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A Study of the Enteric Bacterial Population of the Rio Grande From Cochiti Dam to Bernalillo, New Mexico and of The Water Systems of Cochiti, Santo Domingo and San Felipe Villages

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This thesis, directed and approved by the candidate's committee, has been accepted by the Graduate Committee of The University of New Mexico in partial fulfillment of the requirements for the degree of Master of Science

A STUDY OF THE ENTERIC BACTERIAL POPULATION OF THE RIO GRANDE FROM COCHITI DAM TO BERNALILLO, NEW MEXICO AND OF THE WATER SYSTEMS OF COCHITI, SANTO DOMINGO AND SAN FELIPE VILLAGES

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THESIS

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ABSTRACT

A bacterial survey of fecal coliforms, fecal streptococci, Arizona and Salmonella was made of the waters of the Rio Grande, its adjoining canals and ditches, and of the well water of three pueblos along the Rio Grande in New Mexico. The river and ditch survey consisted of sampling 11 sites from Cochiti Bridge south to the Bernalillo Acequia at Bernalillo, and took place between September, 1971 and November, 1972. Water was collected from six sites in Cochiti Village, five sites in Santo Domingo Village, and seven sites from San Felipe Village between May 25, 1972 and November 14, 1972.

Six Salmonella enteritidis isolations were made including serotypes carrau, newport, belem, muenchen, and bredeney from four sites - Cochiti Bridge, Sili Main Canal, Bernalillo Acequia, and San Felipe Bridge. Two Arizona isolations were made from Cochiti East Side Main Canal and Bernalillo Acequia. All of the isolates were found in July and August when surface water temperatures ranged from 22.8 to 24.0°C.

Fecal coliforms and fecal streptococci were counted using Millipore Filter techniques and the fecal coliform/ fecal streptococcus ratios (FC/FS) were computed. There appeared to be a seasonal pattern; the quantity of both organisms reached their greatest numbers during the

warmer months of May to September. However, the FC/FS ratios had no apparent pattern and showed no correlation other than that Salmonella was not isolated when the ratio was less than 9.7. Samples collected from the pueblos showed contamination at various sites. An attempt was made to find causes of contamination and measures were taken in each pueblo to try and eliminate it.

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INTRODUCTION

This study is concerned with the public health significance of bacteria from extraneous sources such as soil, air, decomposition of various plants and animals, sewage, etc. which are found in water. The topic has received much attention in the last few years (Prescott, Winslow and McCrady, 1945) because potentially pathogenic organisms may be found in water. It was recorded in 1864 (Pelczar and Reid, 1965) that water could transmit disease. Since then, many bacteriological studies of water have been conducted to find sources and kinds of microorganisms important in public health.

The drinking and irrigation water in some places comes from surface water such as rivers, streams and lakes, and is likely to be polluted with domestic and industrial wastes (Pelczar and Reid, 1965). Enteric pathogens enter water via feces and excreta. There are many kinds of fungi, bacteria, protozoa, and viruses present in sewage which are responsible for a variety of diseases such as typhoid and paratyphoid fevers, salmonellosis, shigellosis, cholera, polio, hepatitis, leptospirosis, and others. It is the organisms causing salmonellosis that are reported in this thesis.

Salmonellosis was seldom reported or studied as a disease before 1920 (Cherubin et al., 1969) except for

Salmonella typhi, the etiologic agent of typhoid fever. It became a reportable disease in 1959. Edwards and Galton (1967) indicate a sharp rise in reported human cases from 1962 to 1964. Reported Salmonella isolations from animal sources also increased significantly from 1957 to 1965 which may have contributed to increased rates of typhoid fever and salmonellosis in man.

Weibel et al. (1964) found that from 1946 to 1960, there were 228 known outbreaks of waterborne diseases recorded at the National Office of Vital Statistics. Pelczar and Reid (1965) noted 198 outbreaks (7972 cases) in 1961 alone, but this may not be accurate because of the neglect of state laboratories, physicians, and other authorities to report their findings.

The reported number of cases of disease caused by Salmonella from the year 1963 to 1967 varied from 18,659 to 20,865 with the average being 20,078 reported cases annually (Cherubin et al., 1969). These numbers probably represent only one tenth of the actual number of cases. VanDonsel and Geldreich (1971) say that the baseline infection in the United States is 1%. The National Research Council, Committee on Salmonella (1969) also has some corroborative evidence that the number of cases is grossly underreported. In 1965, there was a waterborne outbreak of gastroeneritis in Riverside, California due to Sal. typhimurium. One hundred human

isolations with 200 cases of gastroeneritis were made known to the Public Health Department. However, epidemiological evidence of 16,000 cases was found (Aserkoff, Schroeder, and Brachman, 1970).

Aside from the suffering, Salmonella is responsible for high costs in medical care, hospitalization, and absence from work. There is also an immense cost in livestock and poultry due to the death of animals, poor milk and egg production, contamination of products, expensive measures of control and testing, and so on. The National Research Council estimates the loss to be around $300,000,000 per year.

According to the National Research Council, Salmonella is the most important communicable pathogen causing disease in the United States (Feigin, 1970). Members of this genus are causative agents for typhoid and paratyphoid fevers and salmonellosis. Typhoid has been brought under control in the United States, but infections caused by other members of the genus have not. Sal. typhimurium is responsible for most infections in man, fowl, and domestic animals. Only 12 serotypes of Salmonella account for 78% of the diagnosed infections with about 20% of the cases being due to Sal. typhimurium (Cherubin et al., 1969). Sal. typhimurium frequency in animals other than man is about 20%. All serotypes of Salmonella may be pathogenic for man, animals, or both.

The six most commonly isolated serotypes of Salmonella are: Sal. typhimurium and Sal. typhimurium var. copenhagen, 29.4% (5803/19723); Sal. heidelberg, 8.4% (1648/19723); Sal. enteritidis, 6.5% (1277/19723); Sal. newport, 6.4% (1263/19723); Sal. infantis, 5.0% (980/19723); and Sal. st. paul, 4.6% (907/19723). Out of all 50 states, Hawaii has the highest Salmonella rate with 85 isolations per 100,000 population, while New Mexico is second with 32 per 100,000. (Nat. Res. Coun., Committee on Salmonella, 1969)

The five most isolated serotypes in New Mexico are Sal. typhimurium, Sal. heidelberg, Sal. newport, Sal. infantis, and Sal. thompson, (Table 13). Four of these serotypes are among the six commonly most isolated in the U.S. The high incidence of isolates of Salmonella in New Mexico is due cause for concern. More information on distribution and occurrence of the organism is necessary. Hopefully, data collected for this thesis will be helpful.

LITERATURE REVIEW

A high incidence of Salmonella can be caused by a number of factors. Domestic and wild animals, birds, and reptiles may carry Salmonella for long periods of time during which their excreta may contaminate water supplies. VanDonsel and Geldreich (1971) indicate that Salmonella infections in animal populations are much higher than in humans. Milone and Watson (1970) present evidence suggesting that the main reservoirs of Salmonella are animals and subsequent transmission to man comes from infected animals or contaminated animal products. In 1956, Bruner stated that the main Salmonella reservoirs in the United States are fowl, swine, and man. Since fowl and other animals acting as reservoirs comprise most of the protein in diets in the United States, it is not surprising that salmonellosis in man often is traced to these animals. Reported frequency of Salmonella isolated from fowl indicates that domestic poultry is the largest single reservoir of these organisms among animals, especially chickens and their eggs and turkeys. Cold-blooded animals also harbor Salmonella. Insects as well as arthropods such as ticks may carry and excrete Salmonella for over two months (Edwards and Galton, 1967).

Salmonella, Shigella and Arizona are the primary genera causing gastroenteritis in domestic animals and man. Rodents and other animals and birds shedding Salmonella and Arizona are potential public health hazards because they may contaminate food and water. Arizona can cause disease of major proportions in turkey flocks (Greenfield and Bankier, 1969). In 1962, Lofton, Morrison, and Leiby noticed a high incidence of human diarrhea and intestinal disorders of unknown etiology in Colorado and decided to learn about the sources of the causative agents and their ecology. A total of 76.9% (453/589) of the small mammals collected yielded enteric bacteria. Six and three-tenths percent (8/127) of the birds and 2.5% (15/589) of the animals were positive for Salmonella. VanDonsel and Geldreich (1971) found 13% of the cattle, 3.5 to 15% of the sheep and 7 to 22% of the swine positive for Salmonella (authors did not state sample size).

The problems in examing water supplies for potentially disease producing organisms are many. These organisms may be sporadic in their entry of water supplies, do not survive long, may be present in small quantities, and are hard to find and isolate, (Pelczar and Reid, 1965). One can test the water directly for the presence of Salmonella, Shigella,

and Arizona but usually the water is examined for the presence of indicator organisms or coliforms. Coliforms include facultatively anaerobic, Gram-negative, non-spore forming rod-shaped bacteria which may ferment lactose with gas formation within 48 hours at 35°C. The genera include Klebsiella, Enterobacter, Serratia, Escherichia, Arizona, Edwardsiella, Citrobacter, and Providencia. Some coliforms are of fecal origin (from intestines of warm-blooded animals) and therefore designated as fecal coliforms (FC), to differentiate from coliforms of non-fecal origin (Standard Methods, 1971).

Fecal coliforms have been isolated from intestines and feces of almost every animal and bird, and are normal inhabitants of man's intestine and therefore his feces. If fecal coliforms are found in water, then it can be assumed that the water is polluted with fecal material along with pathogens which may be present in that material.

Dunlop, Twedt, and Wang (1951) state that a high coliform index is associated with the presence of Salmonella. In 1952, they found a high Salmonella incidence with high FC and fecal streptococcus (FS) counts.

Spino (1966) stated that Salmonella can be isolated from water with a coliform count as low as 2200 organisms/100 mls, and a fecal coliform count of 220 organisms/100 mls.

VanDonsel and Geldreich (1971) found there was frequent isolation of Salmonella only when there were 200 FC or more per 100 mls water and that the survival of Salmonella parallels the survival of fecal coliforms. This suggests that FC might be useful indicators of human pathogens.

Despite 50 years of research on bacterial methods for determining the sanitary quality of water, procedures are still imperfect. Isolation of coliforms is considered satisfactory, but these organisms are so widely distributed that they are found in water not contaminated, they may multiply in water causing inaccuracies in counts, and they can survive for long periods of time, thus, they may not be indicating recent contamination. Houston, in 1899, (Leininger and McCleskey, 1953) noticed the faults of using coliforms and suggested the use of fecal streptococci (FS) as indicators of fecal pollution.

In 1918, Savage and Wood studied the viability of FS in water. They found that the rate of loss of viability of FS paralleled the rate loss of viability of coliforms; however, the FS were less stable than the coliforms. They agreed that FS showed more recent contamination than FC and that a negative test did not necessarily mean there was no pollution, but that a positive test confirmed pollution.

Leininger and McCleskey (1953) studied waters from 6 different sites for one year and found that the difference between heavily polluted drainage canals and fairly clean water was shown more accurately by counts of FS and E. coli together than by the coliform group alone. The ability of enterococci to magnify the difference between clean and polluted water is seen by the coliform/enterococci ratio. Heavily polluted waters have lower ratios while clean water or water with small numbers of enterococci would have very large ratios.

Geldreich (1967) stated that an important use of FS was the relationship of the density of FC and FS in the same samples. The FS have been shown to be present in greater numbers than FC in feces from wild animals, dogs, and cats. In comparison, feces from man and domestic wastes have four times more FC than FS (Geldreich and Kenner, 1969). In human fecal material and domestic wastes, the FC densities are four times greater than the FS, while in livestock, poultry, rodents, and the like, the ratio is less than 0.6 to 0.7. This may be relative to the conditions prevailing in the water or the material sampled, but if the ratio is greater than 4.0, pollution is attributed to domestic wastes. The fecal ratio range from 0.7 to 4.0 has not been adequately investigated. These ratios may be designated as intermediates —

possibly due to old or atypical organisms (VanDonsel and Geldreich, 1971).

There is still disagreement as to whether FS multiply in water. Leininger and McCleskey (1953) experimentally found no increase in the number of coliforms or FS from zero to ten days of storage — only a steady decrease in number. These investigators as well as Slanetz, Bent, and Bartley (1955), Litsky, Mallman, and Fifield (1953) agree that FS are very good indicators of fecal pollution.

Water pollution is important industrially and bacteriologically. A number of investigators have been studying bacterial pollution and have found that few waters are untouched by it.

In 1967, Fair and Morrison tested supposedly high quality surface water from the Cache la Poudre River in Colorado. In one summer they isolated 11 Salmonella and 52 Arizona with a general coliform count of 30/100 ml showing that a mountain stream considered to be high-quality water may be a source of enteric infection. Hendricks and Morrison (1967) studied the aquatic environment associated with the same river and found that it maintained a bacterial population as well as supplied nutrients sufficient to initiate multiplication. They also tested water collected from different depths

and incubated it at different temperatures. Bottom sediment at 16°C was optimum for bacterial growth. No bacterial multiplication occurred at temperatures less than 10°C. They stated that only a small portion of the total bacterial growth occurs in free flowing water, but that extensive growth occurs in bottom sediment, concluding that enteric bacteria can grow in low nutrient, low temperature environments, and that effluent plant materials add enough nutrients to stimulate growth of the bacteria. Lack of recovery of Salmonella from a particular spot does not mean that there was none, but only that it may be on the bottom. Hendricks (1971a) points out the need for a more accurate kind of water sampling which would include sampling bottom sediment. He collected samples from river surface water and from mud and obtained higher recover yield of Salmonella from the sediment during one year. Ninety percent (177/195) of the Salmonella recovered was found in the bottom sediment.

Not only do Salmonella and other organisms persist indefinitely in soil on bottom of rivers, but they persist in dirt, farm soil, and other matter as well. Rudolfs et al. (1950) found that Salmonella (Eberthella) typhosa and other organisms lasted in soil from 24 hours to two years without recontamination, but generally less than 100 days.

Organisms do survive a long time in soils and may remain viable a long time on plants and other objects that come into contact with these organisms. Rudolfs et al. (1950) studied Salmonella on plants. They found that bacteria cannot penetrate plants, but can live on the outside or in decayed parts for seven to ten weeks which will allow these organisms to be eaten by consumers. Survival in soil also is important since stormwater and other runoff is a major intermittent source of bacterial pollution entering waterways (Geldreich, 1967).

Weibel et al. (1964) studied seven kinds of surface waters to see which was responsible for the largest number of disease outbreaks from 1946 to 1960. Untreated ground water caused the greatest number of waterborne disease outbreaks. Geldreich et al. (1968) collected stormwater from gutters of residential streets and suburban business districts, wooded hillsides, rural areas and rainfall. The streets of the residential and business areas and the hillside had a bacterial composition similar to that of runoff from farm fields. The average percent of FC in the 843 stormwater runoff samples was 8.6%, but it did reach 21.1% in the business district. The FC/FS ratios for their study of stormwaters were generally less than 0.7% so pollution most likely

was from warm-blooded animals other than man. VanDonsel and Geldreich (1971) agreed that stormwater runoff contained predominantly bacteria from nonhuman sources. This contamination may be a health hazard since Salmonella may be present. Claudon et al. (1971) examined urban runoff into a lake and found that it was a regular contributor of Salmonella.

Several techniques have been used to sample and process water. A number of investigators (Moore, 1948; Harvey and Price, 1968; Edwards and Galton, 1967; Morahan and Hawksworth, 1969; Vassiliades, Trichopoulos, Papadakis, 1970; and Claudon et al., 1971) agree that using the "Moore Swab" — a large gauze folded so that the water to be sampled flows through it while the organisms become enbedded — is the best technique.

Jameson (1969) used a similar technique. He poured his samples through a cotton plug and then cultured the plug. In 1970, Harvey and Price used modified Moore gauze swabs to monitor Salmonella from its source to human hosts. They showed that the patterns in slaughter houses in Cardiff, Wales can reflect national trends and the swabs are an easy and versatile way to detect organisms in sewers and other piped waters since they can be placed and left to collect organisms for several days. Since contamination in water is intermittent, taking

samples a few minutes apart, or using swabs is better than taking one large sample (Dunlop et al., 1951).

Wells, Morris and Brachman (1971) isolated Salmonella in milk using gauze swabs, a technique so sensitive that Salmonella was found when there was only one organism per liter. Swabs exposed to milk with a concentration of 100 organisms per liter and washed for two hours in noncontaminated milk still remained positive for Salmonella. The only problem Harvey and Price (1970) could foresee was looking for possible mulitple serotypes.

If, however, the water must be collected in a container or the swab must be transported some distance before any testing can be done, proper procedure for holding the sample must be followed. In 1933, Caldwell and Parr showed direct plating of water to be the best method, that any amount of time lapsing before examination was important, and that icing in general contributed to recovery of bacteria. They found very little recovery of organisms other than E. coli after 24 hours.

In 1946, Prescott et al. said that samples should be iced since bacterial multiplication as well as cell death would stop and samples would be more accurate. Cox and Claiborne (1949) found that refrigerated samples showed unchanged coliform numbers for most samples from 0 to 48 hours and sometimes for 96 hours, but that uniced samples fluctuated. In uniced samples, the

coliform number dropped after 24 hours and always by 48 hours.

