STUDY ON
THE INFLUENCE OF CATTLE KEEPING ON THE BACTERIOLOGICAL QUALITY OF DOMESTIC WELL WATER

By
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THESIS

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DECLARATION

I hereby declare that this thesis entitled STUDY ON THE INFLUENCE OF CATTLE KEEPING ON THE BACTERIOLOGICAL QUALITY OF DOMESTIC WELL WATER is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Mannuthy,
Sept. 24, 1981.

George T. Oommen

CERTIFICATE

Certified that this thesis, entitled

STUDY ON THE INCIDENCE OF CATTLE KEEPING ON THE BACTERIOLOGICAL QUALITY OF DOMESTIC WELL WATER

is a record of research work done independently by Sri.George T. Oommen under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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(George T. Oommen)

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Introduction

INTRODUCTION

The increasing incidence of water-borne epidemics and the many deaths from the contamination of water supplies altered the general public outlook to the hazards of bacterial contamination of water. Water pollution is considered as one of the major causes of health hazards resulting in innumerable diseases among people throughout the world. It has been rightly stated that 80 per cent of the disease problems encountered in the developing countries are water-borne and that the best way to improve the health of the public is to ensure the supply of wholesome water. Mere pleasant appearance and palatability of water does not necessarily mean that it is wholesome.

The survey conducted by WHO (1973) revealed that only one half of the urban population in developing countries have drinking water supply in their houses and only 12 per cent of the rural dwellers have reasonable access to safe drinking water. In India 80 per cent of the people live in rural areas and among them only 18 per cent have a reasonable access to safe drinking water.

In many rural areas of the developing tropical countries due to lack of protected water supply people depend on untreated surface or ground waters such as wells, ponds, springs, rivers, streams, lakes and canals for their domestic use and for the maintenance of livestock (Gupta et al. 1978).

Wells are the main source of water supply in Indian villages and towns. Majority of the wells seen in Kerala are shallow type, tapping the subsoil water above the first impermeable layer. Their construction is unsatisfactory and are liable to serious pollution from various sources.

In Kerala majority of the households keep cattle. Cattle manure, though used as a useful fertiliser, is one of the potential pollutants of ground and surface waters. The improper collection and storage of the cattle slurry and litter and heaping the same at or near the sheds or in the vicinity of water sources and its indiscriminate disposal on land poses serious problems of surface and ground water pollution. The cattle manure reach water sources by way of percolation through the soil and surface runoff from the manured fields (Crane et al. 1980). They also contribute to

the spread of diseases and infections.

It is a well established fact that water seeps down through the layers of soil carrying microorganisms and other pollutants from sources of contamination on the surface of and in the soil and subsequently polluting the ground water.

Wells, especially shallow wells, in or near farmyards, cattle sheds, manure piles, compost pits, privies, septic tanks, land manured with animal excreta, etc. are notoriously liable to pollution (Linton, 1965; Moore, 1973; Loehr, 1977). During monsoon, the extent of pollution is very high from the surface washings of the various sources of pollution. This would definitely deteriorate the quality of well water considerably.

The magnitude of pollution of water varies depending on various factors like distance between cattle shed and well, the type of construction of cattle shed and well, arrangement for the disposal of cattle slurry, surface runoff from the manured land into the well, topography of the area, nature of the soil, season, etc.

At present, the wells in the rural areas are often sunk in any convenient spot, without any regard to its

proximity to dangerous sources of pollution. This shows that the rural population is either unaware of the necessity for a sanitary well, protected water supply and environmental hygiene or negligent in keeping their drinking water sources protected from pollution.

There is only limited and scarce information available about the impact of cattle keeping on the bacteriological quality of well water in the households in Kerala or elsewhere in India. The quality of these domestic well waters from the bacteriological point of view is virtually unexplored. In addition, there is scarcity of adequate well water monitoring programmes to investigate these factors at present in the country. Therefore, an extensive bacteriological investigation of the domestic well water is necessary.

The present investigation was conducted at Mannuthy, a village in Trichur district of Kerala State. This is with a view to evaluating the bacteriological quality of domestic well water in the light of its bacterial load, presence of absence of the common indicator bacteria and a sanitary survey of the wells. Yet another objective of the study is to assess whether cattle keeping in the

households has any influence on the bacteriological quality of well waters in this geographical area. Such a study may help in improving the construction and location of both animal sheds and wells and management practices in the disposal of animal excreta.

Review of Literature

REVIEW OF LITERATURE

Hitherto, there are only very few literatures published on a detailed study conducted in India to assess the bacteriological quality of domestic well water and the allied topics such as the type of wells, their location and construction, sources of pollution of water, its detection and control. Information on the impact of cattle keeping on the bacteriological quality of domestic well water is apparently absent. Nevertheless, the available previous reports on the various aforesaid topics are reviewed hereunder.

2.1 Well

2.1.1. Types of well

Wells are the means of tapping ground water. They are the main sources of water supply in Indian villages and towns (Ghosh, 1959; Park, 1971; Gupta et al. 1978).

Most of the wells in India are of shallow type, for they tap water above the first impervious layer in the ground (Park, 1971). Those wells under 100 feet in depth were classified as shallow wells by Steel (1960).

But Christie and Christie (1971) were of the opinion that the depth of the wells depend mainly on the depth of the first impermeable layer, and the deep well in one part of the country might be shallower than a shallow well in another part.

2.1.2. Location

Wells for public water supplies must be located farther from all sources of pollution. Exact distance from a pollution source cannot be prescribed. John (1970) was of opinion that no one set of distance is adequate and reasonable for all conditions of pollution and the safe distance between a well and source of pollution should be based on the local conditions.

Different authors have specified different distances for the wells from the source of pollution. Ghosh (1959) specified a distance of 24 to 30 metres (80 to 100 feet) as a reasonable distance, while according to Moore (1973) it was still higher - 30 to 45 metres (100 to 150 feet). The minimum distance was suggested by Park (1971) which was only 15 metres (50 feet) from the likely source of contamination. He further added that the wells should be located at a higher elevation with respect to the source of contamination.

Many of the workers have warned against the location of wells in the flooding areas and near privies, manure piles, barnyards, cesspools, etc. (Steel, 1960; Moore, 1973; Park, 1971).

2.1.3. Construction

Based on the method of construction of the wells, they are classified by Steel (1960) into dug wells and tube wells. Dug wells are those excavated by hand or machine, generally more than two feet in diameter and not over 50 feet in depth. The lining is usually of concrete or brick, sometimes extent for a depth of 10 feet below the ground surface. Barrell and Rowland (1979) observed some degree of protection by lining the shaft of the wells. The defects in the plinth surrounding wells allow direct seepage from the surface into the shaft of the wells. Considerably lesser pollution was found in the wells those were enclosed at the top.

Moore (1973) considers the most essential features of a dug well as having three feet to six feet diameter, and an impervious casing extending six to ten feet down into the ground and one foot or more above the ground surface, a tight impervious top and protection from all

surface drainage and flooding.

In India dug wells are the commonest type. Two types of dug wells exist in the Indian rural areas: (a) unlined katcha well and (b) pucca well, built of bricks and stones (Park, 1971). The salient features of a sanitary well are discussed in detail by Ghosh (1959), Steel (1960), Park (1971) and Moore (1973).

2.2. Sources of bacterial pollution of well water

It now becomes necessary to attempt to identify the various sources of pollution. The contaminants of ground water are the same as the contaminants of surface supplies. Most of the microorganisms in water derive from air, soil, living and decaying plants or animals and faecal excrements of man and other warm-blooded animals (Kabler, 1968).

Bolton (1961) reported that the necessity of preventing the contamination of water sources arose since the discovery of infectious bacteria.

Ground water normally do not contain bacteria because of the effect of filtration, exposure to unfavourable environment and the time lapse which will eliminate most of them including those of sanitary significance

(Steel, 1960; Wilson and Miles, 1975). They further added that the sanitary quality of shallow well water would be poorer than that of deep wells, where the number of bacteria would be very few, though not absolutely sterile.

Voelker et al. (1960) found that the geological source and type of well are the two important factors in the bacteriological characteristic of well water. They observed that the dug well types range more widely about their mean bacteriological performance than do the more stable, drilled well types.

