on the equipment of a supporting laboratory to investigate hazards or to monitor critical control points.

In order to accomplish these recommendations WHO, including its Regional Offices in the Americas, the Eastern Mediterranean, and in South East Asia have organized and supported, since 1984, a series of studies in different developing countries. As a result of these studies more than fourteen technical papers have been published.

WHO is also making considerable efforts to teach the HACCP concept. In addition to the inclusion of the HACCP topic in the courses mentioned above, in 1987, in the Dominican Republic, with the support of the private sector, WHO/PAHO organized a Regional Course on HACCP, with participants from many countries. Since that date, the Regional Office for the Americas has organized various regional courses in different countries. In 1990, an intensive course on HACCP was also presented in Pakistan. HACCP has been included in various courses on Food Safety organised by WHO with the support of technical cooperation agencies such as GTZ (Germany), DANIDA (Danemark), EACI (Spain). In some of the Regional Workshops organized by WHO and FAO, and within the framework of the Codex Regional Coordinating Committees meetings, the subject has been particularly addressed. Recently, WHO has published a guide on HACCP, intended for use by public health personnel with some training in food microbiology and technology, and who are concerned with the prevention of foodborne diseases. This guide will assist in the planning of food safety and health education activities focused on the types of food commonly prepared and eaten by local populations.

The WHO Regional Office for Europe has been also working on the topic of HACCP. The most important activities are the Consultation on Food Safety in Europe in the 1990s: the HACCP System as the Tool of Choice for Effective Inspection, in Brussels; and the Seminar on HACCP in Food Law Enforcement, held in Budapest in September 1991. A special monograph on HACCP has been prepared with the support and technical assistance of ILSI Europe. More courses and activities on HACCP are programmed during this and future years.

Microbial modelling or Predictive Microbiology. As mentioned above, food microbiologists started to use mathematical modelling of microbial kinetics over 60 years ago. Kilsby refers to the fact that the "botulinum cook", recognised and used internationally, and that is simple and reliable, was obtained with relatively poor experimental data. The 12D concept is still applied, with confidence and success, by the food canning industry all over the world.

Food microbiologists have also been using a 'challenging' model, in an empirical manner, to determine the spoilage activity of microbes on specific foods, and sometimes to try to demonstrate that a bacteria, mold or yeast isolated in a food sample was the cause of the alteration of that commodity, or was the origin of a toxin or another metabolite found in the food. This procedure has also been applied to evaluate the shelf-life, the microbial stability and the safety of foods. But, this test could give answers to only a few controlled intrinsic or extrinsic parameters.
During the Sixties and early Seventies, some researchers called attention to the need 'to study the interactive effects of various parameters on microbial growth and toxin production'. Baird-Parker and Freame, Ohye and Christian, Matches and Liston, Roberts and Ingram, were among these scientists.

The Eighties marked the beginning of very active progress in the field of microbial modelling. Many researchers, specially in the UK, USA, Australia, Canada, and some European countries, published the results of their studies. In the Nineties, governments are showing an increasing interest in support of research on this subject, and the food industry is performing more studies on it. An international conference was recently held in Florida, USA. This conference in Laval, organized by ASEPT, is another good demonstration of the growing interest in predictive microbiology, and its relations with HACCP. However, some professionals are still reticent about accepting the methodology or are sceptical. Hedges expresses the opinion that "confidence in the predictive ability of the model is unjustified". To that statement Cole answered that "we will continue to develop our predictive models, ... because of the power that models provide for the food microbiologist in day-to-day decision making". Riemann, after comparing modelling in food microbiology with modelling in epidemiology, emphasized that "the predictive value of models therefore becomes suspect as is the practice of 'validating' models using the same data that were used to create the parameters of the model".

In discussing predictive modelling we have to take into consideration these opinions and also realize that in addition to the many advantages of the system, there are a few limitations. One is that definitive conclusions about an organism's ability to grow and/or produce toxin in a certain food environment cannot be made, and the other is that maybe the moment is not opportune to extrapolate and make predictions exceeding the limits of the experiments performed. However, the very valuable information that the predictive modelling gives can be very useful to evaluate, for example, the safety of foods which are subject to temperature abuses during the different steps of their production, processing and commercialization. That is why WHO has an increasing interest in the predictive modelling. In a recent technical consultation on food safety and trade in face of the recent epidemic of cholera in the Americas, it was advocated that due to the scarcity of reliable data on survival or growth of *Vibrio cholerae* in foods which in turn creates unjustified trade barriers in international commerce, it is necessary to perform studies using predictive microbiology to obtain more accurate information. This was emphasized again at the International Symposium on 'Cholera on the American Continent', organized by ILSI and other international organizations in Sao Paulo, Brazil, 21-23 May.