The 13th edition of Standard Methods recommends icing samples during transportation if transportation is to be longer than one hour. All stream pollution samples should be held below 10°C with maximum transport time of six hours with processing within two hours. Samples over 30 hours old should never be used.

There really have been only two methods of testing water samples for bacterial pollution. The first is direct inoculation of the water into testing media. This is known as the Most Probable Number (MPN) technique. The second, probably more accurate method, is the use of the Millipore Filter (MF) technique designed by Goetz (1947). There are a number of advantages in using the MF. For example, large quantities of water can be tested at one time, media can be greatly varied with growth interrupted at any stage, plates are not invalid if colony number is less than 30 or more than 300, results can be preserved, time is saved, and it is convenient (Goetz, 1947; Kabler, 1951; Presnell et al., 1954; Jones, 1957).

Information from Microbial Analysis of Water (1969) suggests that the MF procedure be compared to MPN before being used on a routine basis, but that values for MPN

cannot be compared directly to MF counts since there is a 23% positive bias in MPN results and confidence limits for a five tube, three dilution test vary from 31% to 289% of true bacterial density of the sample (report did not state sample size). Presnell et al. (1954) used MPN and MF to examine sea water. They found 87.1% agreement between the two methods with more heavily polluted samples showing closer agreement.

Slanetz et al. (1956) found 20% (authors did not state sample size) of the MPN samples negative for enterococci, but the corresponding MF samples yielded 5 to 370 enterococci/100 mls. In 1960 ORSANCO (Ohio River Valley Water Sanitation Commission) undertook an evaluation of MPN and MF (Streeter and Robertson, 1960). They stated that MF was the best way properly to test water, but great attention must be paid to accurate testing procedures. Correct collection methods, proper media with correct sterilization, and so on was necessary (Streeter and Robertson, 1960, Edwards and Galton, 1967).

The importance of using the correct media for the isolation of these organisms cannot be overemphasized. Tetrathionate broth for the isolation of Salmonella has been recommended by a number of investigators. There are many modifications of the medium; each one has its own advantages and disadvantages. Some of the first

people to study tetrathonate broth were Knox, Gell, and Pollock (1943). They found that tetrathionate is reduced to thiosulfate by most Salmonella and Proteus and that Escherichia, Enterobacter, and a few Salmonella are not able to do this.

By 1948, the use of tetrathionate (Leiguarda et al., 1948) and later with iodine addition (Hajna and Damon, 1956) had been established for collecting sewer water samples.

Tetrathionate and selenite broths (Banwart and Ayres, 1953; Dunlop and Wang, 1953; McCoy, 1962; Bailey and Scott, 1966; Edwards and Galton, 1967; Jawetz et al., 1968; Greenfield and Bankier, 1969; Morahan and Hawksworth, 1969; Taylor and Schelhart, 1968; Edwards and Ewing, 1970; Palumbo and Alford, 1970; Wells et al., 1971) are the media of choice of most investigators.

The traditional temperature for incubating bacterial cultures has been 37°C since it is supposedly the optimum temperature for most bacteria. Earlier investigators (Leiguarda et al., 1948; Dunlop et al., 1951; Jameson, 1961; Wells et al., 1971) used an incubation temperature of 37°C to incubate their primary enrichment media as well as the secondary solid plating media. McCoy (1962) found temperatures higher than 37°C to be harmful in his preliminary experiments, so he did not use them in his

work. However, the majority of investigators recognize the usefulness of incubating suspected Salmonella contaminated water samples at higher temperatures. In 1953, Harvey and Thompson achieved better isolation of Salmonella from selenite with an elevated temperatures. They found 43°C optimal for 24-hour Salmonella enrichment. They proposed that a fixed concentration of a selective agent exerting a bacteriostatic influence on a competitor at 37°C may at a higher temperature be compatible with Salmonella multiplication and exert a bactericidal effect against other organisms. Spino (1966) noted that the London Water Board said that tetrathionate failed to grow Salmonella at 42°C and 43°C, but Salmonella would grow in selenite at 42 and 44°C. He collected water samples in tetrathionate and selenite brilliant green sulfa enrichment media and incubated them for 24 hours at 37 and 41.5°C. He found 41.5°C to be far superior to 37°C for isolating Salmonella. Carlson et al. (1967) tested temperatures of 10, 25, 37, 40, 43, 45, 47, and 49°C in selenite-brilliant green sylfapyridine selective enrichment media. They found better growth of Salmonella at 43°C with poor growth of Citrobacter, Proteus, and coliforms. Many media are not usable at 43°C, but can be altered to function at that temperature. Other investigators (Greenfield and Bankier, 1960; Ionescu, Ienisttea, Ionescu, 1969;

Geissler and Kosters, 1970, and Banffer, 1971) all recommended incubating the various enrichment media at 43°C to suppress the growth of other organisms. The length of time enrichment broth should be incubated before being subcultured onto various plating media to look for typical Salmonella growth is an important factor. Dunlop et al. (1952) recommended six to nine hours, but other investigators believe 18 to 24 hours or 24 to 48 hours (McCoy, 1962; Edel and Kampelmacher, 1969; Morris and Dunn, 1970) to be better. Leiguarda et al. (1948) and Jameson (1961) found that 24 and 72 hours worked well, while Carlson et al. (1967) found new serotypes coming into prevalence even up to 96 hours.

It has been established that the effectiveness of different enrichment broths can only be shown by results on plating media, so proper plating media are very necessary. Many researchers in enteric bacteriology recommend very strongly the use of three media, namely, brilliant green (BG), bismuth sulfite (BiS), and xylose-lysine desoxycholate (XLD). Virtually no recent researcher, in the literature covered, carried out an experiment in which he did not use at least one or more of these three media. Brilliant green seems to be most commonly used followed by bismuth sulfite. Edwards and Ewing (1970) felt that BiS and BG phenol red agars are the best to

use. They acknowledged that there are many other plating media available which give excellent results in the hands of those who invented them, but which have not come into general use. They feel that BiS is necessary for isolation of Sal. typhi or Arizona; however, to isolate the largest possible number of Salmonella serotypes, BG must be used. No single medium can be used for all purposes. XLD has not been available as long as the other two; and reports of its use have appeared in the literature since 1968 (Taylor and Schelhart, 1968).

Suspected colonies picked from secondary selective media plates must be further tested to confirm Salmonella or Arizona. Each group of investigators has their own group of tests. Biochemical characterization is followed by antigenic confirmation. Since most Salmonella are non-lactose fermenters, carbohydrate fermentation tests are generally run (lactose, sucrose, dulcitol, salicin, mannitol, xylose, and others). Taylor and Selliker (1958) showed a need for a much quicker identification — a carbohydrate that only Salmonella could ferment. They devised dulcitol-lactose iron agar and have had much success in using dulcitol as a primary test. Aside from sugars, triple sugar iron agar, urea, indole, lysine decarboxylase, potassium cyanide, and other tests are

necessary to confirm a Salmonella isolation (Edwards and Ewing, 1970).

The next step following biochemical confirmation, is to check the antigenic character of the isolate (Cheng et al., 1971). McCoy (1962) said that polyvalent "O" antiserum is not a good choice to use alone for identification since it agglutinates only 200 of the 700 possible serotypes. Polyvalent "H" serum has antibodies for almost all 700. He recommends further testing the isolate even if it does not agglutinate with the polyvalent sera since it may be a new serotype.

Edwards and Galton (1967) also used a combination of polyvalent "O" and "H" sera to confirm serologically the biochemical testing. They state there are over 900 different serotypes and new ones are being found. Lack of agglutination may occur when Salmonella are present, but agglutination with "O" or "H" is diagnostic (Feigin, 1970). The National Research Council, Committee on Salmonella (1969) say there are over 1300 serotypes of the genus Salmonella found virtually everywhere.

With such ubiquitous organisms as Salmonella, there are many problems in isolating and identifying these organisms contaminating our natural waters. It is also difficult to monitor to see if corrective measures taken are effective. Preventing salmonellosis

and other waterborne diseases is coming to primary importance with water supplies growing less abundant and more crowded conditions making every drop of water valuable.

MATERIALS AND METHODS

Water was sampled from four river sites, seven ditch locations, and 18 different pueblo sites between August 31, 1971 and November 14, 1972. Nineteen collections were made.

River and Ditch Sites

Below is a list of the river and ditch sites sampled. See Fig. 1-4 for sites and Table 1 for dates sampled.

A Cochiti East Side Main Canal eight miles from I-25 on highway to Cochiti Village.

1 Rio Grande at Cochiti Bridge.

NS Sili Main Canal on the road to Sili.

2 Rio Grande at bridge between Sili and Santo Domingo Village.

3 Lower East Side Santo Domingo Acequia on the road to Santo Domingo Village.

C Collection of standing water in a ditch between Sites 2 and 3.

SFD Cochiti East Side Main Canal a short distance from Highway I-25 on the road to San Felipe Village.

4 Rio Grande at San Felipe Bridge.

5 Angostura Arroyo.

6 Angostura-Algodones Diversion Dam.

B Bernalillo Acequia on the road from Bernalillo to Highway I-25.

Pueblo Sites

Collection of samples from wells and indoor water supplies was begun in Santo Domingo at the request of the Indians in the Community Action Program (CAP) office. After reporting the results of my findings in this investigation of the pueblo to the U.S. Public Health Service in Santa Fe, arrangements were made for me to sample Cochiti Village and San Felipe Village as well as more areas in Santo Domingo.

There were 18 sites sampled in these pueblos. All water samples were taken with permission of the Indians living or working at the collection site. See Fig. 5-7 for sites and Table 2 for dates sampled.

Cochiti Village Sites

CPH Cochiti Pump House. Spigot located on pump.

CTru Trujillo residence. Faucet on kitchen sink.

CCAP Cochiti Community Action Program office. Water was from the faucet in the men's room.

CR Rael's Grocery Store. Faucet in restroom.

CG Gallegos residence. Outdoor faucet by the back door.

CH Herrera residence. Faucet on kitchen sink.

Santo Domingo Village Sites

SDPH Santo Domingo Pump House. Spigot located on pump.

SDLR Sink faucet in the Ladies' Room in the Headstart Dining Hall.

SDK Faucet from sink in the Headstart Kitchen and Dining Hall used to serve the children's lunch.

SDsch Faucet in one of the classrooms in the Headstart Grade School.

SDAE Santo Domingo Adult Education Center. Water sampled was a mixture of the water from the sink in the restroom and from the drinking fountain.

San Felipe Village Sites

SFS Sandoval residence. Faucet on kitchen sink.

USPHC U.S.P.H. Clinic. Faucet in one of the examining rooms.

B.I.A. Sch B.I.A. Grade School. Faucet in classroom.

SFPHE San Felipe Pump House East. Spigot on pump.

SFPHS San Felipe Pump House South. Spigot located on pump.

SF251 Chavez residence. Outdoor faucet in yard.

SFD Demerio residence near SFPHE. Outdoor faucet by the front door.

Collection of Samples

The day before sampling was to take place, 100 ml of 10X Difco tetrathionate broth base (Hajna and Damon, 1956) was prepared (boiled) in a two-liter flask and placed in an ice chest at 4 to 5°C overnight. The corresponding iodine-potassium iodide solution also was prepared (5g I, 8g KI in enough water to equal 40 ml; multiplied by the number of samples to be collected). This was put into bottles and also stored overnight with the tetrathionate broth. The ice chests containing the flasks of tetrathionate broth were filled with ice on the day of sampling to keep the sample media cold until the time of inoculation.

The river and ditch samples were collected in a clean plastic bucket dropped into the water from a bridge or overhang at each site. The water in the bucket was hauled up and the temperature of the water was taken and recorded. Forty ml of the iodine solution were added to the 100 ml of the 10X tetrathionate broth and the solution was well mixed. The water in the bucket was stirred and 900 ml were added to the tetrathionate broth to equal one liter. This mixture was hand shaken for a minute (Smyser et al., 1970) and the

flask was put into the car to incubate at ambient temperature until return to the laboratory. Two small 150 ml sterile collecting bottles of each water sample also were collected from the bucket in order to determine FC and FS counts. These bottles were put in the ice chests to be kept cold until return to the lab (Cox and Claiborne, 1949; Caldwell and Parr, 1933; and Standard Methods, 1971). The bucket and graduated cylinder were rinsed out with one 10% Chlorox solution rinse and several sterile water rinses between each sample collected. All samples were collected between 0700 and 1700 hours.

The indoor water samples from faucets were collected in approximately the same way, except that the sterile bottles were inoculated ahead of time with 0.12 ml of 1% sodium thiosulfate (Standard Methods, 1971) and the water was measured directly into a graduated cylinder for the broth or directly into the bottles for FC and FS counts. When available, a butane burner was used to heat the faucet before collection to kill organisms living around the mouth of the faucet. Unfortunately, this was not always possible.

Salmonella and Arizona Isolation and Identification

The tetrathionate broths were incubated in the laboratory at 41°C (Spino, 1966; Smyser et al., 1970).

Samples were taken at 24 (Dunlop et al., 1952), 48 and 72 hours (Leiguarda et al., 1948). The flasks were shaken at the time of sampling and a portion was removed with a sterile Pasteur pipette. A drop was placed on SS, BG, and XLD agar plates (Taylor and Schelhart, 1968; Edwards and Ewing, 1970; Claudon et al., 1971; and Banffer, 1971). One quadrant of each plate was streaked with a sterile swab and the remainder of the plate was streaked for isolation with an inoculating loop. These plates were incubated at 37°C for 24 to 48 hours. Suspected Salmonella and Arizona colonies were picked with a sterile loop and restreaked for isolation on the media from which they were taken to insure pure cultures for biochemical testing. After a growth period of 24 hours, single colonies from the plates were picked and inoculated into triple sugar iron agar (TSI) (Edwards and Ewing, 1970). After 18 hours, alkaline slants with acid butts and acid slants with acid butts with or without gas and H_2S were further checked by inoculation into dulcitol (Taylor and Selliker, 1958) urea, malonate broth, lysine decarboxylase, and citrate (BBL, 1968; Diagnostic Microbiology, 1966; Edwards and Ewing, 1970). See Figure 8. With these media, Salmonella and Arizona (Fig. 9) could be identified biochemically at the 90% confidence level (Table 3).

If the results of the test were positive for Salmonella or Arizona, the organisms were inoculated onto trypticase soy agar (TSA) slants, incubated at 37°C for 24 hours. Suspected Salmonellas were tested with polyvalent "O" antisera by slide agglutination (Edwards and Ewing, 1971). Agglutinating Salmonella (and possible Arizona) were sent to the State Scientific Laboratory Service for group typing. Positive Salmonella was sent to the Center for Disease Control in Atlanta, Georgia for serotyping.

Determination of Fecal Coliforms

The samples collected in sterile bottles for FC enumeration were filtered upon returning to the lab. The amount of water filtered depended upon the turbidity of the water sample as well as the previous count of that sample. A preliminary count was made in August, 1971 to determine the amount of water that would give the best results before regular collecting was begun in September, 1971. The FC count for each site was obtained by filtering 10 ml water. If the counts were high for a particular site, the amount of water filtered was reduced. If the count was low, the amount of water filtered was increased.

One to 100 ml of water were filtered by negative pressure through a 0.45 micron HA Millipore filter.

The filter was then placed on a prefilter soaked with 2 ml of M-FC broth (BBL) in a 60 mm plastic petri dish (Microbial Analysis of Water, 1969; Geldreich et al., 1965). Care was taken to avoid bubbles between the filter and the prefilter (Kabler and Clark, 1952). The petri dishes were placed in a plastic bag which was placed in a plastic box. The box was submerged in a 44.5°C ± 1° water bath for 24 hours. At the end of this time, the numbers of blue colonies were counted using a Quebec Colony Counter and the numbers of organisms per 100 mls were determined.

Determination of Fecal Streptococci

One to 100 mls of water were filtered in the same way as for FC. The filter was then placed on M-enterococcus agar (BBL) and incubated at 37°C for 48 hours (Microbial Analysis of Water, 1969; Slanetz and Bartley, 1957). The red and pink colonies were counted using a Quebec Colony Counter and the numbers of organisms per 100 mls were determined.

With this information, the FC/FS ratios were computed for each sample at each collection. The reports for both river and ditch and pueblo samples were sent monthly to Santa Fe to the Environmental Protection Agency. The results of the samples also

were given to Mr. Ken Bailey of the U.S. Indian Field Health Service. In return, he supplied the state laboratory results for the past year and a half (results of pueblo water supply tests only) for comparison of state data and these results.

On several occasions, Mr. Marcus Coriz accompanied Ken Bailey and me to collect water samples. His samples were sent to the State Laboratory in Clovis, New Mexico. This provided three sets of data for some sites so comparisons could be made (Table 14).