Ground water contamination can spread owing to improperly constructed deep intrusion wells, geological faults and other cracks or crevices of aquifers, and a sudden rise in the subsoil water with wastes resulting in cross contamination of different aquifers containing bacteriological wastes. The entrance of insufficiently filtered water into wells and springs can also contribute to the contamination (Ghosh, 1959; Steel, 1960; Bolton, 1961).

The shallow open wells which are badly constructed and located near barnyards, manure pits, cesspools, privies, etc. often get polluted either from surface

runoff water during rainy season or by flooding of the area or from the contamination of the subsoil (Ghosh, 1959; Steel, 1960; Linton, 1965; Moore, 1973; Park, 1971; Wilson and Miles, 1975; Loehr, 1977).

Linton (1965) has attributed improper staining of the wells as one of the major causes of pollution of wells.

Wells that produce safe water may be contaminated with external sources such as surface water, dirty ropes and buckets and unclean hands (Ghosh, 1959; Purdom, 1980).

The study of infectious hepatitis epidemic in Posen, Michigan by Vogt (1961) revealed that the wells are located too near septic tanks, tile fields and seepage pits. The septic tank effluents travelled down through the cone of filtration of the wells and through the cracks in the rock for great distances with very little change in its characteristics.

A similar incident was observed by Burrows (1968). He found that in the case of shallow wells, typhoid and other bacilli enter the water supply from privies and latrine, via., ground water.

Later on, Viraraghavan and Warnock (1976) reported the incidence of pollution of surface and subsurface waters by the septic tank system in Canada and U.S.A.

The works of Hagedorn et al. (1978) has confirmed that the septic tank effluents from their discharge pits could seep through the soil and contaminate the ground water.

2.3. Animal source of bacterial pollution

The influence of animals on the quality of well water from the bacteriological point of view is virtually unexplored. Not only ponds and streams but wells are also susceptible to faecal contamination and the sanitary quality of the well water is therefore of public health interest and worth investigating.

The various communicable water-borne diseases of man and animals are transmitted by the intestinal and urinary discharges of infected individuals or carriers. These discharges when disposed off carelessly without adequate treatment may contaminate the ground water and endanger well water supplies (Steel, 1960; Jones, 1960).

There are ample evidences from the literature to indicate that various potentially pathogenic bacteria

are present in the excreta of livestock, poultry, cats, dogs and wild animals (Geldreich, 1970; Stuart et al. 1976; Loehr, 1977; Jones, 1980).

Diesch and McCulloch (1966) reported the contamination of water used for recreation, with Leptospira pomona from the urine of infected cattle, swine and wild animals or from drainage of adjacent livestock pasture. There is a controversial report from Moore (1975). According to him there was no evidence that pollution with animal wastes adds to the water organisms pathogenic to man, but since animal coliforms cannot readily be differentiated from human coliforms, this has little practical significance.

The movement of animal wastes into surface and ground waters is often cited as a major factor contributing to the pollution of available water in many regions.

Bolton (1961) in his survey of an area where livestock were raised has shown that animal offals were a constant source of bacterial pollution of water supplies. He further added that the indiscriminate storage of wastes on ground surface was one of the common sources of ground water contamination.

Morrison and Fair (1966) reported runoff from the watersheds surrounding a stream, where there were no pollution from domestic sewage, was the most important source of bacterial contamination.

The same investigators made a study to determine the load of bacterial pathogens in the high quality surface water. The presence of potentially pathogenic bacteria was the result of contamination by wild animals and cattle (Fair and Morrison, 1967).

Van Donsel et al. (1967) reported non-human sources of bacterial contaminants in urban runoff water. Kabler (1968) observed that more faecal coliform and faecal streptococci in urban runoff indicate probable source of faecal pollution as dogs, cats, rodents and other animals.

Land application of dairy manure has been a traditional method of waste disposal. Barker and Sewel (1972) studied the effect of surface spreading of dairy manure, surface runoff and ground water quality in terms of the bacteriological load and observed that the results were far exceeding the normal permissible criteria. Similar observation was made by Crane et al. (1980). They found that the animal manure applied on land could be a major

contributor of agricultural nonpoint pollution.

Runoff from manured fields and effluents from animal waste disposal lagoons could affect the quality of surface and ground waters (Janzen et al. 1974; Loehr, 1977). The investigations of Girevolo et al. (1979) on the effect of anaerobic swine waste lagoons on ground water quality indicated that seepage entered ground water from the lagoons.

Loehr (1977) from the examination of rural water supply in Missouri, U.S.A., reported that livestock production is a natural contributor to bacterial load in surface waters as the waste materials at the sites of animal habitations form the main contaminating source.

The investigations on Salmonella pollution of surface waters by Smith et al. (1978) revealed that sewage and farm effluents were the main source of pollution.

Information available on the impact of cattle grazing operations on the quality of water indicates that pollution problems are usually associated with increased sediment or bacterial counts in runoff water (Robbins et al. 1972; Milne, 1976).

Kunkle and Weiman (1967) investigated the effect of cattle and sheep grazing on mountain water quality and found that the faecal coliform counts were high.

2.4. Bacteriological examination of water

Many bacteria are found in water. Most of them are of no sanitary significance; some are indicators of faecal pollution but are harmless, few others are pathogenic. Bacteria are most widely used as a guide to water quality (Steel, 1960).

The pollution of water with faecal material presents hazard to health since faecal material from either human or animal sources may contain pathogenic microorganisms. Bacteria constitute over 30 per cent of the total wet volume of faeces and counts of 10^{10} anaerobe and 10^{8} aerobe per gram wet weight of faeces are regularly reported (Dvison and James, 1978).

The method for the bacteriological examination of water is designed to provide an index of faecal contamination. Escherichia coli (E. coli) and other coliforms are abundant in the faeces of man and animals as they normally inhabit the intestinal tract. The coliforms and E. coli, therefore, are useful indicators of faecal

contamination of water (Hammer, 1977; Wilson et al. 1979). According to Sundstrom and Klei (1979) the most important criterion used in classifying a water as 'polluted' is based on the microbial count and the faecal coliform (FC) and faecal streptococci (FS) group organisms.

2.4.1. Standard plate count (SPC)

The standard plate count provides an estimate of general bacterial purity of water. It may serve as useful indices of changing sanitary conditions, particularly when past records are available (Moore, 1973; Mistreich and Lechtman, 1980). The results of SPC are by themselves of little value in estimating the hygienic quality of a water. By this test the total number of viable bacteria in a water sample can be estimated and thus is a useful supplementary test. It also gives an indication of the amount and type of organic matter present in the water (Cruickshank et al. 1975). Most of the saprophytic bacteria grow at 22°C while those growing at 37°C would be parasites of man and animals derived from soil, excreta or sewage. Therefore, a higher count at 37°C relative to the count at 22°C indicated pollution with animal or human excreta (Campbell, 1979).

Water samples of appropriate volume in nutrient agar plates were incubated either at 20° ± 0.5°C for 48 ± 3 h or 35° ± 0.5°C for 24 ± 2 h and the results were expressed as the number of bacteria per millilitre (Moore, 1973). Geldreich et al. (1975) examined the bacteriological quality of potable water, bottled water and emergency water supplies. The SPC of the samples were determined using the pour plate method and incubated the plates at 35°C ± 0.5°C for 24 to 48 h. But Cruickshank et al. (1975) prescribed incubation temperature as 37°C for one day and the other as 20 to 22°C for three days. They were against the practice of extending the incubation period at 37°C for one day into two days, because after two days certain saprophytic bacteria capable of growing more slowly at 37°C might develop into visible colonies.

Wilson and Miles (1975) suggested standard nutrient agar for the enumeration of the organisms. Cruickshank et al. (1975) and Barrell and Rowlands (1979) also used the standard plate count agar for the enumeration of the total viable bacteria in water.

According to Moore (1973) uncontaminated well waters can be expected to yield plate counts not greater than

100 to 300 colonies per ml. But Campbell (1979) and Wistreich and Lechtman (1980) limit the SPC to 100 per ml as a standard. According to Gilereas (1975) no definite standard can be set for the total count but usually it should be less than 500/ml.

2.4.2. Indicator bacteria

Certain bacteria which are commensals of the intestinal tract of man and animals are denoted as indicator bacteria. Their presence in food and water is a clear indication of its contamination with faeces. According to Buttiaux and Mossel (1961) organisms selected as indicators should possess specificity, i.e., the bacteria should occur in the intestinal tract, in high numbers in the faeces, possess resistance to external environment and permit easy and reliable detection.