WHO is also trying to promote the idea that it would be useful if some researchers perform predictive modelling studies on ethnic foods, street foods, and foods prepared at domestic level in developing countries, in order to obtain a good information on the behaviour of the pathogenic microorganisms contaminating these types of foods. In the future, maybe it will be advisable for WHO to organize a consultation on predictive modelling and food safety.

7
'Uncommon' vehicles

(Some examples)

Table 1  BOTULISM

<table>
<thead>
<tr>
<th>Food</th>
<th>Country</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spread-cheese with onions</td>
<td>Argentina</td>
<td>1974</td>
</tr>
<tr>
<td>Palm heart ('palmitos')</td>
<td>Argentina</td>
<td>1974</td>
</tr>
<tr>
<td>Potato salad (from baked</td>
<td>USA</td>
<td>1978</td>
</tr>
<tr>
<td>potatoes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sauteed onions</td>
<td>USA</td>
<td>1983</td>
</tr>
<tr>
<td>Chopped garlic</td>
<td>USA</td>
<td>1985</td>
</tr>
<tr>
<td>Hazelnut yogurt</td>
<td>UK</td>
<td>1989</td>
</tr>
<tr>
<td>'Fresh' fish</td>
<td>USA</td>
<td>1990</td>
</tr>
</tbody>
</table>

(Hawaii)

Table 2  SALMONELLOSIS

<table>
<thead>
<tr>
<th>Food</th>
<th>Country</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate ('coins')</td>
<td>Canada/USA</td>
<td>1985-86</td>
</tr>
<tr>
<td>Beans sprouts</td>
<td>UK</td>
<td>1988</td>
</tr>
<tr>
<td>Rattlesnake meat</td>
<td>USA</td>
<td>1990</td>
</tr>
<tr>
<td>Cantaloupes</td>
<td>USA/Canada</td>
<td>1991</td>
</tr>
</tbody>
</table>
Table 3
VIRAL INFECTIONS

<table>
<thead>
<tr>
<th>Food</th>
<th>Virus/Country</th>
<th>Country</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen raspberries 46</td>
<td>Hepatitis A</td>
<td>UK</td>
<td>1989</td>
</tr>
<tr>
<td>Bread 44</td>
<td>Hepatitis A</td>
<td>UK</td>
<td>1991</td>
</tr>
<tr>
<td>Orange Juice 47</td>
<td>Norwalk like</td>
<td>Australia</td>
<td>1991</td>
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</tbody>
</table>

"Records"
(some examples)

Table 4
BACTERIAL ORIGIN

<table>
<thead>
<tr>
<th>Disease</th>
<th>Biggest</th>
<th>Deadliest</th>
<th>Year</th>
<th>Place</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonellosis 48</td>
<td>200 000</td>
<td></td>
<td>1985</td>
<td>USA</td>
</tr>
<tr>
<td>Listeriosis 49</td>
<td>142</td>
<td>48</td>
<td>1985</td>
<td>USA</td>
</tr>
<tr>
<td>Botulism E 50</td>
<td>84</td>
<td>18</td>
<td>1991</td>
<td>Egypt</td>
</tr>
<tr>
<td>Cholera 51</td>
<td>600 000</td>
<td></td>
<td>1991</td>
<td>Worldwide</td>
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</tbody>
</table>

Table 5
VIRAL ORIGIN

<table>
<thead>
<tr>
<th>Disease</th>
<th>Biggest</th>
<th>Year</th>
<th>Place</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis A 52</td>
<td>292 000</td>
<td>1988</td>
<td>China</td>
</tr>
<tr>
<td>Gastroenter.</td>
<td>&gt; 3 500</td>
<td>1991</td>
<td>Australia</td>
</tr>
<tr>
<td>Norwalk-like 47</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6  MARINE TOXINS

<table>
<thead>
<tr>
<th>Disease</th>
<th>Biggest</th>
<th>Deadliest</th>
<th>Year</th>
<th>Place</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paralytic Shellfish</td>
<td>187</td>
<td>26</td>
<td>1987</td>
<td>Guatemala</td>
</tr>
<tr>
<td>poisoning (PSP)</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciguatera</td>
<td>57</td>
<td></td>
<td>1987</td>
<td>Cuba</td>
</tr>
<tr>
<td>PSP</td>
<td>&gt;300?</td>
<td></td>
<td>1989</td>
<td>C.America/M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mexico</td>
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A guide to identifying hazards and assessing risks associated with food preparation and storage

Frank L. Bryan
Director, Food Safety Consultation and Training,
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World Health Organization
Geneva
1992
EVALUACIONES POR ANALISIS DE PELIGROS EN PUNTOS CRITICOS DE CONTROL

Guía para identificar peligros y evaluar riesgos relacionados con la preparación y la conservación de alimentos

Frank L. Bryan
Director, Centro de Consulta y Adiestramiento sobre Inocuidad de los Alimentos
Lithonia, Georgia, Estados Unidos de América

Organización Mundial de la Salud
Ginebra
1992
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