Figure 1. Map of Survey Area from Cochiti Diversion to Santo Domingo Village

CD	Cochiti Diversion
CWSA	Cochiti West Side Acequia
CV	Cochiti Village
CESMC	Cochiti East Side Main Canal
CESA	Cochiti East Side Acequia
CPG	Cochiti Pueblo Grant
VF	Village Feeder
RG	Rio Grande
	Pena Blanca Lateral Canal #1
PBLC-2	Pena Blanca Lateral Canal #2
PBRDL	Pena Blanca Riverside Drain and Levee
SMC	Sili Main Canal
SA	Sili Acequia
UESSDA	Upper East Side Santo Domingo Acequia
UWSSDA	Upper West Side Santo Domingo Acequia
LESSDA	Lower East Side Santo Domingo Acequia
LWSSDA	Lower West Side Santo Domingo Acequia
SDRDWL	Santo Domingo Riverside Drain West and Levee
SDV	Santo Domingo Village
SDESL	Santo Domingo East Side Levee
SDPG	Santo Domingo Pueblo Grant
GL	Galisteo Levee
GA	Galisteo Arroyo
AL	Augustine Lateral
ILL	Island Levee and Lateral
PL	Pueblo Lateral
ML	Majada Lateral
MD	Majada Drain
RPL	Rock Point Lateral
SW	Seguro Wasteway
SF	Seguro Feeder
PaL	Paulita Lateral
RL	Rivera Lateral
TF	Trujillo Feeder

Legend:

Main Canal
Lateral
Acequia
Interior Drain
Riverside Drain and Levee

Sites:

A = Cochiti East Side Main Canal at Highway.
1 = Rio Grande at Cochiti Bridge.
NS = Sili Main Canal on the Road to Sili.
2 = Rio Grande at Sili Bridge.
3 = Lower East Side Santo Domingo Acequia.
C = Standing water in a ditch between sites 2 and 3.

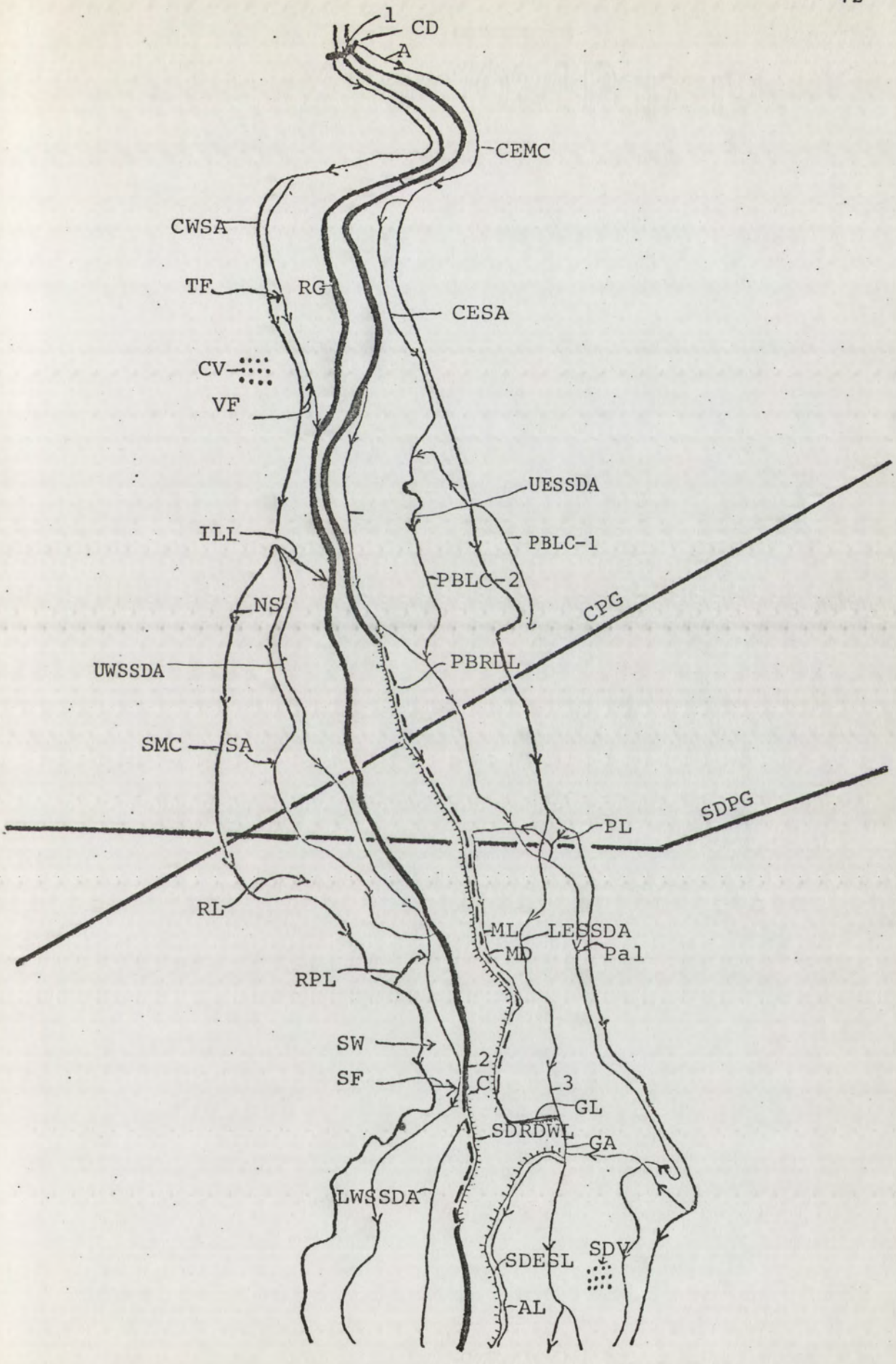

1
CD
A
CEMC
CWSA
TF
RG
CESA
CV
VF
UESSDA
ILI
PBLC-1
PBLC-2
NS
CPG
UWSSDA
PBRDL
SMC
SA
SDPG
PL
RL
ML
LESSDA
MD
Pal
RPL
SW
2
SF
C
3
GL
SDRDWL
GA
LWSSDA
SDESL
SDV
AL

Figure 2. Map of Survey Area from Santo Domingo Village to San Felipe Village

CESMC	Cochiti East Side Main Canal
LWSSDA	Lower West Side Santo Domingo Acequia
LESSDA	Lower East Side Santo Domingo Acequia
SDWSL	Santo Domingo West Side Levee
SDESL	Santo Domingo East Side Levee
SDD	Santo Domingo Drain
SDRDEL	Santo Domingo Riverside Drain East and Levee
SDPG	Santo Domingo Pueblo Grant
SRDCG	Santa Rosa De Cubero Grant
SMC	Sili Main Canal
SFESA	San Felipe East Side Acequia
SFPG	San Felipe Pueblo Grant
NWFA	N.W. San Felipe Acequia
SFV	San Felipe Village
SWSFA	S.W. San Felipe Acequia
ARDL	Algodones Riverside Drain and Levee
RG	Rio Grande
AL	Augustine Lateral
AlL	Alto Lateral
RL	Railroad Lateral
SL	Santiago Lateral
ML	Monica Lateral
EL	Elota Lateral
CL	Cubero Lateral
TL	Tongue Lateral
AnL	Angostura Lateral
AlgL	Algodones Lateral
VF	Valencia Feeder
BW	Borrego Wasteway

Legend:

Main Canal
Lateral
Acequia
Interior Drain
Riverside Drain and Levee

Sites:

SFD = Cochiti East Side Main Canal on the road to San Felipe.
4 = Rio Grande at San Felipe Bridge.

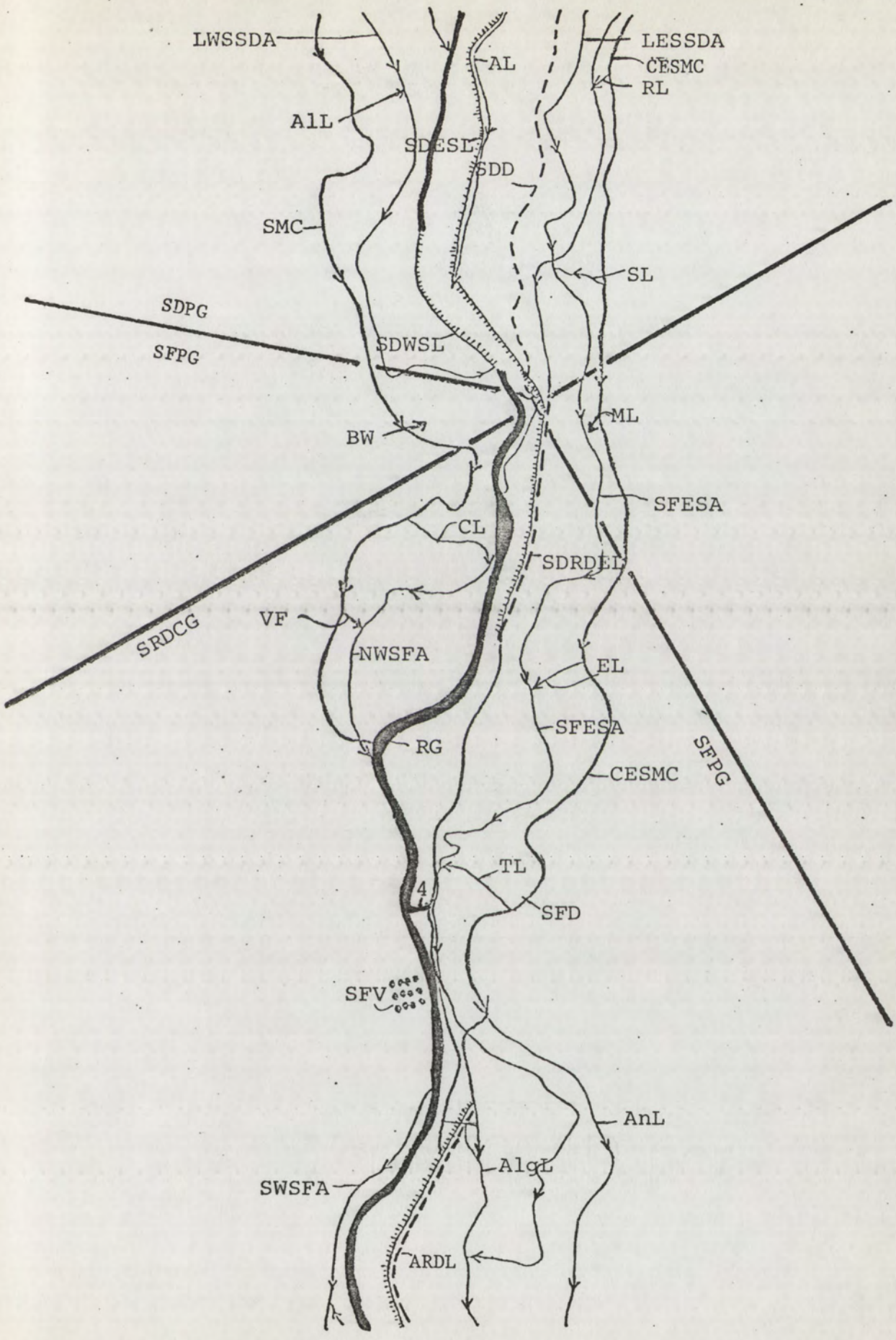
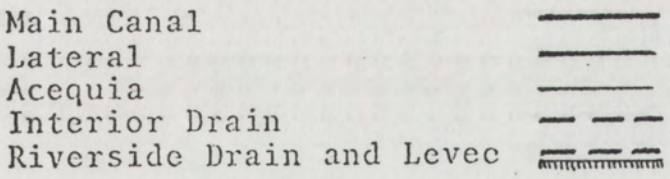

LWSSDA
AL
LESSDA
CESMC
RL
AlL
SDESL
SDD
SMC
SL
SDPG
SFPG
SDWSL
ML
BW
SFESA
CL
SDRDEL
VF
SRDCG
NWSFA
EL
SFESA
RG
SFPG
CESMC
TL
4
SFD
SFV
AnL
AlgL
SWSFA
ARDL

Figure 3. Map of Survey Area from San Felipe Village to Llanito

SWSFA	S.W. San Felipe Acequia.
SFPG	San Felipe Pueblo Grant.
SFRDL	San Felipe Riverside Drain and Levee.
SFC	San Felipe Canal.
A	Angostura.
AnA	Angostura Arroyo.
ARDL	Algodones Riverside Drain and Levee.
A1A	Algodones Acequia.
SAA	Santa Ana Acequia.
JR	Jemez River.
RG	Rio Grande.
SPG	Sandia Pueblo Grant.
AMC	Albuquerque Main Canal.
BRDL	Bernalillo Riverside Drain and Levee.
YL	Yeso Lateral.
YD	Yeso Drain.
TD	Turrieta Drain.
TA	Turrietta Arroyo.
LA	Lomita Arroyo.
L	Llanito.

Legend:



Sites:

5 = Angostura Arroyo.
6 = Angostura-Algodones Diversion Dam.

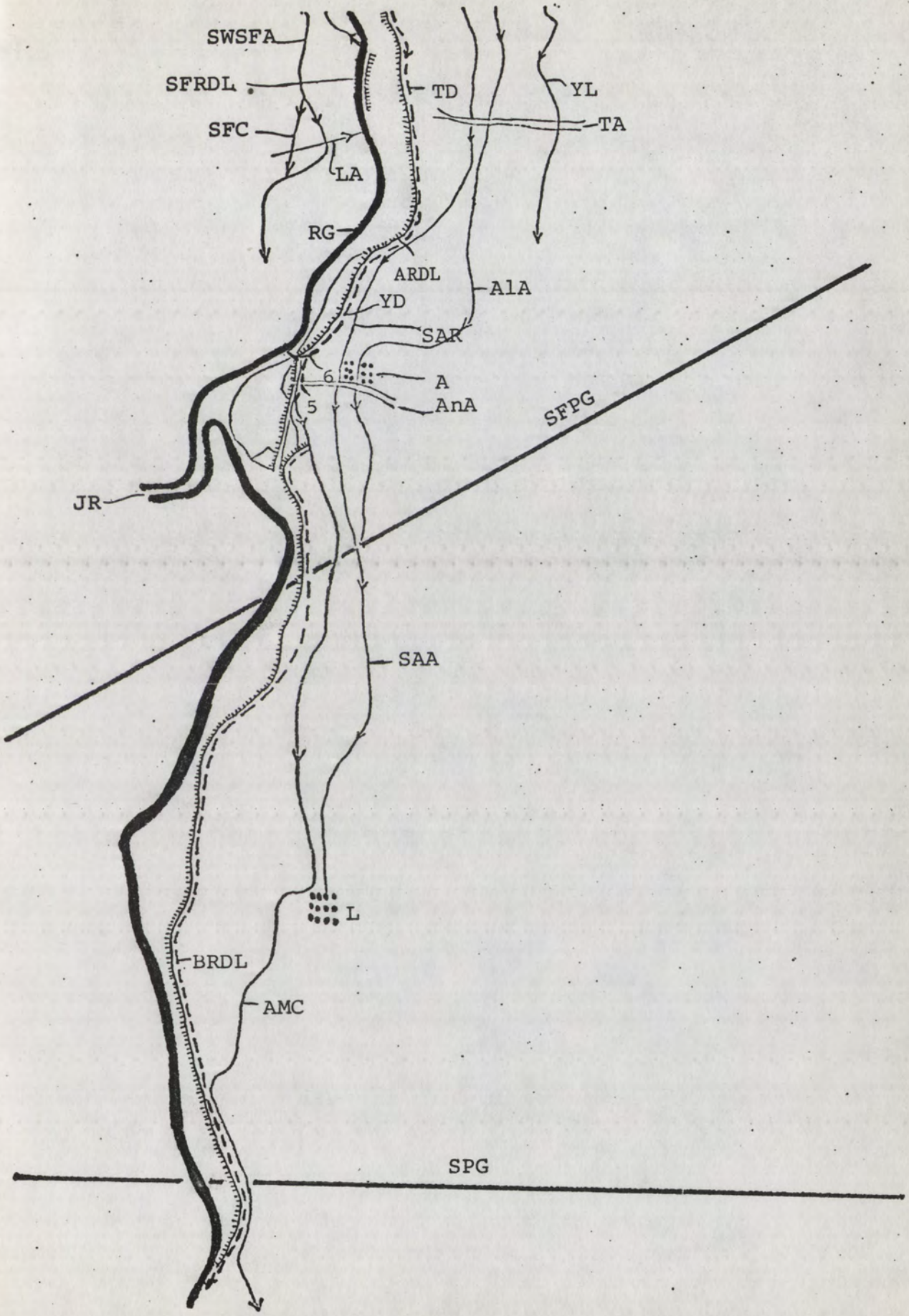

SWSFA
SFRDL
SFC
LA
RG
TD
YL
TA
ARDL
A1A
YD
SAR
6
A
5
AnA
SFPG
JR
SAA
L
BRDL
AMC
SPG

Figure 4. Map of Survey Area at Bernalillo

BA	Bernalillo Acequia
SA	Sandia Acequia
SAA	Santa Ana Acequia
AMC	Albuquerque Main Canal
BRD	Bernalillo Riverside Drain
BL1	Bosque Lateral No. 1
BL2	Bosque Lateral No. 2
BG	Bernalillo Grant
SFG	San Felipe Grant
SPG	Sandia Pueblo Grant
RG	Rio Grande
JR	Jemez River
Be	Bernalillo
S44	State 45
US 85	U.S. 85
A	Angostura

Legend:

Main Canal
Lateral
Acequia
Interior Drain
Riverside Drain and Levee

Site:

B = Bernalillo Acequia

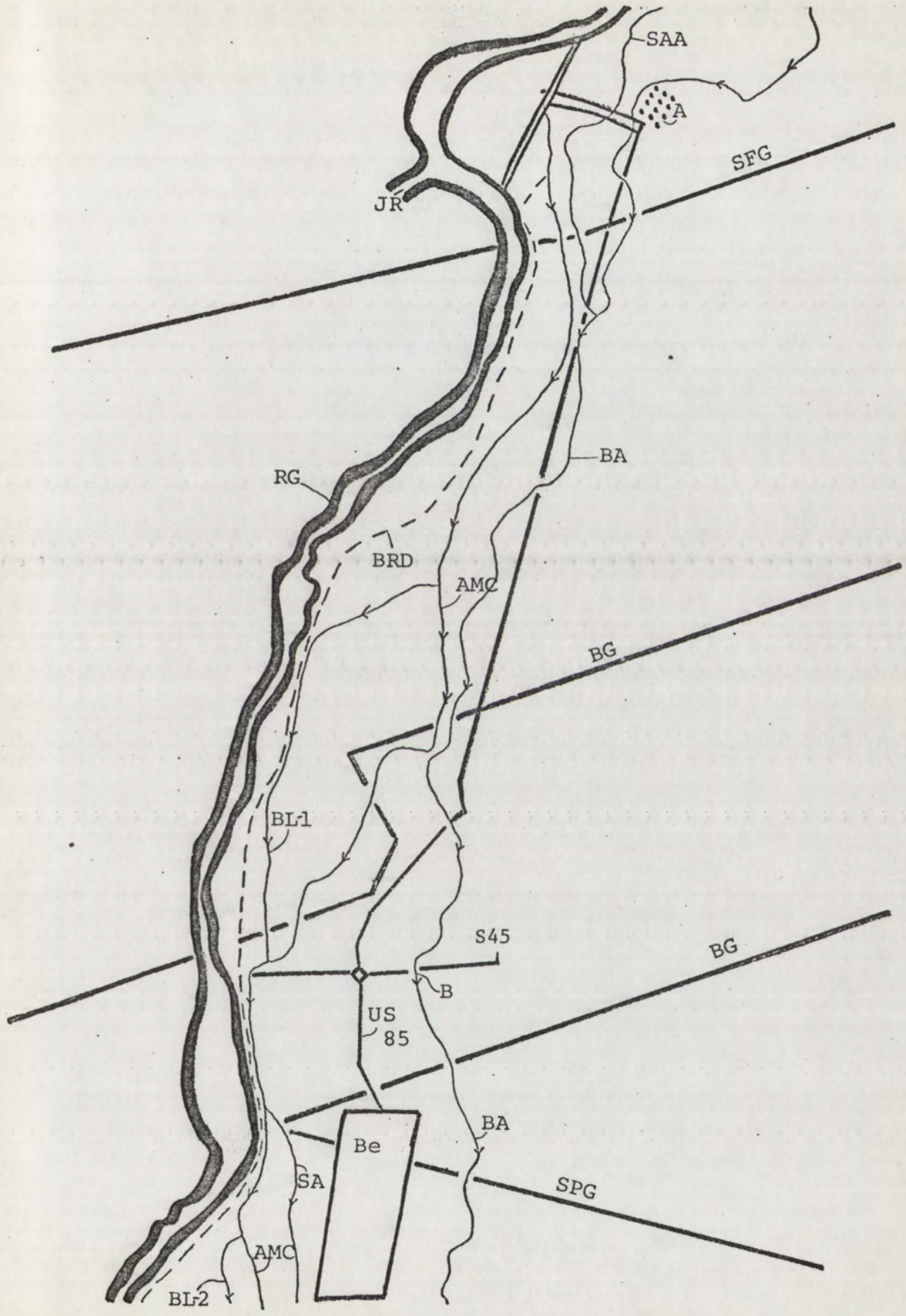

SAA
A
SFG
JR
BA
RG
BRD
AMC
BG
BL-1
S45
BG
B
US
85
BA
Be
SA
SPG
AMC
BL-2

Table 1. Sampling Dates of Rio Grande, Canal and Ditch Sites

Dates	Sites										
	A	1	NS	2	3	C	SFD	4	5	6	B
8-17-71		x		x	x			x	x	x	
8-31-71		x		x	x			x	x	x	
9-14-71		x		x	x			x	x	x	
9-28-71		x		x	x			x	x	x	
10-12-71		x		x	x			x	x	x	
11-9-71		x		x				x	x	x	
12-7-71		x		x				x	x	x	
1-17-72		x		x				x	x	x	
2-18-72		x		x				x	x	x	
3-10-72		x		x				x	x	x	
3-30-72	x	x		x	x	x		x	x	x	x
4-28-72	x	x		x	x	x		x	x	x	x
5-25-72	x	x		x	x			x	x	x	
6-23-72	x	x		x				x	x	x	
7-28-72	x	x	x	x	x			x	x	x	x
8-17-72	x	x	x	x	x		x	x	x	x	x
8-28-72	x	x	x	x	x			x	x	x	x
9-15-72		x	x	x				x			
11-14-72		x		x				x	x	x	

Legend. A = Cochiti East Side Main Canal, 1 = Rio Grande at Cochiti Bridge, NS = Sili Main Canal, 2 = Rio Grande at bridge between Sili and Santo Domingo Village, 3 = Lower East Side Santo Domingo Acequia, C = Standing water between 2 and 3, SFD = Cochiti East Side Main Canal on road to San Felipe, 4 = Rio Grande at San Felipe Bridge, 5 = Angostura Arroyo, 6 = Algodones-Angostura Diversion Dam, B = Bernalillo Acequia at Bernalillo. (Fig. 1-4). x indicates a collection was made.

Figure 5. Map of Sites in Cochiti Village

ST = Septic tank and tile field installations
1 = 100,000 gallon water tank
2 = New well
3 = Old windmill cross connecting into water system

Legend:

Road system
Old water system
New water system
Sewerage

Sites:

CPH = Cochiti Pump House.
CTru = Trujillo residence.
CCAP = Cochiti Community Action Program Office.
CR = Rael's Grocery Store.
CG = Gallegos residence.
CH = Herrera residence.

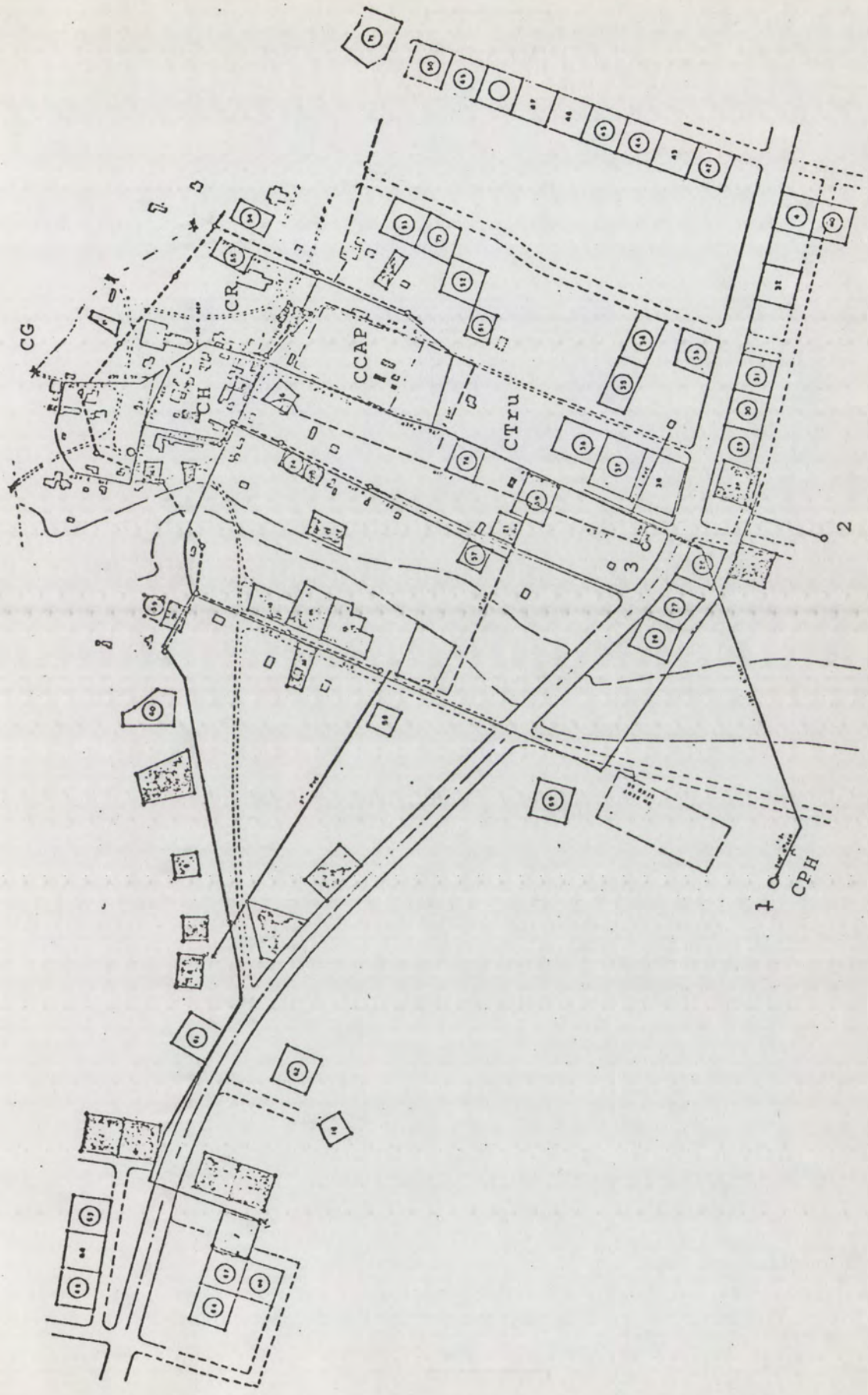

CG
CR
CCAP
CTru
CH
CPH
1
2
3

Figure 6. Map of Sites in Santo Domingo Village

1 Santo Domingo Community Action Program Office
2 200,000 gallon water tank
3 Old 26,000 gallon water tank (no longer in use)
4 To San Felipe
5 To Cochiti
6 New Pump House

Legend:

Road System
Railroad
Irrigation System

Sites:

SDPH = Santo Domingo Pump House.
SDLR = Ladies Room in Headstart Dining Hall.
SDK = Headstart Kitchen and Dining Hall.
SDS = Headstart Grade School.
SDAE = Santo Domingo Adult Education Center.

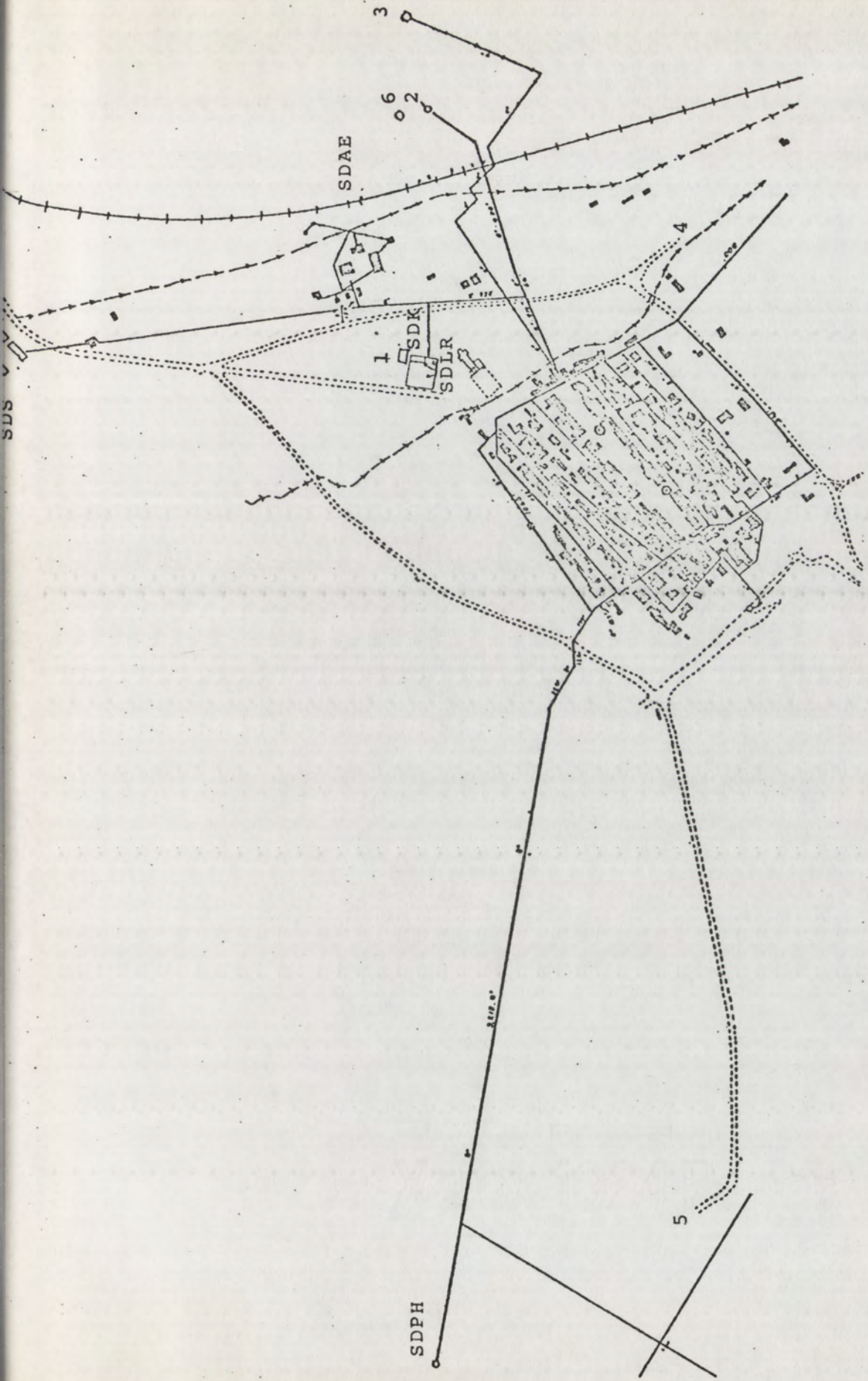
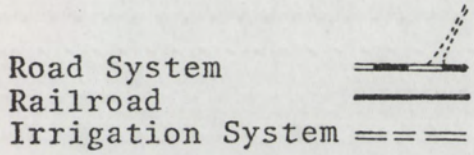

3
6
2
SDAE
4
1
SDK
SDLR
5
SDPH

Figure 7. Map of Sites in San Felipe Village

1 50,000 gallon steel water reservoir.
2 200,000 gallon steel water reservoir.
3 New Pump House, 1½ miles east of SFPHE

Legend:



Sites:

SFS = Sandoval residence.
USPHC = U.S.P.H. Clinic.
BIASch = B.I.A. Grade School.
SFPHE = San Felipe Pump House East.
SFPHS = San Felipe Pump House South.
SF251 = Chavez residence.
SFD = Demerio residence.
4 = Rio Grande at San Felipe Bridge