Moterman et al. (1974) recommended total coliforms, faecal coliforms, faecal streptococci and SPC at 35°C as the commonly accepted bacterial indicators of pollution of water. Coliforms, faecal streptococci and Clostridium perfringens (Cl. perfringens) were considered the common indicator bacteria and pathogens

(Cruickshank, 1975; Harrigan and McCance, 1976). Verstraete and Voets (1975) made a detailed survey of the different microorganisms which could be used as indicators of environmental hygiene. He established that faecal coliforms, faecal streptococci, Cl. perfringens, enteroviruses and Pseudomonas aeruginosa could be used as indicator organisms in drinking water, recreational water and swimming pools.

In United States E. coli belonging to the coliform group has commonly served as the indicator microorganism signalling the probable presence of pathogens. But in Europe faecal streptococci is also used to indicate bacterial contamination (Purdom, 1980).

2.4.3. Coliforms and Escherichia coli

A brief review of the scientific basis for the use of coliform group as a pollution indicator will assist in establishing the various interpretations. Escherich (1885) observed that Bacillus coli (E. coli) not only occurred in high densities in faeces but also was frequently associated with the typhoid bacillus and, therefore, that it might be used as an indicator of faecal contamination. The coliform bacilli are the most

reliable indicators of faecal pollution of water. The research investigations on the significance of faecal coliform bacteria in the environment have demonstrated that this pollution indicator system has an excellent positive correlation with warm-blooded animal faecal contamination (Geldreich, 1970).

The coliform organisms include both faecal or intestinal and nonfaecal organisms. Typical of the first group is E. coli and of the second, Enterobacter aerogenes. E. coli has its habitat in mammals, birds and in some cold-blooded animals while the nonfaecal type is found in soil, on fruit, leaves, grains and many other places in nature (Moore, 1973). But Geldreich (1970) detected a few faecal coliforms on vegetations and were derived from animal manure or night soil used as fertilizer or by contact with contaminated insects. Insects that spend part of their life cycle associated with animal dung may be expected to transmit variable number of faecal coliform in low densities. Currently there is no satisfactory method available for differentiating human faecal coliforms from those of animal origin (Hammer, 1977; Sundstrom and Klei, 1979).

Burrows (1968) was of the opinion that the presence of coliform bacteria of domestic animals in water does not carry the same implication as that of human origin.

According to Steel (1960) the coliforms die at a logarithmic rate and a few individuals exist for weeks or months in fresh water. Salle (1973) reported the multiplication of coliform on organic materials such as leather washers, wood, swimming pool ropes, jute packing and even in water pipes. The presence of large number of coliforms of single type in water obtained from wells, springs or a single distribution system was suggestive of multiplication of coliforms on the above materials. Cooke (1974) observed that E. coli did not multiply in natural water but only survive for a limited time. Patterson et al. (1974) observed that E. coli type I died out in the soil faster. But Thomas and Druce (1955) reported that E. coli could persist in surface soils underlying cow manure for 20 weeks. A faecal coliform culture had a more rapid die-off than a nonfaecal variety at 22°C as has been reported by Geldreich et al. (1968). Guy and Small (1977) studied the survival of faecal coliform of bovine origin in drainage water and soil stored at five, 10, 15 and 20°C under laboratory conditions. In drainage

water the number of coliforms had more than doubled after 48 h at all temperatures. But in the effluent irrigated soil there was a decrease at five, 10 and 15°C up to four days but an increase of 30 per cent was found at 20°C.

It has been reported frequently that coliform number in water can increase quite significantly, especially in warm polluted water (Deaner and Kerri, 1969; Dutka, 1973; Evison and James, 1974). Evison and James (1978) observed the regrowth of coliforms in polluted tropical waters, where the temperature exceeded 20°C.

All the available evidence showed that E. coli type I as the most frequently isolated organisms from the human and animal intestine (Sojka, 1965). In heavily polluted surface water the faecal coliform component usually falls between 10 per cent to 35 per cent of the total coliform (Kabler, 1968). On an examination of livestock faecal discharge, Geldreich (1970) ascertained that non-aerogenic coliforms come to only 0.4 per cent of the total coliforms.

Geldreich et al. (1962) studied soil from a wide

variety of areas and observed that the density of E. coli in soil was proportional to the degree of faecal contamination. Kuneto et al. (1973) investigated the relationship between the degree of pollution and the type of coliforms in fresh water and found that the number of total bacteria, coliforms and E. coli increased parallel to the degree of pollution.

The American Public Health Association (1953) recommended brilliant green bile (BGB) broth and eosin methylene blue (EMB) agar for the detection of coliforms and E. coli.

Maloney et al. (1961) used lactose broth and BGB broth to determine the most-probable-number (MPN) of coliforms. Using BGB broth a close approximation of the coliform count in farm pond waters was obtained.

Bhatta (1966) employed BGB broth for the evaluation of coliforms and EMB agar for E. coli from water. Hall et al. (1967) used MPN method for the detection of coliforms using BGB broth. They also studied the growth characters of E. coli on EMB agar.

McFeters et al. (1974) identified purified cultures of coliform bacteria by their cultural characteristics

such as growth in BGB broth, characteristic colonies on EMB agar and IMViC reaction. Cruickshank et al. (1975) suggested BGB broth two per cent for the use of Mijkman test.

Wilson and Miles (1975) have commented that no perfect medium has yet been devised that will enable all coliform organisms to develop and produce gas, while suppressing the growth or at least the gas production of other organisms.

Enumeration of the total coliform bacterial population by the fermentation tube procedure has been used by microbiologists for some sixty years as an indicator of water quality. This technique is still used for monitoring the quality of potable drinking water supplies through out the world. According to Standard Methods (APHA, 1971) only MPN and membrane filter techniques are accepted methods for coliform enumeration. The results of the fermentation tube technique are reported in terms of the probable number of coliform bacilli per 100 ml. According to Wilson and Miles (1975) the count is best referred to as presumptive coliform count (PCC).

The presumptive coliform count or multiple tube technique can be used to enumerate the coli-aerogenes group. Results of the test are expressed as the MPN of coliforms since the count is based on the statistical analysis of sets of tubes in a series of serial dilutions. MPN is the definition related to a sample volume of 100 ml (Hammer, 1977). The coliform counting by MPN procedure was recommended by APHA (1953), Malaney et al. (1961), APHA (1971), Cruickshank et al. (1975) and Harrigan and McCance (1976) using BGB broth.

Cruickshank et al. (1975) and Oblinger and Coburger (1975) preferred five tube method to three tube method for the determination of MPN.

The elevated temperature test, which is used to differentiate E. coli from that of nonfaecal coliforms, is found to be superior to all other procedures with the additional advantage of simplicity and a testing time of only 24 h at 44.5°C (Clark and Kabler, 1964).

Sojka (1965) also recommends Eijkman test for the detection of E. coli type I. Geldreich (1970) found out the occurrence of faecal coliforms in water and measured it by a faecal coliform test that was

based on lactose fermentation at 44.5°C. He emphasises the need for incubation in a water-bath carefully controlled at 44.5°C ± 0.2°C. Eijkman test is usually employed to detect E. coli, which depends on the ability of the organism to produce gas at 44°C when grown in bile salt lactose peptone water (Cruickshank et al. 1975).

2.4.4. Faecal streptococci (FS)

According to Hartman et al. (1966) faecal streptococci is broadly divided into enterococcus group and viridans and nonhaemolytic groups. Diebel (1964) studied most of the streptococci that have been isolated from faeces and identified as belonging to the group D streptococci and include three principal species, viz., Streptococcus faecalis, Streptococcus faecium and Streptococcus bovis. Geldreich (1970) considers S. bovis and Streptococcus equinus of limited sanitary significance as their survival time in the external environment is low.

Medrek and Litsky (1959) consider enterococci and E. coli as indicators of faecal pollution. Buttiaux (1959) compared the presence of coliforms and faecal

streptococci in water and concluded that group D streptococci are excellent indicators of faecal contamination. Bartley et al. (1960) isolated faecal streptococci from water, sewage and faeces and considered them as the best indicators of pollution even in the absence of coliforms.