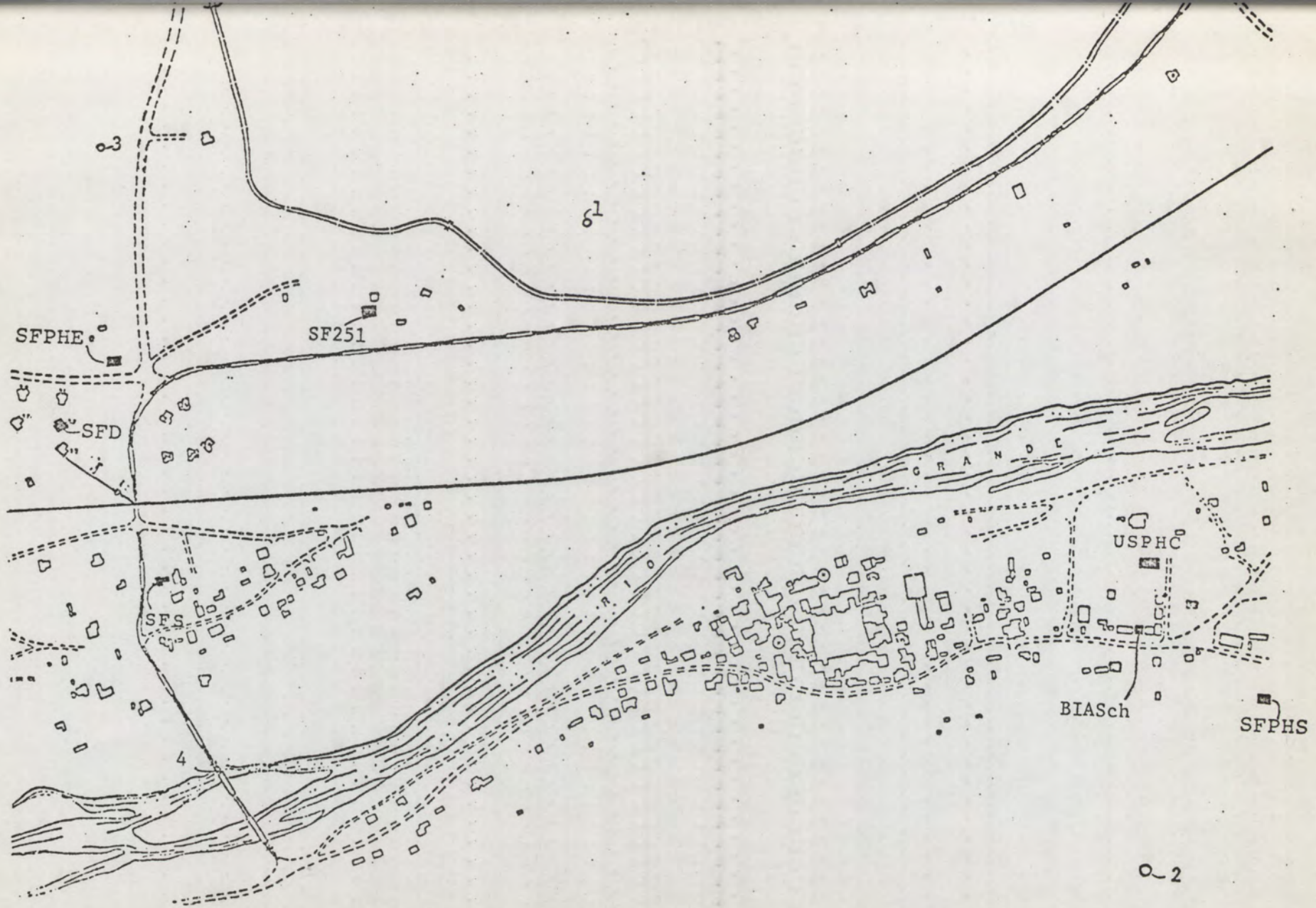

3
1
SFPHE
SF251
SFD
SFS
USPHC
BIASch
SFPHS
4
2

Table 2. Sampling Dates of Pueblo Sites

	Sites																	
	Cochiti Village						Santo Domingo Village					San Felipe Village						
Dates	CPH	CTru	CCAP	CR	CG	CH	SDPH	SDLR	SDK	SDS	SDAE	SFS	USPHC	BIA Sch	SFPHE	SFPHS	SF251	SFD
5-25-72							x	x										
6-23-72							x	x	x		x							
7-28-72								x	x									
8-17-72							x	x	x									
8-28-72	x	x					x	x	x	x		x	x	x				
9-13-72	x		x	x	x			x	x	x					x		x	x
11-14-72	x		x			x	x	x	x						x	x		

Legend. CPH = Cochiti Pump House, CTru = Trujillo residence, CCAP = Cochiti Community Action Program Office, CR = Rael's Grocery Store, CG = Gallegos residence, CH = Herrera residence. SDPH = Santo Domingo Pump House, SDLR = Ladies Room sink in Headstart Dining Hall, SDK = kitchen sink in Headstart Dining Hall, SDS = classroom faucet in Headstart Grade School, SDAE = Santo Domingo Adult Education Center, SFS = Sandoval residence, USPHC = U.S.P.H. Clinic, BIA Sch = B.I.A. Grade School, SFPHE = San Felipe Pump House East, SFPHS = San Felipe Pump House South, SF251 = Chavez residence, SFD = Demerio residence.

x indicates a collection was made.

FIGURE 8. Isolation and identification scheme for *Salmonella* from water samples. BG = brilliant green agar, XLD = xylose lysine desoxycholate agar, SS = *Salmonella*-*Shigella* agar, TSI = triple sugar iron agar, K/K = alkaline slant/alkaline butt, K/A = alkaline slant/acid butt, A/A = acid slant/acid butt.

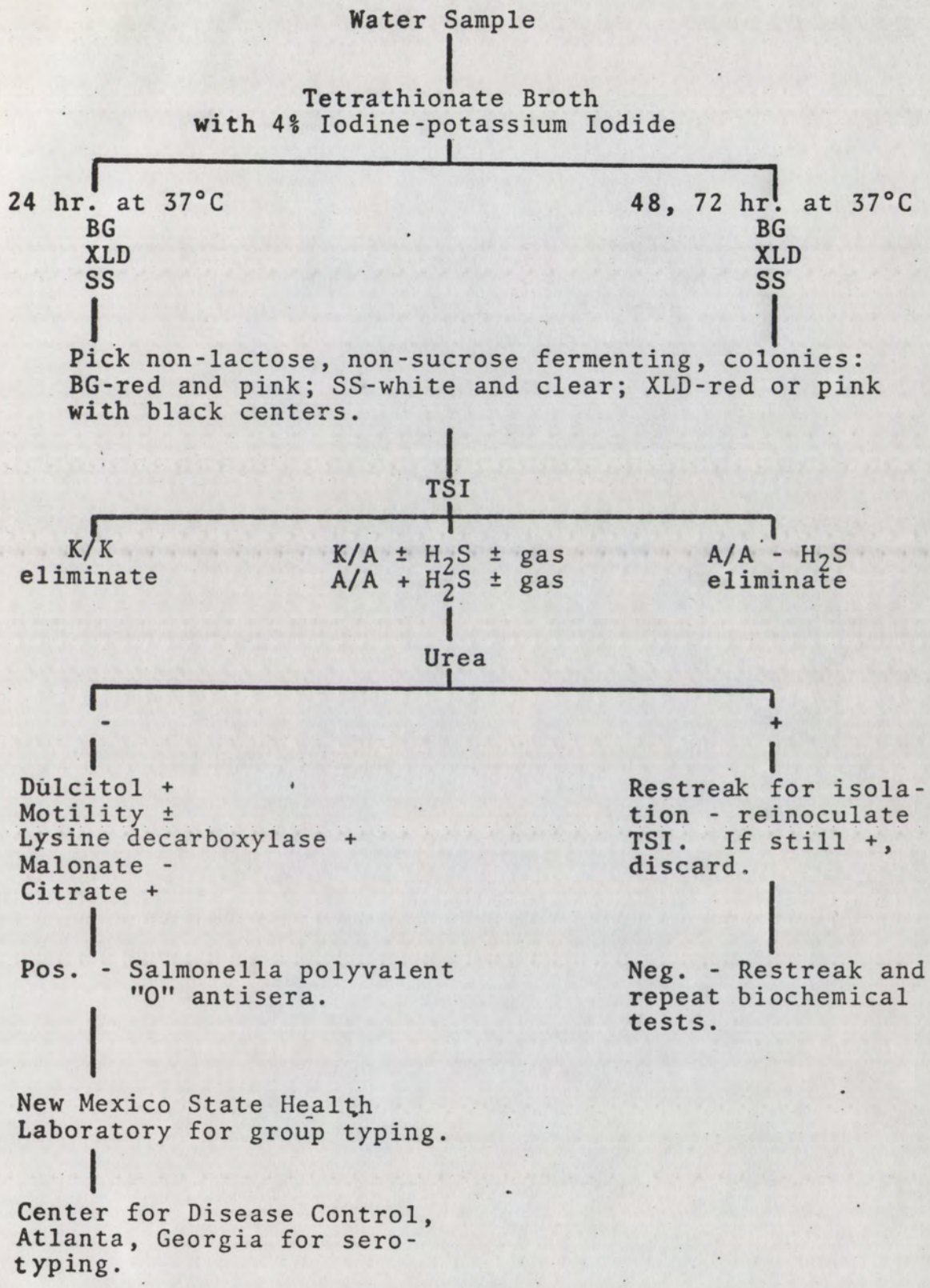

Water Sample
Tetrathionate Broth
with 4% Iodine-potassium Iodide
24 hr. at 37°C
BG
XLD
SS
48, 72 hr. at 37°C
BG
XLD
SS
Pick non-lactose, non-sucrose fermenting, colonies:
BG-red and pink; SS-white and clear; XLD-red or pink
with black centers.
TSI
K/K
eliminate
K/A ± H2S ± gas
A/A + H2S ± gas
A/A - H2S
eliminate
Urea
-
+
Dulcitol +
Motility ±
Lysine decarboxylase +
Malonate -
Citrate +
Restreak for isola-
tion - reinoculate
TSI. If still +,
discard.
Pos. - Salmonella polyvalent
"O" antisera.
Neg. - Restreak and
repeat biochemical
tests.
New Mexico State Health
Laboratory for group typing.
Center for Disease Control,
Atlanta, Georgia for sero-
typing.

FIGURE 9. Isolation and identification scheme for *Arizona* from water samples. BG = brilliant green agar, XLD = xylose lysine desoxycholate agar, SS = *Salmonella*-*Shigella* agar, TSI = triple sugar iron agar, K/K = alkaline slant/alkaline butt, K/A = alkaline slant/acid butt, A/A = acid slant/acid butt.

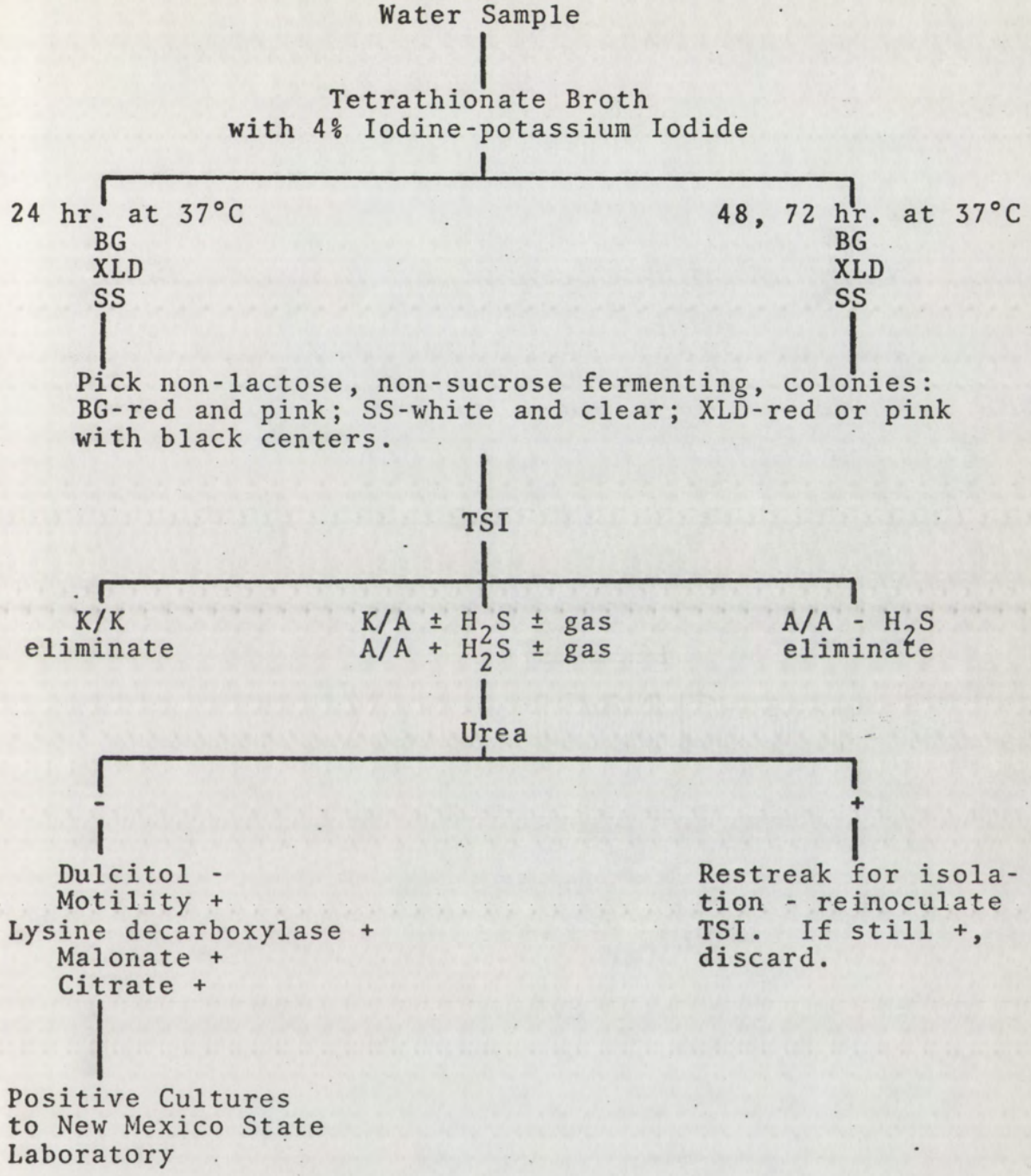

Water Sample
Tetrathionate Broth
with 4% Iodine-potassium Iodide
24 hr. at 37°C
BG
XLD
SS
48, 72 hr. at 37°C
BG
XLD
SS
Pick non-lactose, non-sucrose fermenting, colonies:
BG-red and pink; SS-white and clear; XLD-red or pink
with black centers.
TSI
K/K
eliminate
K/A ± H2S ± gas
A/A + H2S ± gas
A/A - H2S
eliminate
Urea
-
+
Dulcitol -
Motility +
Lysine decarboxylase +
Malonate +
Citrate +
Positive Cultures
to New Mexico State
Laboratory
Restreak for isola-
tion - reinoculate
TSI. If still +,
discard.

Table 3. Summary of Biochemical Tests of a Selected Group of Enteric Bacteria (from Edwards and Ewing, 1972). (continued)

Organism	Confidence Level	Mot	U	Cit	H_2S	LD	Mal	Dul	Glu	Lac	Suc
Salmonella enteritidis	99	V	-	V	V	V	-	V	+	-	-
	95	V	-	V	V	+	-	+	+	-	-
	90	+	-	V	+	+	-	+	+	-	-
Salmonella typhi	99	+	-	-	V	+	-	V	+	-	-
	95	+	-	-	V	+	-	V	+	-	-
	90	+	-	-	+	+	-	V	+	-	-
Salmonella choleraesuis	99	+	-	-	V	V	-	V	+	-	-
	95	+	-	-	V	V	-	-	+	-	-
	90	+	-	-	V	+	-	-	+	-	-
Arizona hinshawii	99	+	-	V	V	+	V	-	+	V	V
	95	+	-	+	+	+	V	-	+	V	-
	90	+	-	+	+	+	+	-	+	V	-
Citrobacter freundii	99	V	V	V	V	-	V	V	+	V	V
	95	+	V	V	V	-	V	V	+	V	V
	90	+	V	+	V	-	V	V	+	V	V
Klebsiella pneumonia	99	-	V	V	-	V	V	V	+	V	+
	95	-	V	+	-	+	+	V	+	+	+
	90	-	+	+	-	+	+	V	+	+	+
Klebsiella ozaenae	99	-	V	V	-	V	V	-	+	V	V
	95	-	V	V	-	V	-	-	+	V	V
	90	-	-	V	-	V	-	-	+	V	V
Klebsiella rhinoschleromatis	99	-	-	-	-	-	V	-	+	-	d
	95	-	-	-	-	-	+	-	+	-	d
	90	-	-	-	-	-	+	-	+	-	d

Table 3. Summary of Biochemical Tests of a Selected Group of Enteric Bacteria (from Edwards and Ewing, 1972) (continued)

Organism	Confidence Level	Mot	U	Cit	H_2S	LD	Mal	Dul	Glu	Lac	Suc
	99	V	V	+	-	-	V	V	+	V	V
Enterobacter cloacae	95	V	V	+	-	-	V	V	+	V	+
	90	V	V	+	-	-	V	V	+	V	+
	99	V	V	V	-	V	V	V	+	V	+
Enterobacter aerogenes	95	+	-	V	-	+	V	-	+	V	+
	90	+	-	+	-	+	V	-	+	+	+
	99	V	V	-	-	+	-	-	+	-	V
Enterobacter hafniae	95	V	-	-	-	+	-	-	+	-	V
	90	+	-	-	-	+	-	-	+	-	V
	99	V	V	V	-	V	V	-	+	V	+
Enterobacter liquefaciens	95	V	-	V	-	V	V	-	+	-	+
	90	V	-	+	-	V	-	-	+	-	+
	99	V	V	V	-	+	V	-	+	V	+
Serratia marcescens	95	+	V	+	-	+	-	-	+	-	+
	90	+	V	+	-	+	-	-	+	-	+
	99	V	V	V	-	-	V	-	V	V	V
Pectobacterium sp.	95	V	V	V	-	-	V	-	V	V	V
	90	V	V	V	-	-	V	-	V	V	V
	99	V	V	V	V	-	-	-	+	-	V
Proteus vulgaris	95	V	V	V	V	-	-	-	+	-	V
	90	+	+	V	+	-	-	-	+	-	+
	99	V	V	V	V	-	V	-	+	V	V
Proteus mirabilis	95	+	V	V	V	-	-	-	+	-	V
	90	+	V	V	+	-	-	-	+	-	V

Table 3. Summary of Biochemical Tests of a Selected Group of Enteric Bacteria (from Edwards and Ewing, 1972)

Organism	Confidence Level	Mot	U	Cit	H_2S	LD	Mal	Dul	Glu	Lac	Suc
	99	V	V	-	-	-	V	-	V	-	-
Proteus morganii	95	V	+	-	-	-	-	-	+	-	-
	90	V	+	-	-	-	-	-	+	-	-
	99	V	+	V	-	-	V	-	+	V	V
Proteus rettgeri	95	V	+	+	-	-	-	-	+	V	V
	90	+	+	+	-	-	-	-	+	-	V
	99	V	-	V	-	-	-	-	+	-	V
Providence alcalifaciens	95	+	-	+	-	-	-	-	+	-	V
	90	+	-	+	-	-	-	-	+	-	V
	99	V	-	V	-	-	V	-	+	V	V
Providence stuartii	95	V	-	+	-	-	-	-	+	-	V
	90	V	-	+	-	-	-	-	+	-	V
	99	V	-	-	-	V	-	V	+	V	V
Escherichia coli	95	V	-	-	-	V	-	V	+	V	V
	90	V	-	-	-	V	-	V	+	+	V
	99	-	-	-	-	-	-	V	+	-	V
Shigella sp.	95	-	-	-	-	-	-	V	+	-	V
	90	-	-	-	-	-	-	+	+	-	V
	99	V	-	-	+	+	-	-	+	-	-
Edwardsiella tarda	95	+	-	-	+	+	-	-	+	-	-
	90	+	-	-	+	+	-	-	+	-	-

Legend. Mot = motility, U = urease production, Cit = growth on Simmon's Citrate, H_2S = hydrogen sulfide production, LD = lysine decarboxylase, Mal = malonate utilization, Dul = acid ± gas from dulcitol, Glu = acid ± gas from glucose, Lac = acid ± gas from lactose, Suc = acid ± gas from sucrose. + = positive, V = variable at that confidence limit, - = negative, d = delayed reaction.

RESULTS

The region from Cochiti Dam to Bernalillo is hilly and sandy with low bushes and desert plants covering it. There are many cattle roaming freely and in the areas immediately adjacent to the pueblos, there are fields where corn, chilies, and other crops are grown. There are quite a few drainage ditches, irrigation ditches, and canals weaving in and out of the entire vicinity and, of course, the Rio Grande runs southward through the whole sampling area. Many of these ditches drain into the river and cattle, horses, dogs, and other animals have easy access to the river and ditches.

Temperature

The average temperatures of the river and ditch sites varied from 2.5°C on December 7, 1971 to 24°C in August 28, 1972 (Figure 11). The average temperature at which *Salmonella* was isolated was 22.9°C (Table 4).

Fecal Coliforms and Fecal Streptococci

River and ditch FC varied from zero (several dates) to 735,000 (September 15, 1972 (Tables 5 and 6). The highest counts occurred in the warmest months of July, August and September. *Salmonella* and *Arizona* were isolated with FC counts ranging

from 12,300 to 400,000/100 ml of sample of water (Tables 5 and 6).

River and ditch FS varied from zero (several dates) to 26,000 (August 23, 1972) (Tables 7 and 8). The highest FS counts occurred in July, August, and September. Salmonella and Arizona were isolated when counts ranged from 684 to 11,600.

The FC/FS was computed where possible and ranged from zero to 1144.7! No Salmonella or Arizona were isolated at a ratio of less than 9.7 (Table 9). Five serotypes of Salmonella were isolated at 4 sites and 2 Arizona were isolated from two sites (Table 4).

Cochiti Village

Working with state personnel made it possible to get into some of the other pueblos and do some collecting. The first samples of Cochiti Village were taken on August 28, 1972 from the Cochiti pump house and the kitchen sink of the Trujillo residence. The pump house water had low counts, but the Trujillo household water had 282 fecal coliforms per 100 ml. Two weeks later, the pump house, the Cochiti Community Action Program office men's room sink, Rael's Grocery Store, and the Gallegos residence water were sampled. All four of these water samples had an unacceptably high FC count (Table 10). The last collection was done on November 14,

1972. Only the pump house and the Herrera residence water were sampled. They still had high FC counts. No FS were isolated.

The water system of the Cochiti Pueblo was chlorinated for two months (October-December, 1972) until the pump burned out. The system was flushed, sediment cleaned out, chlorinated and flushed again.

An old windmill cross connected onto the old water system (Figure 5). It was found to be contaminated. The valve was shut off by state authorities and the line was removed. As of January 4, 1973, the water still was contaminated. The pump house water count was too numerous to count (TNTC), the men's room water at the CAP was 10/100 ml, and the Herrera residence was 37/100 ml.

Santo Domingo Village

Sampling was begun May 25, 1972 at the request of the Indians. They asked that I sample the Headstart School kitchen and restrooms, classrooms, and other places, but only enough sampling media were prepared for one site so the water pump in the pump house was chosen for sampling. The result was 54 fecal coliforms/100 mls water which is considerably over the state allowable limit (Table 10). One month later the Headstart Dining

Hall kitchen and ladies' room, the pump house, and Adult Education Center water fountain were sampled. The latter two sites showed no coliform count, but the ladies' room and the kitchen sink both showed 126 FC/100 mls. At this point, state authorities were contacted. Mr. Ken Bailey, the Indian Field Health representative offered to make collections with me so he could see for himself and then make comparisons between the two sets of data. Samples were collected until September 16, 1972 at which time the counts were still high. The last sampling was done on November 14, 1972, at which time all counts were zero. I should add that in FC testing, there were large numbers of "non-fecal" bacteria present. FS were isolated only once on July 28, 1972 in the ladies' room of the Headstart Dining area (Table 11).

As a result of this testing, some changes were made in the water system of the Pueblo. The Indians had been complaining of the terrible taste of their drinking water — some even went so far as to haul water from Santa Fe. The water tank (200,000 gallon) was checked and found to have iron and magnesium deposits 18 inches deep on the bottom. It was supposed to have been flushed yearly, but this flushing had not been done for four years. Plans have been presented for a new well near the larger tank site (Figure 6) and for some changes in

the water distribution system in the pueblo since Santo Domingo is expanding and present water systems will be inadequate (Starr, 1972).

San Felipe Village

Sampling of San Felipe Village was begun on August 28, 1972. The sites sampled were the BIA Grade School, U.S.P.H. Clinic, and the Sandoval residence. The water from the school and clinic had low counts — 3 to 4/100 ml, but the water from the Sandoval house site had nearly one-half million organisms/100 ml. The house had layers of dead flies lying over every possible spare surface in the kitchen and food preparation area had quite a few live ones, too. On September 15, the East pump house and the Demerio residence, which are close together, were sampled. Both had high counts (Figure 7). The Chavez residence had zero organisms. The last collection was made on November 14, 1972, from the East and South pump houses. The counts were zero.

Table 4. Isolation of *Salmonella* and *Arizona*, FC and FS numbers with FC/FS ratios, and water temperature on date of isolation

Date	Site	Arizona or *Salmonella*	FC	FS	FC/FS	Water Temperature
7-28-72	1	*Sal. carrau*	12,300	796	15.5	23°C
	NS	*Sal. newport*	<150,000	1,056	142.1	23°C
	B	*Sal. belem*	<150,000	684	219.3	26°C
8-17-72	B	*Sal. muenchen*	25,000	2,160	11.6	23°C
8-28-72	A	*A. hinshawii*	55,500	1,850	30.0	22°C
	4	*Sal. bredney*	100,000	10,300	9.7	27°C
	B	*Sal. belem* *A. hinshawii*	400,000 400,000	11,600 11,600	34.5 34.5	27°C 27°C

Legend. 1 = Rio Grande at Cochiti Bridge, NS = Sili Main Canal, B = Bernalillo Acequia, A = Cochiti East Side Main Canal, 4 = Rio Grande at San Felipe Bridge.

Table 5. Fecal Coliform Counts of Rio Grande Sites

Dates	Sites				
	1	2	4	6	Average
8-17-71	720	180	20	0	230
8-31-71	1,420	920	1,240	900	1,120
9-14-71	0	0	0	0	0
9-28-71	1,393	40	390	0	456
10-12-71	2,512	4	56	4	644
11- 9-71	2,152	22	206	0	595
12- 7-71	1,428	0	2	0	358
1-17-72	778	2	1	0	195
2-18-72	1,322	272	14	0	402
3-10-72	582	610	526	0	430
3-30-72	0	376	116	38	132
4-28-72	472	110	0	58	160
5-25-72	110	44	TNTC	354	169**
6-23-72	410	472	652	472	502
7-28-72	12,300*	87,000	2,775	5,670	26,936
8-17-72	3,000	3,000	7,210	31,500	11,172
8-28-72	56,000	38,000	100,000*	130,000	81,000
9-15-72	42,000	735,000	52,500		276,500
11-14-72	6,000	6,000	5,740	0	4,438

Legend. 1 = Rio Grande at Cochiti Bridge, 2 = Rio Grande at Sili Bridge, 4 = Rio Grande at San Felipe Bridge, 6 = Algodones-Angostura Diversion Dam, * = Salmonella or Arizona isolated, a blank space indicates no collection was made. All numbers represent numbers of organisms/100 mls, ** = average computed without site 4 for that date.
On 9-14-71, counts of 0 may be due to water bath temperatures of 51°C instead of 44.5°C.

Table 6. Fecal Coliform Count of Canals and Ditch Sites

Date	Sites A	NS	C	3	SFD	5	B	Average
8-17-71				0		0		0
8-31-71				1450		1060		1255
9-14-71				0		0		0
9-28-71				420		3		212
10-12-71				12		4		8
11-9-71						0		0
12-7-71						0		0
1-17-72						0		0
2-18-72						2		2
3-10-72						0		0
3-30-72	682		0	154		38	60	187
4-28-72	168		2	112		50	88	84
5-25-72	TNTC			104		378		241**
6-23-72	0					420		210
7-28-72	15,750	<150,000*		1225		<150,000	<150,000*	93,395
8-17-72	10,000	1500		1590	2970	10,560	25,000*	8603
8-28-72	55,500*	38,000		16,000		190,000	400,000*	139,800
9-15-72		4200						4200
11-14-72						0		

Legend. A = Cochiti East Side Main Canal
C = Collection of Standing Water
SFD = Cochiti East Side Main Canal on road to San Felipe
B = Bernalillo Acequia at Bernalillo
NS = Sili Main Canal
3 = Lower East Side Santo Domingo Acequia
5 = Angostura Arroyo
* = Salmonella or Arizona Isolated
** = average computed without site A for that date

A blank space indicates no collection was made. All numbers represent numbers of organisms/100 mls.

TNTC = too numerous to count

On 9-14-71, counts of 0 may be due to water bath temperatures of 51°C instead of 44.5°C.

Table 7. Fecal Streptococal Counts of Rio Grande Sites

Dates	Sites				
	1	2	4	6	Average
8-17-71	35	69	60	0	41
8-31-71	1,500	2,030	2,200	1,860	1,898
9-14-71	200	0	0	376	149
9-28-71	423	TNTC	420	13	285
10-12-71	1,376	844	952	52	806
11- 9-71	424	0	288	0	178
12- 7-71	244	0	0	2	62
1-17-72	156	0	0	2	39
2-28-72	286	116	36	0	109
3-10-72	74	92	112	0	70
3-30-72	0	110	56	14	45
4-28-72	200	54	8	80	86
5-25-72	0	0	1	0	0
6-23-72	382	382	334	340	360
7-28-72	796*	76	36	30	235
8-17-72	220	550	3,830	9,450	3,512
8-28-72	2,180	8,000	10,300*	26,100	29,145
9-15-72	11,000	29,000	27,000		22,333
11-14-72	300	326	260	0	222

Legend. 1 = Rio Grande at Cochiti Bridge, 2 = Rio Grande at Sili Bridge, 4 = Rio Grande at San Felipe Bridge, 6 = Angostura-Algodones Diversion Dam, * = Salmonella or Arizona isolation, a blank space indicates no collection was made. All numbers represent numbers of organisms/100 mls, TNTC = too numerous to count.

Table 8. Fecal Streptococcus Counts of Canal and Ditch Sites

Date	Sites A	NS	C	3	SFD	5	B	Ave.
8-17-71				0		0		0
8-31-71				2040		1500		1770
9-14-71				0		0		0
9-28-71				TNTC		0		
10-12-71				180		0		90
11-9-71						0		0
12- -71						0		0
1-1 -72						0		0
2-18-72						12		12
3-10-72						0		0
3-30-72	60		18	50		20	52	40
4-28-72	162		18	154		104	126	114
5-25-72	136			0		0		45
6-23-72	0					348		174
7-28-72	814	1056*		36		2552	684*	1028
8-17-72	280	230		485	380	8240	2160*	1963
8-28-72	1850*	1080		3920		13,200	11,600*	6330
9-15-72		0						0
11-14-72						0		0

A = Cochiti East Side Main Canal, NS = Sili Main Canal, C = Collection of Standing water, 3 = Lower East Side Santo Domingo Acequia, SFD = Cochiti East Side Main Canal on road to San Felipe, 5 = Angostura Arroyo, B = Bernalillo Acequia, * = Salmonella or Arizona isolation, a blank space indicates no collection was made. All numbers represent numbers of organisms/100 mls. TNTC = too numerous to count.

Table 9. FC/FS Ratios for River and Ditch Sites

Date	Sites A	1	NS	2	3	4	SFD	5	6	B
8-17-71		20.6		2.6		0.3			0	
8-31-71		0.9		0.5	0.7	0.6		0.7	0.5	
9-14-71				0						
9-28-71		3.3				0.9				
10-12-71		1.8		.005	0.007	0.1			0.1	
11- 9-71		5.1				0.7			0	
12- 7-71		5.9		0						
1-17-72		5.0								
2-18-72		4.9		2.4		0.4	0.17		0	
3-10-72		7.9		6.6		4.7			0	
3-30-72	11.4	0		3.4	3.1	2.1	1.9		2.7	1.2
4-28-72	1.0	2.4		2.0	0.7 0.1		0.5		4.1	0.7
5-25-72	110.3									
6-23-72		1.1		1.2		2.0	1.2		1.4	
7-28-72	19.4	15.5*	142.1*	1144.7	34.0	77.1	58.8		189.0	219.3*
8-17-72	35.7	13.6	6.5	5.5	3.3	1.9	7.8		3.3	11.6*
8 28-72	30.0*	25.7	35.2	4.8	4.1	9.7*	1.3		5.0	34.5*
9-15-72		3.2		25.4		1.9	14.4			
11-14-72		20.0		18.4		22.1				

Legend. A = Cochiti East Side Main Canal, 1 = Rio Grande at Cochiti Bridge, NS = Sili Main Canal, 2 = Rio Grande at Sili Bridge, 3 = Lower East Side Santo Domingo Acequia, C = Standing water between 2 and 3, SFD = Cochiti East Side Main Canal on road to San Felipe, 4 = Rio Grande at San Felipe Bridge, 5 = Angostura Arroyo, 6 = Angostura-Algodones Diversion Dam, B = Bernalillo Acequia at Bernalillo, * indicates isolation of Salmonella or Arizona.

Table 10. Fecal Coliform Counts of Pueblo Sites

Date	Cochiti Village						Santo Domingo Village							San Felipe Village				
	CPH	CTru	CCAP	CR	CG	CH	SDPH	SDLR	SDK	SDS	SDAE	SFS	USPHC	BIA Sch	SFPH E	SFPH S	SF 251	SFD
5/25							54											
6/23							0	126	126		0							
7/28								388	352									
8/17							430	1810	356									
8/28	2	16					9	476,528	6	12		459,185	4	6				
9/15	14		10	10	46			277	80	158					46		0	14
11/14	514		48			68	0	0	0						0	0		

Legend. CPH = Cochiti Pump House, CTru = Trujillo residence, CCAP = Cochiti Community Action Program Office, CR = Racl's Grocery Store, CC = Gallegos residence, CH = Herrera residence.
SDPH = Santo Domingo Pump House, SDLR = Ladies Room Sink in Headstart Dining Hall, SDK = kitchen sink in Headstart Dining Hall, SDS = classroom faucet in Headstart Grade School, SDAE = Santo Domingo Adult Education Center.
SFS = Sandoval residence, USPHC = U.S.P.H. Clinic, BIA Sch = B.I.A. Grade School, SFPHE = San Felipe Pump House East, SFPHS = San Felipe Pump House South, SF251 = Chavez residence, SFD = Demerio residence.
Blank spaces indicate no collection was made. All numbers represent numbers of organisms/100 mls.

Table 11. Fecal Streptococcal Counts of Pueblo Sites

Date	Cochiti Village						Santo Domingo Village							San Felipe Village				
	CPH	CTru	CCAP	CR	CG	CH	SDPH	SDLR	SDK	SDS	SDAE	SFS	USPHC	BIA Sch	SFPH E	SFPH S	SF 251	SFD
5/25							0											
6/23							0	0	0		0							
7/28								2	0									
8/17							0	0	0									
8/28	0	0					0	0	0	0		0	0	0				
9/15	0		0	0	0			0	0	0					0		0	0
11/14	0		0			0	0	0	0						0	0		

Legend. CPH = Cochiti Pump House, CTru = Trujillo residence, CCAP = Cochiti Community Action Program Office, CR = Rael's Grocery Store, CG = Gallegos residence, CH = Herrera residence.
SDPH = Santo Domingo Pump House, SDLR = Ladies Room Sink in Headstart Dining Hall, SDK = kitchen sink in Headstart Dining Hall, SDS = classroom faucet in Headstart Grade School, SDAE = Santo Comingo Adult Education Center.
SFS = Sandoval residence, USPHC = U.S.P.H. Clinic, BIA Sch = B.I.A. Grade School, SFPHE = San Felipe Pump House East, SFPHS = San Felipe Pump House South, SF251 = Chavez residence, SFD = Demerio residence.
Blank spaces indicate no collection was made. All numbers represent numbers of organisms/100 mls.