Although the faecal streptococci show little tendency to regrow their rapid death rate in temperature greater than 20°C seems to diminish their value as indicators in tropical waters (Evison and James, 1974).

The result of the studies by Hanes et al. (1964) and Kaushik and Bewtra (1965) showed that the enterococcous group may be a better indicator of recent faecal contamination of water than the coliform bacterial group. Geldreich (1970) Sundstrom and Klei (1979) and Purdom (1980) recommended faecal streptococci as an indicator of recent faecal contamination of water.

The findings of Geldreich and Kenner (1969) showed that faecal streptococci densities were significantly higher than faecal coliform densities in all warm-blooded animals' faeces examined, except that of humans. The application of this to some extent is useful in determining the source of faecal pollution in water.

Cooper and Ramadan (1955), Kjellander (1960), Bartley et al. (1960), Kenner et al. (1960), Ramadan and Sabir (1963) and Geldreich (1970) and many others have demonstrated that the presence of typical *S. faecalis* strains would indicate pollution of human origin while the presence of *S. bovis* would point to pollution of animal origin. *S. faecium* was also more predominantly found in animal faeces.

Different workers have used different media for the detection and enumeration of faecal streptococci in water.

Ramandan and Sabir (1963) employed potassium tellurite, sodium azide and thallium acetate broth as the primary isolation media for the recovery of faecal streptococci. But none of the media yielded 100 per cent recovery. Gupta et al. (1978) also used sodium azide broth for the enumeration of faecal streptococci in drinking water from natural sources.

Kenner et al. (1960) cultivated streptococcus from faeces on KF streptococcus medium (KF agar). For the cultivation and enumeration of streptococci in surface waters also Kenner et al. (1961) used KF agar and the

colonies of faecal streptococci appear after 48 h of incubation at 34 to 36°C with red or pink centre.

Geldreich and Kenner (1969) employed KF agar for the recovery of faecal streptococci. They claim that all faecal streptococcus biotypes including S. bovis and S. equinus could be recovered.

Pavlova et al. (1972) reported about the highest recovery of faecal streptococci from natural sources using KF agar and Pfizer selective enterococcus (PSE) agar.

Azide blood agar and streptocel agar are intended for the isolation and enumeration of streptococci in general, whereas, KF agar and M-entrococcus agar are primarily intended for the faecal streptococci or the enterococcus group (Oblinger, 1975).

Brodsky and Schiemann (1976) recovered significantly greater number of faecal streptococci from water using KF agar than did TTC agar based on mean confirmed counts. These investigators found that when incubated at 35°C the KF agar plates showed better recovery of a wide variety of streptococcal biotypes than at 45°C, but with a lower selectivity. Bissonnette et al. (1975)

and Sandhu et al. (1979) employed KF streptococcus agar as a selective medium for the enumeration of S. faecalis.

Medrek and Barnes (1962) noted that S. bovis was the predominant faecal streptococci in the intestine of sheep and cattle. Bovine faecal material contains such higher population of S. bovis (25 per cent of the total faecal streptococcus).

Doran and Linn (1979) suggested that the choice of media is important for the proper enumeration of faecal streptococci in water, where faecal materials from different sources are involved.

Ramadan and Sabir (1963) and Tilton and Litsky (1967) have emphasized the use of triphenyltetrazolium chloride (TTC) reduction test for the identification and classification of S. faecalis and its variants. The tetrazolium test serves as a reliable tool in detecting faecal streptococci with low redox potentials which, in general, are of animal origin.

The ratio of faecal coliform to faecal streptococci (FC/FS) has been used to delineate between human and animal pollution. The application of faecal coliform

faecal streptococci ratio was first proposed by Geldreich et al. (1964). They pointed out that ratio greater than four indicates a human source, but the studies of McFeters et al. (1974) indicates that the ratio can decrease well below four during exposure of the bacteria to water. Geldreich and Kenner (1969) feels that FC/FS ratio is valid only during the 24 h immediately following the discharge of bacteria into the receiving stream. Therefore, the use of the ratio in the case of streams and wells has been questioned.

According to Geldreich (1970) FC/FS ratio of the faeces of certain warm-blooded animals are as follows: man - 4.3; cattle, sheep and poultry - 0.104 to 0.421; and wild animals - 0.0008 to 0.043. The ratios between 0.7 and 4.0 may indicate situations where cattle are localised close to sampling or overflow points as reported by Doran and Linn (1979). Water with FC/FS ratio greater than four indicates pollution from domestic waste water, and ratio less than 0.7 indicates nonhuman animal wastes. Hodges (1977) was of the opinion that FC/FS ratio greater than four indicates human source of contamination and less than 0.5 for contamination by cattle, swine, sheep and poultry;

thus a ratio above 2.5 indicates primarily human contamination, one below 1.0 indicates primarily animal source of contamination, and one in between indicates a relatively even mixture.

2.4.5. Clostridium perfringens

Clostridium perfringens is a commensal inhabitant of the animal and human intestine. It is an opportunistic pathogen and produce diseases. They form spores and having high resistance when present without other intestinal bacteria in water indicate past pollution.

Cl. perfringens grow at an optimum temperature range 35-37°C. Normal density of Cl. perfringens is about 10^4 per gram wet weight of faeces of man and animals. It occours in the soil, sewage and air and is therefore a common environmental contaminant (Cruickshank et al. 1975).

Evison and James (1978) reported that Cl. perfringens is used for the detection of intermittent pollution of temperate and tropical waters.

Bonde (1966) in Denmark has reached the conclusion that the coliform test is less valuable than a test for

Cl. perfringens for faecal pollution. But Willis (1956) earlier concluded that the anaerobes in the water supply do not provide an important index of faecal pollution.

According to Marshall et al. (1965) tryptone sulphate neomycin (TSN) agar gave the maximum count of Cl. perfringens in a lesser incubation period, when compared to sodium sulphite polymixin sulphadiazine (SPS) agar. The addition of thioglycollate buffer enhances the anaerobiasis and therefore it is added when TSN agar is used, under aerobic conditions.

Harmon et al. (1971) compared statistically SPS, TSN and Shahidi-Fergusson-Perfringens (SFP) agar and concluded that TSN agar was the most selective of the three media. Though SPS and TSN agars have almost similar selectivity TSN agar yielded significantly lower recoveries.

Gibbs and Freame (1965) used sulphadiazine and polymixin as selective antibacterial agents for the isolation of Cl. perfringens. Jayne and Williams (1973) tried D. cycloserin and neomycin to suppress the group D streptococci during the isolation of Cl. perfringens.

Cl. perfringens is actively saccharolytic and ferments sugars with gas production. Labee and Duncan (1975) used starch, glucose or maltose in the culture media and obtained a 100 fold increase in the recovery rate of the organism.

2.5. Sanitary survey

Judgement as to the sanitary quality of a water supply is based on the information obtained from two sources: field survey of the water source and laboratory examination of collected samples. The sanitary survey determine the presence or absence of possible sources of pollution. The laboratory examination indicate whether the collected sample of water contain substances or organisms that are indicative of pollution (Steel, 1960; Moore, 1973).

Sandhu et al. (1979) conducted a sanitary survey of the area, in addition to the bacteriological analysis of the rural potable water samples that he collected.

2.6. Season

The monthly variations in the bacterial content of waters depend chiefly on the temperature and the rainfall (Wilson and Miles, 1975).

Burrows (1968) has stated that the microorganisms in the air and soil have relatively ready access to bodies of water under certain conditions as during and immediately after heavy rain.

Voelker et al. (1960) studied the seasonal bacteriological variations in well water and has indicated that season of the year was a major factor controlling the number of bacteria in a well. They got higher counts in the warmer months of the year and concluded that a significant difference in bacterial quality by season exists. But Barrell and Rowland (1979) got a dramatic increase in counts throughout the rainy season rather than in summer. A similar result was obtained by Viraraghavan and Warnock (1976). They found that the bacterial concentration declined in summer and early fall in the ground water. According to Kabler (1968) all coliforms survive longer in cold water than at warmer temperatures. Hagedorn et al. (1978) revealed that the load of faecal indicator bacteria increased in the ground water during heavy rain fall.

Materials and Methods

MATERIALS AND METHODS

3.1. Description of the study site and sanitary survey

The investigation was carried out in the seventh and ninth wards of Ollukkara Panchayat in Trichur district, Kerala State. Geographically, the study site is situated at longitude 76°, 16" east and at latitude 10°, 32" north and the altitude of the place is 22.25 m above the sea level. The site occupies an area of approximately 0.25 km^2 and it comes under the agro-climatic zone of central midland, where the type of soil is laterite (Bureau of economics and Statistics, 1978).

From the seventh and ninth wards, forty households having wells were randomly selected for the present study. A sanitary survey was also conducted on August 23, and 24, 1981 with the help of a ready made proforma to gather information regarding the wells, cattle keeping, presence or absence of possible sources of pollution and nature of the soil (the proforma is given in the appendix).

Based on the data obtained from the sanitary survey and the type of construction, the selected wells

were put in three categories, viz., pucca, pucca-katcha and katcha. Each of the above categories was further subdivided into two groups on the basis of the presence and absence of cattle keeping.

Based on the presence or absence of the parapet, platform, lining or casing and plastering, the wells were grouped as follows: pucca (+ + + +), pucca-katcha (+ +/- + +/-) and katcha (-/+ -/+ - -). The number of wells in each subgroup is given below: pucca well with cattle keeping (W_1A_1) - 4; pucca well without cattle keeping (W_1A_0) - 3; pucca-katcha well with cattle keeping (W_2A_1) - 8; pucca-katcha well without cattle keeping (W_2A_0) - 8; and katcha well with cattle keeping (W_3A_1) - 8; katcha well without cattle keeping (W_3A_0) - 9.

3.2. Collection of water samples for bacteriological examination

The bacteriological examination of water from all the forty wells was carried out from March 1, 1981 to August 31, 1981, for a period of six months embracing both the summer and south-west summer monsoon. The samples were collected either in the morning or in the

evening and the frequency of collection during summer and monsoon was one each from all the wells.

Clean, sterile, wide-mouthed 250 ml reagent bottles with ground glass stoppers and overlapping rims were used for the collection of water samples. Water in none of the wells were cholorinated or treated in any manner. The sample was taken in the bottle from the pail, which was used for hauling water from the respective wells. The pails were thoroughly cleaned and sterilized by means of a blow lamp. The sterilized and cooled buckets were then lowered into the wells and water was drawn. The collection bottles were then placed on a clean cloth and filled with water by pouring from the pail. They were filled up to 2 cm below the stopper.

After the collection of the water samples, the bottles were kept on ice in a thermocol chest, and brought to the laboratory and stored at 5 to 10°C till laboratory analysis could be completed. All samples were processed and incubated within one hour after sampling.

The temperature of the samples was recorded at the time of collection and pH was determined in the laboratory

using a pH meter (Photovolt), to provide a physical evaluation of the waters under investigation.

3.3. Bacteriological analysis

The samples were bacteriologically examined for the standard plate count at 35°C, presumptive coliform count, Escherichia coli, faecal streptococci and Cl. perfringens counts.

The complete processing of the samples was done under an absolutely sterile condition in an inoculation cabinet irradiated with ultraviolet light. The glass-wares used for the study were sterilised at 160°C for 60 to 90 min. All the media used were Hi-media products (Hindustan Dehydrated Media, Bombay, India), unless otherwise specified. The composition of the media and reagents prepared in the laboratory are given in the appendix. The media were prepared at the time of its use and sterilised in autoclave for 15 minutes at 121°C. Petri dishes (Corning) 100 mm outer diameter with the side wall of the bottom 17 mm high were used for the plate counts.

3.3.1. Standard plate count

The sample bottle was shaken twenty five times with rapid rotary motion and serial decimal dilutions

up to 10^{-4} of the sample were made using sterile phosphate buffer (prepared in the laboratory). The SPC was determined by the pour-plate method.

One ml each of the original water sample and the first dilution or any two consecutive dilutions were aseptically transferred to separate sterile petri dishes. Duplicate plates for each dilution were also prepared.

To each plate, approximately 12 to 18 ml of the liquefied plate count agar (standard methods agar) at a temperature of 43° to 45°C was added. The agar and the inoculum were mixed immediately by a combination of to-and-fro shaking and circular movements of the petri dishes. The plates were inverted after the setting of the agar and incubated at 35°C for 24 h (APHA, 1971).

After the incubation, selected a dilution which yielded fewer than 300 colonies and greater than 30 colonies per plate. The arithmetic mean of the colony counts from both the plates at the chosen dilution was multiplied by the dilution factor in order to obtain the SPC at 35°C. This is expressed as the number of

bacteria per milliliter of water.

3.3.2. Presumptive coliform count (Multiple tube technique)

The total number of coliform bacilli in water was determined according to the Standard Methods for the Examination of Water and Waste Water (APHA, 1971) using five fermentation tubes of brilliant green bile broth two per cent per dilution and three dilutions per sample. The following amounts of water were added: five 10 ml quantities each to 20 ml of double strength medium, five 1 ml quantities each to 5 ml of single strength medium and five 0.1 ml quantities each to 5 ml single strength medium.

The inoculated fermentation tubes were incubated for 48 h at 35°C and were examined for gas production in the Durhem tubes and turbidity. The number of positive tubes for each serial dilution were recorded. The result of the test was expressed as the most probable number of coliforms per 100 ml of water. From the probability tables, the MPN index for the various combinations of positive tubes were obtained (Hammer, 1977).

3.3.3. Escherichia coli count

Eosin methylene blue agar, Levine, was used for the quantification of E. coli in water by pour-plate method. One ml each of the undiluted water sample was mixed with the media in duplicate plates, and incubated at 35°C for 24 h. The plates were examined for colonies with greenish metallic lusture in reflected light, which were considered as E. coli. The arithmetic mean of the counts from the duplicate plates were recorded as the number of E. coli per milliliter of water.

In order to identify the E. coli type I colonies from others on EMB agar, Eijkman test was conducted. Few random E. coli colonies from the EMB agar plates were subcultured in 5 ml fermentation tubes containing single strength BGB broth two per cent. Before inoculation, the tubes were warmed to the incubation temperature. The inoculated tubes were incubated at 44.5°C for 24 h in an air incubator. The formation and presence of gas in any amount in the Durham tubes along with turbidity of the broth was considered positive for the presence of E. coli type I, faecal, in the water samples.

3.3.4. Faecal streptococci count

For the enumeration of faecal streptococci in water KF streptococcus agar was used and the procedure was the same as in the case of E. coli count. The molten KF streptococcus agar was cooled to 60°C and added 10 ml of filter-sterilised 1 per cent 2, 3, 5-triphenyl-tetrazolium chloride solution per litre of the medium and mixed thoroughly The media is then allowed to cool to 45°C and used for making pour plates. The plates were incubated for 48 h at 35°C and colonies with a red or pink or pale pink centre were counted as faecal streptococci. The count denoted the number of faecal streptococci per milliliter of water.

3.3.5. Clostridium perfringens count

Tryptone sulphite neomycin (prepared in the laboratory) agar was used for the detection and counting of Cl. perfringens in water using deep layer tubes. One ml quantities of the original water sample were dispensed into duplicate tubes (corning, 25x200 mm).

As the medium was used under aerobic conditions, one litre of the liquefied medium cooled to about 47°C was mixed homogeneously with 25 ml of a buffered

thioglycollate solution, sterilized by filtration. About 30 ml of this medium was mixed with the inoculum in the tube.

Sterilized liquid paraffin was poured over the media in the tubes to form a seal of 2.5 cm thickness in order to create an anaerobic condition. The tubes were incubated at 37°C for 48 h.

Cl. perfringens formed black colonies, which were counted and recorded as the number of Cl. perfringens per milliliter of water.

3.4. Statistical analysis of the data

The data obtained from the bacteriological analysis of water in the different types of wells in both summer and monsoon and the sanitary survey were statistically analysed according to the standard techniques of Snedecor and Cochran (1967).

Results

RESULTS

The results of the bacteriological analysis of water coupled with the sanitary survey of pucca, pucca-katcha and katcha wells in households with and without cattle keeping are furnished hereunder.

4.1. Sanitary survey

The sanitary survey of the wells in the households in the area of investigation brought to light various information. The water samples collected for the bacteriological analysis come from forty wells of three different categories; pucca, pucca-katcha and katcha. All of them were shallow dug wells which were not at all considered sanitary as they were uncovered. The wells were dug in laterite soil with varying depth from 6 to 10 m and average depth 8.4 m. Seventy per cent of the wells had a depth of 8 to 10 m. The depth of laterite soil was only up to 9.5 m beyond which was rock.