DISCUSSION

The Upper Rio Grande Basin in New Mexico extends from the New Mexico-Colorado boundary down to the area of Cochiti Dam (Figure 10). By 1800, overgrazing significantly decreased natural vegetation cover in the Upper Rio Grande Basin and dramatically increased soil erosion. The waters of the Rio Chama and Rio Grande have been affected ever since. Practically all surface water produced in the basin is from mountain areas to the east and west of the Rio Grande within the northern two-thirds of the basin. Nearly all of the Rio Grande perennial tributaries are upstream from Otowi Bridge, near Los Alamos, and runoff is primarily from snow melt. The tributaries and almost all of the Rio Grande except one stretch near Espanola have chemical and biological conditions suited to a variety of beneficial uses. Population growth, rural development, etc. may cause problems in water management, but at present, problems are relatively few. Upper Rio Grande Basin plan objectives are: to aid the Water Quality Division of the New Mexico Environmental Improvement Agency (EIA) in implementing present water quality standards and regulations; to provide an abatement plan for point sources with known unsatisfactory effluents; to establish a basis for program and implementation planning for

maintenance of water quality in the basin; and to establish projections for future water use and waste loads and their impact (New Mexico E.I.A., 1973).

The water use in the basin is directly related to economic activity. Seventy percent of all depletions are due to irrigation, while urban and domestic uses are only 5%.

Present data indicate that except for one stretch of the Rio Grande below Espanola at Otowi Bridge (Table 12) other portions of the Rio Grande and Rio Chama have a bacterial quality better than required by State standards. The high bacterial count at Otowi Gaging Station, directly related to the population concentration in the Espanola Valley, is due to relative contributions from inadequate municipal plants, numerous individual septic tanks, and irrigation return flows. The Rio Chama which meets the Rio Grande above Espanola has a high fecal coliform count when it leaves Colorado, but this count is greatly reduced or is zero by the time it reaches Espanola, so it does not contribute to the pollution. Even upon the completion of an adequate regional waste treatment facility for the Espanola area, pollution of the Rio Grande below Espanola will still occur because of the Upper Rio Grande Basin as a whole. Extensive monitoring of the Rio Grande and surrounding drainages around Espanola should begin

immediately and should be continued regularly. Analysis and documentation of the relative importance of various waste loads and their geographical origin is essential to any effective water quality management in that area (New Mexico EIA, 1973).

The Middle Rio Grande Conservancy District comprises lands along the Rio Grande from Cochiti to the headwaters of the Elephant Butte Reservoir.

As Cochiti Reservoir begins to fill, the Rio Grande will be altered permanently, hopefully improving flood and sediment control further downstream. With existing data, there is no way of determining accurately the relative importance of any factors or of specific geographical locations in relation to the future water quality of the Rio Grande or Cochiti Reservoir. Therefore, adequate monitoring of water quality and waste load capacity is a significant water quality problem and should start as soon as the reservoir is filled in 1975. Data projections indicate problems for Cochiti Reservoir because of nutrient loading. It is not known whether eutrophication will occur, but it is possible, so bacterial contamination will be very important (New Mexico EIA, 1973).

Samples collected for my research were collected from the Upper Rio Grande Basin and in the Middle Rio Grande Conservancy District (Fig. 10).

High bacterial counts at Site 1 may be from Espanola or other sources. Santa Fe dumps its effluent into the Santa Fe River which joins the Rio Grande about one-half mile below Cochiti Dam — below Site 1 but above Site 2 (Fig. 10), so counts at Site 1 are not due to Santa Fe directly. From Tables 5 and 6, a slight difference in FC counts from September through May can be seen, but not during the warm months of June through August. There is a difference in FS counts with those at Site 1 generally higher. During the warm months of 1972, the FS counts were much higher at Site 6 than at sites upstream. If FS counts are an indication of human fecal contamination, then, probably contamination of the river and irrigation ditches occurred somewhere below San Felipe Pueblo, possibly at the Angostura Riverside Levee and Drain. Further downstream at Site 4, the bacterial counts were about the same as those of Sites 1 and 2. At Site 6, the FC and FS counts are much lower until the warm months of July and August. September figures are not available since September 14 there was a tremendous rainstorm flooding out many of the sites making them inaccessible. It was difficult to notice any differences in FC or FS between the different canal and ditch sites partly because of sporadic collections.

There are several factors that may influence the range of high and low organism counts. Differences in organism counts seem to be related to temperature. FC and FS counts at almost all river and ditch sites greatly increased between June 23, 1972 and July 28, 1972. The counts were high until some time between September 15 and November 14, 1972. June, July, and August were the months with the highest average water temperature, the highest FC and FS counts, and the most Salmonella and Arizona isolations (Fig. 11). Salmonella incidence generally peaks during the warm months with the highest numbers from July to October or November and the lowest numbers from January to March (Kitrell and Furfari, 1963; Weibel et al., 1964; Aserkoff et al., 1970; Feigin, 1970; Morbidity and Mortality, 1971-1972; Stetler, 1973).

Precipitation is another factor to consider. The months of highest precipitation around Cochiti were July and August in 1971 and August, September and October in 1972. At Bernalillo, July, August and October were the months with the highest precipitation in 1971, and August and September in 1972 (Table 13). Fecal coliform and FS counts were highest in July, August and September. In dry periods, bacterial pollution can be high, but during the rainy season, this pollution begins high due to turbulence and dirt runoff and then becomes low because of dilution

(Prescott et al., 1946; Leininger and McCleskey, 1953; and Geldreich, 1967). Other authors (Weibel et al., 1964, Claudon et al., 1971) have suggested runoff (associated with high precipitation) waters to be important sources of bacterial contamination while Miner et al. (1967) did a study to determine whether Salmonella is present in feedlot runoff and what public health significance it might have. They found 100% (26/26) of these isolations to be Salmonella. They serotyped 10 of these and all were S. enteriditis ser. infantis which can cause gastroenteritis.

A very important factor which ties in very closely with runoff and precipitation is irrigation. Irrigation is begun the first week in March each year and is stopped the first week in November. There is a marked change in the river. When irrigation is begun, the river becomes very muddy. It clears when irrigation is terminated. The organism counts from this study did not increase significantly when irrigation was started. In fact, organism counts from the ditches and canals themselves are low in comparison to the numbers obtained in July-September from these sites when the water temperatures are highest. This was a somewhat unexpected finding since irrigation causes extra dirt to be carried into the river possibly increasing the chance of Salmonella and other organisms being carried with it. Also, animals (cows,

horses, swine, dogs, and fowl) have access to the river and even easier access to irrigation ditches. Bruner, 1956, showed these animals to be frequent Salmonella carriers and Morrison and Fair (1966) showed that cattle located in irrigation areas do contribute to coliform numbers in streams. I expected the ditch counts to be higher than the river counts in general, to show that they do contribute to the river counts, and to find more Salmonella there than in the river. The organism counts, did decrease in general when irrigation was terminated, although that coincided with a temperature drop.

There are other factors to consider when looking at the FC and FS counts of the river. The northernmost site sampled and the most southern were approximately 20 miles apart. Many things can change in the river during such a distance. Construction of the Cochiti Dam was begun in September 1971. I have no samples of the sites near there before construction started, but the organism counts may have been much lower then. Construction at San Felipe Bridge was begun on August 10, 1972. There is an increase in FC counts, but because of the timing, it is difficult to ascertain whether the increase is due to construction which caused the water at Site 4 to be very turbulent and muddy or to the seasonal effect.

On the basis of results obtained, it appears that water temperature is the most important factor affecting FC and FS numbers, as well as Salmonella and Arizona isolations. It is not possible to determine the exact effects of precipitation, irrigation, and runoff on the bacterial population of the river from this study. Hendricks and Morrison (1967) have shown that water temperature may affect bacterial growth. Some enteric bacteria such as Salmonella grow in water. Large amounts of protein were synthesized at 16°C and some was synthesized at 5°C. They also found that numbers of several enteric organisms showed a net increase after six days when the water temperature was 12°C. The same experiment repeated when water temperatures were 5°C showed cell death in two days. Therefore, if organisms can persist and increase in number from runoff and other factors, this might be one explanation of higher bacterial numbers in summer months.

The FC/FS ratio has been lauded by some authors as a good indicator of pollution. Supposedly, ratios above 4.0 indicate human pollution while those of 0.7 or less do not. The FC/FS ratios in this study ranged from 0 to 1144.7! It is difficult to see a pattern in these ratios (Table 9) other then an increase in the ratios during July and August. Salmonella or Arizona

were never isolated when the ratio was less than 9.7.

Of the three pueblos sampled, Santo Domingo was sampled longer so more samples were taken from each site. There were both fecal and nonfecal bacteria in that water system.

Faucets were heated with a butane burner to eliminate fomite contamination of the water for most collections. The collections made before those made with Ken Bailey were taken with no burner, so faucet contamination on those dates (collections before 8/28/72) is possible especially in bathroom and classroom sinks where children recently playing in the dirt have just washed their hands and most likely had touched the faucet. However, water was allowed to run for at least 1 minute before samples were collected to allow for removal of these organisms and to eliminate the possibility that organisms collected on the faucet may have gotten into the water near the faucet and have flowed back down the pipes.

The first collection at Santo Domingo showed the pump house to be contaminated with 54 organisms/100 mls water. Other sites were tested and showed unacceptably high FC counts (Table 10). According to Fig. 6, the water for Santo Domingo Village is stored in two water tanks— a 200,000 gallon new water tank and an old 26,000 gallon

water tank. (The 26,000 gallon tank is no longer used.) The 8" line from the 200,000 gallon tank joins a 6" line which almost surrounds the entire pueblo and joins an 8" line leading from the pump house. The pump in the pump house was the first site sampled and was found to have unacceptably high FC counts on three occassions. The Headstart Dining Hall kitchen and ladies' room tanks also had high counts during the entire sampling period except for the last collection when temperatures had dropped considerably. These two sites obtain their water from a 2" line which apparently comes from the 200,000 gallon tank. The Headstart School (also having high FC counts) also receives water from the same line. The Adult Education Center also receives water from this line, but there is a network of other 2" lines leading to that site. Perhaps bacterial contamination has been kept out of those joining lines.

Even with water line maps, it is difficult to locate definite sources of comtamination. It is possible that the contamination originally came from the pump house and went to the storage tanks to be distributed all over the pueblo. However, a new well has been drilled on the mesa 1200 feet east of the 200,000 gallon tank. This pump house will be in use by the end of June 1974. The new well is much deeper than the old one and the

water from it is of far superior quality. The old pump house west of the pueblo will be kept operable and used in emergencies only. The 200,000 gallon tank was the one cleaned out. It had not been flushed for 4 years and contained 18" of iron and magensium deposits on the bottom. The deposit was not checked for FC, coliforms or bacterial numbers. The tank has not been reflushed since late summer of 1972, but it will be when the new pump house is in use. After that, the tank will not need flushing every year since the water quality is better with little or no iron or magnesium to build up deposits.

The cause of contamination could have arisen at the storage tank or a number of places along the lines. Since all sites tested were outside the pueblo proper, this may be something to investigate. It is unfortunate that I was not able to sample inside the pueblo to see if the water was also contaminated.

On September 6, the Tribal Officials were contacted and asked to okay chlorination of the water system as well as provide some men to help clean the water tank. On September 8, 1972, the water system and well were chlorinated. However, my collections on September 15, show that there was still contamination. The contamination was gone by November 14, although this may have been due to temperature changes as well as to chlorination.

There is still some debate in the Indian Field Health Service whether to chlorinate or not. They would like to set up a chlorination system on all wells whether it is needed or not. There will be chlorination facilities on the new well at Santo Domingo so that chlorination can be started. The system will be maintained by a maintenance man from Santo Domingo.

Cochiti Village also has a contamination problem with the water supply. The first sampling of the village showed contaminated water at the Trujillo residence. The pump house only had 2 FC/100 ml, but on the second sampling, the pump house, C.A.P. men's room sink, and Rael's Grocery store, which are all on a direct 6" line from the pump house, had FC counts unacceptably high as did the Gallegos residence which is an a 4" line that branches off the 6" line that serves preceeding three sites (Fig. 5).

Even in November, after the temperatures dropped, the pump house had 514 FC/100 ml, the C.A.P. men's room sink had 48 FC/100 ml, and the Herrera residence, which is also on a 4" line branching from the same 6" line from the pump house, had 68 FC/100 mls.

A 3" water line from an old windmill was found to be cross-connected to the main community supply serving the Trujillo and other households. It was disconnected

from the distribution system (Fig. 5). However, there may be lines existing that are unknown to authorities and serve to contaminate the water system further. On one day of my sampling, two men from one of the state organizations were digging in the middle of one of the roads because someone had told them that there was an illegal line hooked up into public lines somewhere in that vicinity. There have been no problems with contamination since the windmill was closed off except for one problem associated with a break in the line. A new well has been drilled, not because of poor quality water but, because they need a larger system. Both wells will be in use.

The pump house and water tank were cleaned, and the entire system was chlorinated and flushed. The water tank was cleaned and flushed again in January, 1973, and chlorination equipment and supplies were obtained. A meeting was set up in January for the Tribal Council to be informed of the advantages of chlorination. A small outbreak of Shigella helped convince the Council to implement chlorination. As of March 20, 1974, the chlorination system was set up and is in operation. There have been no complaints. The system is monitored daily. Before June 7, 1974, Cochiti will also have an experimental fluoridation system, too.

Of the three pueblos I sampled, San Felipe had the "cleanest" water system. Collections were made only on three occassions, but with the exception of the Sandoval household, the FC counts generally were lower than the other two pueblos.

Sites USPHS, and BIA Sch are served by the south pump house while the other sites used the east pump house. There does not appear to be any particular pattern to the contamination. The cause of contamination was investigated by the state authorities, but was not found. In September, 1972, the water tank was cleaned of sediment and the system was flushed and chlorinated. My data for September 15, 1972 show high counts in two places, but these were down to zero by November (although this may have been due to cold temperatures). A contract has been let for a new well to be located on the east side of the pueblo. It is about 1½ miles east of the east pump house and has been drilled 625 feet deep (into Albuquerque aquifer). The water quality is far superior than that from the east pump house.

Humans may be one other factor which may play some role in river contamination. Most of the Indian pueblos have oxidation ponds or lagoons installed and supervised by the B.I.A., P.H.S., or the Department of Interior. The three pueblos sampled for this thesis all had lagoons.

No comtamination from these supposedly reaches the Rio Grande, or any of the adjoining ditches or canals.

However, the river may be polluted by the irrigation and drainage ditches which run through the yards or lands of many residences in the pueblos. It is very difficult to obtain information on sewage and plumbing facilities for individual residences since frequently this is handled by the families or by tribal officials. Salmonella and Arizona were isolated from canals that flow through the pueblo and Indian lands (Table 4). I witnessed children, their pets, and numerous animals in these ditches and canals and therefore suspect that these potentially pathogenic organisms could be picked up by the children, and pets, spread to the family, and carried back to the ditches and eventually to the river.

Dumping sewage into the river or ditches is prohibited by state law (New Mexico E.I.A., 1973), but it is almost impossible to enforce. If a family with an irrigation ditch or drainage canal running through their property want to dump or drain their waste into it, they can. Regulations concerning water lines, sewage disposal, etc., are written by the pueblo officials, but 90% of them are not enforced (Dreschler, 1974).

If a violation of regulations is noted by pueblo authorities, legal action can be taken. If a pipe is

responsible for the drainage and it is below the level of the water, it would not be noticed. It would be impossible to patrol all the ditches, canals, and drains to check on this type of pollution.

Dr. Phillip Peterson from the Public Health Clinic in San Felipe stated that in summer months, especially in August and September, they have so many patients with diarrhea, much of which might be Salmonella caused, that patients are treated symptomatically since the necessary money, equipment or staff to check each patient for salmonellosis or shigellosis is unavailable. He reiterated that it was such a common event there that people expected to have diarrhea in the summer and that many people did not even bother to seek treatment—especially the older ones (Dr. P. Peterson, 1972). To educate the Indians as well as other people of New Mexico is a very difficult and slow process. Aside from a language barrier, resistance to change still exists. Instruction concerning personal hygiene and sanitation might not be effective.

Salmonella can cause serious disease, so the finding of Salmonella and Arizona in these waters indicates a real danger. Monitoring the water bacteriologically could be a purposeful endeavor and perhaps

efforts ought to be increased to see that it is done. Salmonella is in the waterways from Cochiti to Bernalillo, and according to three recent theses (Medina, 1973, Dominguez, 1972, Stetler, 1973) Salmonella is quite plentiful from Bernalillo to Bernardo, New Mexico.