Only 65 per cent of the wells had a lining built of either granite or laterite which extended to a depth of 2.45 m (mean 1.2 m). This depth is only one sixth of the minimum recommended standard (6 m). Cement plastering of the lining was done only in 30.5 per cent of the wells.

The others were left without plastering, leaving spaces in between the stones. These spaces were found widened by the penetration of the roots of nereby trees. During monsoon, water co ld percolate through these spaces into the wells. The growth of vegetation on the lining was also noticed in all wells.

All the lined wells had a parapet which ranged in height 0.3 to 1 m above the ground surface. But only 61.5 per cent were having the standard height of 0.7 to 0.75m.

None of the forty wells were having a cement concrete platform of one metre width all around the well. Forty five per cent of the wells had only a rough concrete platform or plinth of about one metre width on one side of the well where people used to stand for drawing water. Platforms of all wells were poorly constructed and many of them formed puddles.

Drains were absent for all wells, but one. Practice of washing clothes and kitchen utensils, washing of animals near the wells and bathing on the platform were common. This resulted in water-logging in the cone of filtratio and the surrounding ground of the wells. During monsoon the storm water also aggravated the water-logging.

However, storm water runoff into katcha wells without parapets was not observed in this area.

Water was drawn by hand from the wells using coir ropes and galvanized iron buckets. After drawing water the buckets were kept either on the platforms or in the puddle or on parapets or on the ground in the case of wells without platforms.

Measurement of the water level in the wells indicates that the water table was very high and its depth ranged 0.4 to 5.6 m (mean 2.45 m), during monsoon.

Majority of the wells were situated near the pollution sources such as cattle sheds, manure pits and latrines, either at the same level of the source of pollution or at the higher or lower level. The average distance of the wells from cattle sheds and the manure pits were 10.2 m (0.5 to 24 m) and 9.9 m (0.25 to 24 m), respectively. The latrines were located at a distance of 14.25 m (2.5 to 28.5 m) from the wells.

The construction of the cattle sheds were not at all according to any standards. The floors of the sheds were either concrete (35%) or stone-laiden(40%) or just soil (25%). In cattle sheds with stone-laiden floor, small

puddles were also present in between the stone. The cattle slurry was disposed in the manure pits either adjacent to the sheds or its vicinity. None of the sheds were having drains.

The latrines in the households were three types, viz., pit latrines, (45%) research-cum-action (R-C-A) (direct) type (45%) and septic tanks (10%). The pits were not lined and plastered.

The temperature of water in the wells was generally in the range 26 to 29°C. The pH of water was always alkaline (7.3 to 8.2).

In 60 per cent of the households the branches of the neroby trees overhung the wells and as a result the dead leaves fall into the wells and increase the organic content of the well water. This also mask the sunlight into wells.

4.2. Bacteriological analysis

4.2.1. Bacterial density

The standard plate count, presumptive coliform count, counts of *E. coli*, faecal streptococci and *Cl. perfringens* in the various types of wells in summer and monsoon are given in Tables 1 to 6. The bacteriological quality of

water in these wells was evaluated by comparison with the recommended water quality standards of APHA (1971). The bacterial density from all the forty wells exceeded the standards revealing the signs of contamination with faecal materials of warm-blooded animals.

The SPC for all the wells fell within the range 4×10^1/ml to 2.9×10^6/ ml. A considerable increase in the SPC was noticed during monsoon. The increase from 4×10^1 to 3.7×10^4/ml in summer to 6×10^1 to 2.9×10^6/ml in monsoon vividly indicating the influence of season on the total bacterial load of water.

The MPN of coliforms ranged 23 to 16,000/100 ml of water. Coliforms were present in 100 per cent of the water samples. Only 10 per cent of the domestic wells had less than 100 coliforms/100 ml during summer while in monsoon the percentage rose up to 17.5 per cent. None of these well waters did meet the health standard cited for unpiped rural supplies which restricts as not more than 10 coliforms/100 ml (Cruickshank et al. 1975).

The information furnished in Tables 1 to 6 indicates that during summer 87.5 per cent, 60 per cent and 80 per cent of wells did harbour E. coli, faecal streptococci and

Cl. perfringens, respectively. During monsoon they were 95 per cent, 65 per cent and 67.5 per cent.

All the wells had shown the presence of at least one or more of the indicator bacteria during both seasons. This indicates that all the wells in the area were invariably contaminated with faecal matter, either recently or remotely. The E. coli count ranged zero to 285/ ml faecal streptococci - zero to 400/ml and Cl. perfringens - zero to 24/ml of water. Except in three wells all others showed a Cl. perfringens count less than 10/100 ml of water.

The mean bacterial counts in water from the different categories of wells with and without cattle keeping in summer and monsoon are summarized in Table 7.

The E. coli colonies from the EMB agar plates were subjected to Eijkman test and was found producing gas at 44.5°C indicating them as E. coli type I.

The bacterial counts of the domestic well waters were statistically analysed. For the analysis the $\log_{10}$ of the SPC and MPN of coliformswere taken, as their counts varied very widely. The data were analysed to assess the influence of cattle keeping and the type of

construction of the wells on the bacteriological quality of water. The seasonal variations in the bacterial counts and the correlation of various characteristics of wells, cattle keeping and latrines were also considered. The contribution of latrines to the total bacterial load and indicator bacteria were also computed.

4.2.2. Influence of cattle keeping

Analysis of data of bacterial counts during summer in all three types of wells with and without cattle keeping are given in Tables 8, 10 and 12. There was no significant difference between wells with and without cattle keeping. Similar data was analysed for monsoon and no significant difference in bacteriological quality of water in wells with and without cattle keeping was observed (Tables 9, 11 and 13).

4.2.3. Type of construction of wells

The bacterial counts of water in pucca, pucca-katcha and katcha wells were analysed season-wise with a view to understanding whether the type of construction of the wells has any influence on the bacteriological quality of well water during the two seasons.

Comparison of the bacterial counts in the three

different types of wells without cattle keeping during summer (Table 14) showed that only Cl. perfringens count in pucca wells were significantly different from that in the other two types of wells. The count was more in pucca wells. No significant difference was noticed in the bacterial counts of all three types of wells without cattle keeping during monsoon (Table 15).

In respect of all the three types of wells, where cattle keeping was present the data showed that the Cl. perfringens was significantly higher in pucca wells than in the other two types of wells during monsoon (Table 17). But no significant difference was noted during summer (Table 16). This shows that the type of construction of well has got no significant influence on the bacterial counts except that of Cl. perfringens.

4.2.4. Seasonal variations in the counts

The influence of summer and monsoon on the bacterial density of well water was also computed.

Table 18 shows that the SPC, E. coli and Cl. perfringens counts in both seasons were significantly different. The SPC was more in monsoon, whereas E. coli and Cl. perfringens were lesser during monsoon (Table 7).

4.2.5. Correlation of characteristics of wells, latrines and cattle keeping on the bacterial counts

Multiple linear regression analysis of the data was conducted in order to measure the correlation of the various independent variables of wells, latrines and cattle kee ing on the SPC, presumptive coliform count and counts of E. coli, FS and Cl. perfringens.

The different characteristics or variables studied were the following: type and depth of wells, depth of lining, type and location of cattle sheds and its distance from the well, number of cattle, location of the disposal of cattle slurry and its distance from the well and type, location and distance of latrines from the wells. These characteristics were ranked for the statistical analysis; higher the pollution potential, higher the rank allotted.

The Tables 19 and 19a show the correlation of all the aforesaid characteristics on the SPC, PCC, counts of E. coli, FS and Cl. perfringens during summer and their regression coefficients, respectively. Corresponding values for monsoon are given in Tables 20 and 20a.

It was found that all the eleven independent variables (the characteristics) were significantly correlated with

the SPC and MPN of coliforms during summer and FS during monsoon.

Out of these characteristics, the depth of well and type of latrine were positively correlated with the SPC by 0.712 and 0.526, respectively. As the depth of the well increased the SPC also increased. Similarly wells in households with pit latrines had the highest SPC. The SPC of well water was lower in households with R-C-A-type latrines and least in those with septic tanks (Table 19a).

The FS count increased correspondingly to the depth of the wells and all factors together could account for 78 per cent of the variations in faecal streptococci count (Table 20a). A negative correlation of FS count with the distance of the latrines from the wells was also noticed.

The correlation of the bacterial counts of water with the characteristics of the wells and latrines in households without cattle keeping during both seasons is presented (Tables 21, 21a, 22 and 22a).

It was found from the analysis that neither the latrines nor the wells had any significant correlation on the bacterial counts in the water during summer (Table 21).

During monsoon the characteristics of wells and

latrines had a significant correlation with the PCC. The type of latrine and its distance from the wells were strongly related to the coliform count by 0.622 and 0.587, respectively. Presumptive coliform count was influenced by the characteristics of wells and latrines to the tune of 58 per cent (Tables 22 and 22a).

In order to assess the contributions from cattle keeping and latrines separately, the same type of analysis was resorted to. For this the bacterial counts of water during summer in all the twenty wells with cattle keeping were considered. The characteristics were significantly correlated with the SPC and MPN of coliforms and could explain for 60 per cent and 52 per cent of the variations in SPC and MPN, respectively (Tables 23 and 23a).

Along with the above characteristics, where cattle keeping was also considered(Table 19a) the influence on SPC was increased to 77 per cent and that on MPN to 80 per cent.

During monsoon significant correlation was observed only with SPC. The depth of the well was correlated with SPC by 0.5874 (Table 24). Here also there was a negative correlation between the distance of the latrines from the

wells and FS count (Tables 20a and 24a). But the overall correlation was not significant probably due to multi-collenearity of the various characteristics.

As per the Tables 19 to 24 is seen that the contribution to all the bacterial counts is influenced more by the latrines than by cattle keeping.

4.3. Faecal coliform-faecal streptococci ratio

The FC/FS ratios in water from all the wells in both seasons are given in table 25. The ratios in water from wells with cattle keeping ranged from 0.00045 to 18.0 with an average of 6.543 in summer and 0.15 to 33.5 with an average of 5.809 in monsoon. The ratios in water from wells without cattle keeping were relatively lower than that from the households with cattle keeping. In summer the ratio averaged 4.456 with a range of 0.045 to 16.5. In monsoon the range was from 0.185 to 3.0 with an average of 1.579. The wide variations in the FC/FS ratios obtained for all wells indicating pollution from animal or human or mixed origin.

Tables

Table 1. Bacterial counts in water from pucca wells with cattle keeping

Season	No.of wells	SPC*	MPN**	E.coli*	FS*	Cl. perfringens*
Summer	1	1,025	920	74	0	2
	2	1,265	1,600	15	1	3
	3	1,810	3,500	11	2	2
	4	2,060	9,200	18	1	0
Monsoon	1	795,000	3,500	6	0	6
	2	121,500	2,400	5	1	2
	3	7,750	16,000	21	3	2
	4	3,650	16,000	20	132	11

* Bacterial counts expressed as organisms per ml

** Coliforms per 100 ml

Table 2. Bacterial counts in water from pucca wells without cattle keeping

Season	No.of wells	SPC*	MPN**	E.coli*	FS*	Cl. perfringens*
Summer	1	95	940	0	0	1
	2	1,645	9,200	99	6	24
	3	1,275	350	17	3	6
Monsoon	1	360	5,400	2	1	0
	2	140,000	1,100	1	2	1
	3	340,000	9,200	9	0	2

* Bacterial counts expressed as organisms per ml

** Coliforms per 100 ml

Table 3. Bacterial counts in water from pucca-katcha wells with cattle keeping

Season	No. of wells	SPC	MPN	*E. coli*	FS	*Cl. perfringens*
Summer	1	540	700	0	0	1
	2	2,280	9,200	2	0	5
	3	2,970	350	1	0	2
	4	1,890	2,400	0	1	0
	5	1,410	5,400	13	12	4
	6	320	70	6	0	2
	7	415	16,000	5	16	5
	8	19,000	1,600	285	9	8
Monsoon	1	665	130	23	3	1
	2	287,500	16,000	26	19	7
	3	2,895,000	90	1	3	2
	4	425	31	9	2	0
	5	8,500	16,000	6	3	1
	6	955,000	1,300	1	0	0
	7	134	33	6	1	0
	8	825,000	490	3	0	1

Table 4. Bacterial counts in water from pucca-katcha wells without cattle keeping

Season	No. of wells	SPC	MPN	*E. coli*	FS	*Cl. perfringens*
Summer	1	720	2,400	23	4	1
	2	2,550	2,400	28	0	0
	3	800	1,600	12	5	5
	4	2,000	79	0	0	0
	5	1,500	3,500	13	1	4
	6	2,430	250	37	0	0
	7	7,650	9,200	74	35	1
	8	420	920	6	0	2
Monsoon	1	176,000	5,400	2	1	2
	2	180,000	3,500	5	0	1
	3	1,000	2,200	3	1	0
	4	2,795,000	80	2	2	0
	5	2,000	9,200	4	0	0
	6	110,000	16,000	5	27	3
	7	360	170	2	6	3
	8	1,055,000	330	2	0	0

Table 5. Bacterial counts in water from katcha wells with cattle keeping

Season	No. of wells	SPC	MPN	*E. coli*	FS	*Cl. perfringens*
Summer	1	42	23	5	0	3
	2	125	3,500	10	1	2
	3	390	46	1	1	3
	4	2,875	16,000	220	89	5
	5	37,600	1,600	20	275	0
	6	2,570	2,400	60	13	0
	7	3,250	920	14	1	20
	8	600	130	2	0	6
Monsoon	1	56	31	5	0	2
	2	175	31	0	1	0
	3	595	49	6	5	2
	4	1,490	16,000	1	1	0
	5	261,500	2,400	16	0	2
	6	31,600	2,400	9	0	0
	7	36,500	16,000	67	2	3
	8	11,450	920	17	0	3

Table 6. Bacterial counts in water from katcha wells without cattle keeping

Season	No. of wells	SPC	MPN	*E. coli*	FS	*Cl. perfringens*
Summer	1	880	9,200	7	5	5
	2	2,580	280	16	3	7
	3	29,300	5,400	18	400	7
	4	300	1,600	18	0	0
	5	420	1,600	8	0	1
	6	705	2,400	0	0	2
	7	80	5,400	1	3	3
	8	895	9,200	5	5	6
	9	2,490	16,000	64	145	2
Monsoon	1	2,500,000	490	3	4	2
	2	260	330	3	1	2
	3	234,500	9,200	13	19	3
	4	36,800	280	5	2	1
	5	11,250	2,400	7	0	2
	6	390	5,400	2	0	0
	7	355	5,400	0	1	0
	8	14,500	2,400	5	0	1
	9	1,040	5,400	6	2	1

Table 7. Mean bacterial counts in water from wells with and without cattle keeping in different seasons

		Summer			Monsoon		
		Pucca	Pucca-katcha	Katcha	Pucca	Pucca-katcha	Katcha
With cattle keeping	SPC	1,540	3,603	5,932	231,975	621,528	42,921
	MPN	3,805	4,465	3,077	9,475	4,259	4,729
	E. coli	30	52	42	13	9	17
	FS	1	10	63	45	5	2
	Cl. perfringens	2	4	7	5	2	2
Without cattle keeping	SPC	1,005	2,259	4,183	160,120	539,920	311,011
	MPN	3,497	2,544	4,076	5,233	4,610	3,477
	E. coli	58	28	17	4	3	6
	FS	5	11	94	2	7	5
	Cl. perfringens	10	3	4	2	2	2

Table 8. Analysis of the bacterial counts of water in pucca wells with and without cattle keeping in summer

Bacterial counts	Source	DF	SS	MS	F
SPC	Cattle keeping	1	0.2807	0.2807	1.41
	Error	5	0.9977	0.1995	
MPN	Cattle keeping	1	0.1147	0.1147	0.35
	Error	5	1.6262	0.3252	
E. coli	Cattle keeping	1	144.0476	144.0476	0.09
	Error	5	8269.6667	1653.9333	
FS	Cattle keeping	1	6.8571	6.8571	1.71
	Error	5	20	4	
Cl. perfringens	Cattle keeping	1	126.2976	126.2976	2.12
	Error	5	297.4167	59.4833	

Table 9. Analysis of the bacterial counts of water in pucca wells with and without cattle keeping in monsoon