The annual New Mexico Health Laboratory reports show 535 Salmonella of different serotypes isolations plus 27 Salmonella typhi isolations for 1971, 351 Salmonella plus 12 Salmonella typhi for 1972, and 432 Salmonella plus 14 S. typhi for 1973 for New Mexico. My sampling sites all fell into two counties--Sandoval and Bernalillo. In 1971, 36% (194/535) of the Salmonellae were isolated from Bernalillo county as were 22% (6/27) of the S. typhi. In 1972, 66% (266/399) of the Salmonella and 17% (2/12) S. typhi were isolated from this county. In 1973, 67% (289/432) of the Salmonella and 50% (7/14) of the S. typhi were from Bernalillo county. The incidence in Sandoval county was much lower. For 1971, only 0.93% (5/535) Salmonellae were isolated, 17% (5/28) for 1972, and 0.69% (3/432) for 1973 with no S. typhi isolations (N.M.P.H.L., 1971-73).

Of the five serotypes isolated, three are among the 20 most frequently isolated serotypes in New Mexico during 1971-1972 (Table 14). These three serotypes comprised 50% (3/6) of the Salmonella I isolated. The other two serotypes were not isolated in New Mexico during 1971-72.

There seemed to be no particular difference between isolations from the ditches and the river except that Arizona was isolated from the ditches and not the river, while the Salmonella was isolated from both However, with just six Salmonella isolations and two Arizona isolations, no conclusions could be drawn.

There were many problems with these studies. It was difficult to obtain representative samples of the water. All the samples from the river, ditches and canal sites were collected from a bridge with a bucket. This method allowed for collecting surface water only and there is evidence of a much higher bacterial number in the sediment (Hendricks, 1971b). However, there would have been problems if sediment were collected, since it is extremely hard to Millipore filter. Sediment quickly clogs the filter and counting colonies growing on mud is much harder.

There is much evidence that Moore swabs or mulitple samples would be much more accurate than just collecting a bucketful of water. It certainly would have been better to collect the well samples in this way, but for this study it was not feasible to implement these procedures because of lack of time and funds.

Lack of equipment or improperly functioning equipment influenced the results obtained. The incubators frequently

failed to maintain the proper temperature, unwanted organisms such as Proteus and Pseudomonas flourished in tetrathionate broth below 41°C, or all the FC were killed (September 14, 1972) by a water bath temperature of 51°C instead of 44.5°C.

BG, XLD, and SS were used in the study on the basis of literature (most of which states the BG is the best plating media for Salmonella isolation). Biochemical tests were limited to those that seemed to eliminate most other closely related organisms (Difco Laboratory Chart). The panel of tests-reactions in TSI, malonate, dulcitol, lysine decarboxylase, urease, and growth in KCN were the biochemical tests employed; 90, 95, and 98% confidence limits showed that these media were good choices (Table 3).

Since there were so many canals, levees, drains and, feeding into the river at various places, it would have been even more helpful and interesting to have FS and FC counts from additional sites to try and pinpoint specific sources of contamination. Once particular canals or ditches were shown to have high counts, they could have been monitored at various places. More useful information concerning bacterial contribution could have been obtained, resulting in some definitive action taken.

SUMMARY

Salmonella and *Arizona* were isolated from the Rio Grande and its adjoining canals and ditches. Five serotypes of *Sal. enteriditis* were isolated. *Salmonella* and *Arizona* were isolated from July 28 to August 28, 1974.

The period from July to September, 1972, showed the warmest water temperatures, the highest fecal coliform and fecal streptococcus counts and the only *Salmonella* and *Arizona* isolations which may indicate a seasonal pattern.

The water systems of three pueblos were studied from May to November, 1972. Contamination was observed at various sites in all three. No particular pattern of contamination was established.

ADDENDUM

Water samples from various sites in Cochiti, Santo Domingo, and San Felipe villages had been taken for a number of years and were sent to the New Mexico Scientific Laboratory Services in Clovis, New Mexico. These samples had shown no contamination, yet, people at Santo Domingo had become sick on the water (Starr, 1972). On three separate occasions, collections from various sites in the pueblos were made in triplicate. One set was sent to the laboratory in Clovis, one set was run at the laboratory in Santa Fe by the Indian Field Health representative, and one set was run in the Biology Department at UNM exactly as all other samples collected for this thesis were run. The results from the Indian Field Health Laboratory and those from UNM agreed most of the time while those from Clovis were somewhat different. These results illustrate the inherent difficulties -- even with standardized methodology -- of replication and quantification of microbiological sampling and testing. (Table 15) All three sources used the same MF techniques, but the handling of samples before the actual testing was different. Because of the discrepancy, state authorities did look into the methods of shipping samples. No changes were implemented. Samples are shipped in glass bottles in individual cardboard containers, uniced, on the same day they are collected.

Figure 10. Upper Rio Grande Basin, Rivers and Tributaries

Cities:

E = Espanola
C = Chama
T = Taos
SF = Santa Fe
LA = Los Alamos

Rivers:

RP = Rio de los Pinos
RB = Rio Brazos
RC = Rio Chama
RG = Rio Grande
RR = Red River
RT = Rio Taos
SFR = Santa Fe River
JR = Jemez River

Creeks:

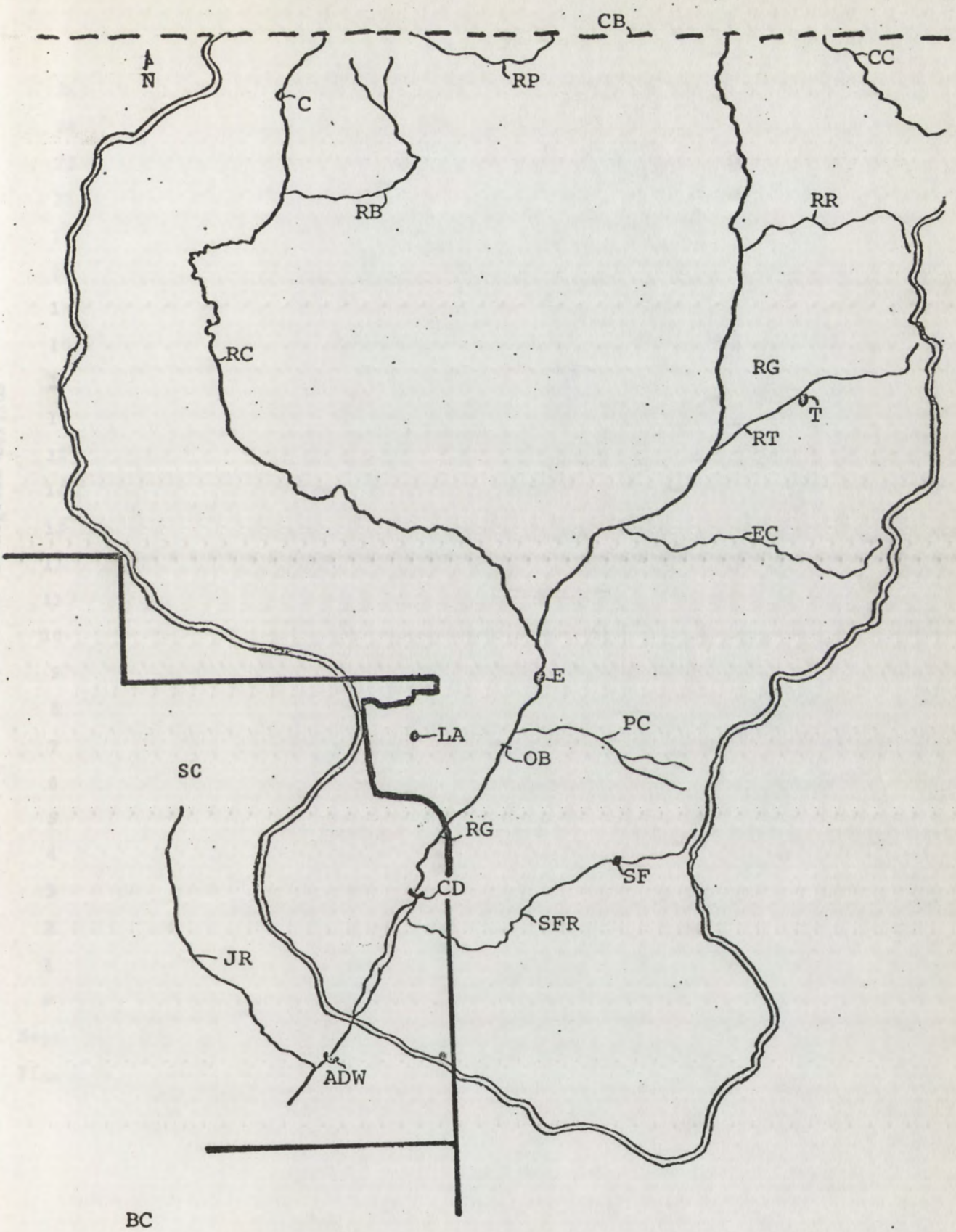
CC = Costilla Creek
EC = Embudo Creek
PC = Pojoaque Creek

Miscellaneous:

CB = Colorado Border
OB = Otowi Bridge
ADW = Angostura Diversion Works
CD = Cochiti Dam
SD = Sandoval County
BC = Bernalillo County

Legend:

Creeks
Major Rivers
Rivers
U.R.G. Basin Boundaries
County Boundaries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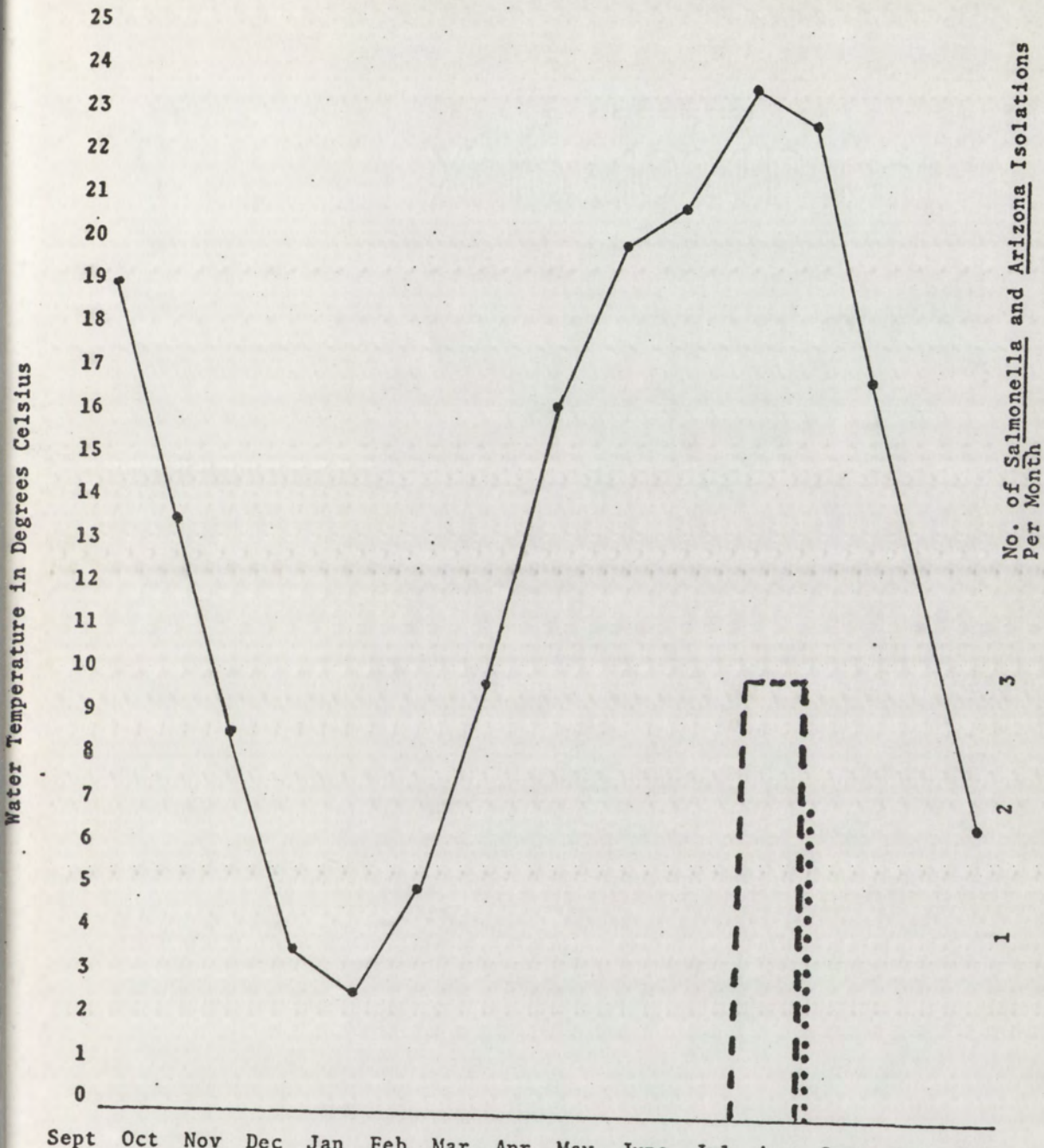

Figure 11. Seasonal Patterns of Salmonella (----) and Arizona (....) isolations and water temperatures (•——•) for the Rio Grande and adjoining canals and ditches from Cochiti Bridge to Bernalillo, New Mexico.

Table 12. Bacterial Water Quality of the Rio Grande at Otowi Bridge, New Mexico

Date	Total Coliform Count	FC	FS	FC/FS
8- 4-71	600	120	0	
9- 9-71				
10- 6-71	est. 4000	920	50	18.4
11- 3-71	42	0	0	
12- 9-71				
1-13-72	480	30	40	0.75
2- 9-72	>300	300	10	30.0
3-14-72	990	990	70	14.1
4- 6-72	est. 800	350	40	8.75
5- 3-72	600	340	30	11.3
6- 6-72	70	<10	10	1
7-12-72	73			
8- 3 72				
9- 6-72		2300		

Samples were analyzed at the State Public Health Laboratory in Albuquerque, New Mexico by Millipore Filter technique.

Blanks indicate that even though a water sample was taken on that date, bacterial studies were not conducted.

Table 13. Average Monthly Precipitation at Cochiti and at Bernalillo, New Mexico

1971

	J	F	M	A	M	J	J	A	S	O	N	D
Cochiti	.8	.2	.2	.8	.2	.1	2.9	1.8	1.3	1.2	.6	1.5
Bernalillo	.66	.26	.18	1.10	.41	.05	2.9	1.1	.9	1.4	.5	1.3

1972

	J	F	M	A	M	J	J	A	S	O	N	D
Cochiti	.2	.1	.1	.0	1.0	.9	1.0	2.0	3.1	2.6	.9	.7
Bernalillo	.4	.1	.3	.0	.8	.6	.5	1.8	1.9	---	.6	.9

Legend. --- indicates missing.

Data when to tenths are from a gage that only records precipitation amounts to the nearest 0.1 inch.

Table 14. The Twenty Most Common Salmonella Serotypes Reported by the New Mexico Scientific Laboratory Services for the Years 1971 and 1972

	Serotype	Total	Percent
1.	Typhimurium	141	29.4%
2.	Heidelberg	59	12.3%
3.	Newport*	51	10.6%
4.	Infantis	36	7.5%
5.	Thompson	30	6.3%
6.	Anatum	17	3.6%
7.	Java	12	2.5%
8.	Enteritidis	12	2.5%
9.	Senftenberg	11	2.3%
10.	Oranienberg	11	2.3%
11.	Saint paul	11	2.3%
12.	Bredeney*	10	2.1%
13.	Meunchen*	8	2.1%
14.	Poona	7	1.5%
15.	Javiana	6	1.2%
16.	Derby	6	1.2%
17.	Montevideo	5	1.0%
18.	Bareilly	5	1.0%
19.	Manhattan	5	1.0%
20.	Give	4	0.8%

* Serotypes isolated by L. Fogleman

Table 15. Comparison of Data for Fecal Coliform Counts

	8-28-72			9-15-72			11-14-72			1-4-73		
	1	2	3	1	2	3	1	2	3	1	2	3
Cochiti Village												
CPH	2	2	0	14			514	TNTC	0		TNTC	0
CTru	16	14	0									
CCAP				10			48	27	0		10	
CR				10								
CG				46								
CH							68	730	0		37	0
Santo Domingo Village												
SDPH	9	16	0									
SDLR	476,528	20	TNTC	277	not available	not available	0	0	0			
SDK	6	12	TNTC	80			0	0	0			
SDS	12			158								
San Felipe Village												
SFS	459,185											
USPHC	4	1	0									
BIA Sch	6	1	0									
SFPHE				46			0		0			
SFPHS							0		0			
SF251				0								
SFD				14								

Legend. CPH = Cochiti Pump House, CTru = Trujillo residence, CCAP = Cochiti Community Action Program Office, CR = Rael's Grocery Store, CG = Gallegos residence, CH = Herrera residence.
SDPH = Santo Domingo Pump House, SDLR = Ladies Room Sink in Headstart Dining Hall, SDK = kitchen sink in Headstart Dining Hall, SDS = classroom faucet in Headstart Grade School,
SFS = Sandoval residence, USPHC = U.S.P.H. Clinic, BIA Sch = B.I.A. Grade School, SFPHE = San Felipe Pump House East, SFPHS = San Felipe Pump House South, SF251 = Chavez residence, SFD = Demerio residence.
Blank spaces indicate no collection was made. All numbers represent numbers of organisms/100 mls.

1 = results obtained by L. Fogleman.
2 = results obtained by Ken Bailey in Indian Field Health Lab.
3 = results obtained by New Mexico Scientific Laboratory Services in Clovis, New Mexico.

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