Bacterial counts	Source	DF	SS	MS	F
SPC	Cattle keeping	1	0.0671	0.0671	0.04
	Error	5	8.7433	1.7487	
MPN	Cattle keeping	1	0.1105	0.1105	0.54
	Error	5	1.0245	0.2049	
E.coli	Cattle keeping	1	138.8571	138.8571	2.63
	Error	5	264.0	52.8	
FS	Cattle keeping	1	1866.8571	1866.8571	0.73
	Error	5	12812.0	2562.4	
Cl.perfringens	Cattle keeping	1	30.9643	30.9643	2.73
	Error	5	56.75	11.35	

Table 10. Analysis of the bacterial counts of water in pucca-katcha wells with and without cattle keeping in summer

Bacterial counts	Source	DF	SS	MS	F
SPC	Cattle keeping	1	0.0013	0.0013	0.01
	Error	14	3.3993	0.2428	
MPN	Cattle keeping	1	0.0609	0.0609	0.12
	Error	14	7.3656	0.5261	
E.coli	Cattle keeping	1	885.0625	885.0625	0.17
	Error	14	73142.875	5224.4911	
FS	Cattle keeping	1	16.0	16.0	0.17
	Error	14	1309.75	93.5536	
Cl. perfringens	Cattle keeping	1	12.25	12.25	2.33
	Error	14	73.75	5.2679	

Table 11. Analysis of the bacterial counts of water in pucca-katcha wells with and without cattle keeping in monsoon

Bacterial counts	Source	DF	SS	MS	F
SPC	Cattle keeping	1	0.1492	0.1492	0.06
	Error	14	36.0781	2.577	
MPN	Cattle keeping	1	1.0756	1.0756	1.11
	Error	14	13.5374	0.967	
E. coli	Cattle keeping	1	156.25	156.25	3.22
	Error	14	678.75	48.4821	
FS	Cattle keeping	1	2.25	2.25	0.04
	Error	14	872.75	62.3393	
Cl. perfringens	Cattle keeping	1	0.5625	0.5625	0.15
	Error	14	50.875	3.6339	

Table 12. Analysis of the bacterial counts of water in katcha wells with and without cattle keeping in summer

Bacterial counts	Source	DF	SS	MS	F
SPC	Cattle keeping	1	0.00	0.00	0.00
	Error	15	10.1741	0.6783	
MPN	Cattle keeping	1	2.2729	2.2729	3.70
	Error	15	9.2041	0.6136	
E. coli	Cattle keeping	1	2924.5621	2924.5621	1.04
	Error	15	42001.5556	2800.1037	
FS	Cattle keeping	1	870.1176	870.1176	0.06
	Error	15	213626.0	14241.733	
Cl. perfringens	Cattle keeping	1	6.1838	6.1838	0.27
	Error	15	348.875	23.2583	

Table 13. Analysis of the bacterial counts of water in katcha wells with and without cattle keeping in monsoon

Bacterial counts	Source	DF	SS	MS	F
SPC	Cattle keeping	1	0.4969	0.4969	0.28
	Error	15	26.8257	1.7884	
MPN	Cattle keeping	1	0.804	0.804	1.0
	Error	15	12.0017	0.0001	
E. coli	Cattle keeping	1	443.7655	443.7655	1.93
	Error	15	3457.7639	230.5176	
FS	Cattle keeping	1	18.6283	18.6283	0.89
	Error	15	314.4306	20.962	
Cl. perfringens	Cattle keeping	1	0.1176	0.1176	0.09
	Error	15	20.6	1.3333	

Table 14. Analysis of the bacterial counts in water from different types of wells without cattle keeping in summer

Bacterial counts	Source	DF	SS	MS	F
SPC	Types of wells	2	0.422	0.211	0.58
	Error	17	0.1575	0.3622	
MPN	Types of wells	2	0.9114	0.4557	1.20
	Error	17	6.4479	0.3793	
E.coli	Types of wells	2	1281.1028	640.5514	0.87
	Error	17	12509.0972	735.8292	
FS	Types of wells	2	16337.875	8168.9375	0.94
	Error	17	147155.875	8656.2279	
Cl. perfringens	Types of wells	2	166.0083	83.0042	3.77*
	Error	17	374.5417	22.0319	

* Significant at 5% level

Means

T_1 - 10.333; T_2 - 1.635; T_3 - 3.667;

Critical difference for comparison between

T1 and T_2 - 6.705; T_1 and T_3 - 6.603; T_2 and T_3 - 4.812

Table 15. Analysis of the bacterial counts in water from different types of wells without cattle keeping in monsoon

Bacterial counts	Source	DF	SS	MS	F
SPC	Types of wells	2	2.1934	1.0967	0.52
	Error	17	35.5904	2.093	
MPN	Types of wells	2	0.3364	0.1682	0.34
	Error	17	8.302	0.4884	
E. coli	Types of wells	2	13.1861	6.5931	0.69
	Error	17	161.7639	9.5155	
FS	Types of wells	2	29.5194	14.7597	0.28
	Error	17	895.4306	52.6724	
Cl. perfringens	Types of wells	2	0.325	0.1625	0.12
	Error	17	22.875	1.3456	

Table 16. Analysis of the bacterial counts in water from different types of wells with cattle keeping - summer

Bacterial counts	Source	DF	SS	MS	F
SPC	Types of wells	2	0.1344	0.0672	0.14
	Error	17	8.4136	0.4949	
MPN	Types of wells	2	1.2103	0.6052	0.88
	Error	17	11.748	0.6911	
E.coli	Types of wells	2	394.8	197.4	0.03
	Error	17	110905.0	6523.8235	
FS	Types of wells	2	9846.675	4923.3375	1.23
	Error	17	67799.875	3988.2279	
Cl. perfringens	Types of wells	2	27.05	13.525	0.67
	Error	17	345.5	20.3235	

Table 17. Analysis of the bacterial counts in water from different types of wells with cattle keeping - monsoon

Bacterial counts	Source	DF	SS	MS	F
SPC	Types of wells	2	4.1665	2.0832	0.98
	Error	17	36.0567	2.121	
MPN	Types of wells	2	3.8562	1.9281	1.79
	Error	17	18.2834	1.0755	
E. coli	Types of wells	2	134.05	67.025	0.27
	Error	17	4238.75	249.3382	
FS	Types of wells	2	3205.45	1602.725	2.08
	Error	17	13103.75	770.8088	
Cl. perfringens	Types of wells	2	45.0	22.5	3.65*
	Error	17	104.75	6.1618	

* Significant at 5 % level

Means

T_1 - 5.25; T_2 - 1.5; T_3 - 1.5.

Critical difference for comparison between

T_1 and T_2 - 3.207; T_1 and T_3 - 3.207; T_2 and T_3 - 2.619.

Table 18. Analysis of the seasonal variations in the bacterial counts in water

Bacterial counts	Source	DF	SS	MS	F
SPC	Seasons	1	23.6341	23.6341	19.50*
	Error	78	945214	1.2118	
MPN	Seasons	1	0.0906	0.0906	0.13
	Error	78	52.53	0.6735	
E. coli	Seasons	1	9658.0125	9658.0125	5.67*
	Error	78	132831.375	1702.9663	
FS	Seasons	1	7742.1125	7742.1125	2.33
	Error	78	259640.775	3328.7279	
Cl. perfringens	Seasons	1	82.0125	82.0125	5.83*
	Error	78	1097.475	14.0702	

* Significant at 5% level

Means		Critical difference for comparison between
SPC	T_1 - 3.099; T_2 - 4.186.	T_1 and T_2 - 0.49
E. coli	T_1 - 30.2 ; T_2-8.225.	T_1 and T_2 - 18.383.
Cl. perfringens	T_1 - 3.75; T_2-1.725.	T_1 and T_2 - 1.669

Table 19. Influence of the characteristics of wells, cattle keeping and latrines on the bacterial counts during summer

Bacterial counts (y)	Source	DF	SS	MS	F
SPC	Regression	11	6.5824	0.5984	2.44[a]
	Error	8	1.9651	0.2456	
MPN	Regression	11	10.3976	0.9452	2.95[a]
	Error	8	2.5608	0.3201	
E. coli	Regression	11	50032.7311	4548.43	0.19
	Error	8	61267.069	7658.383	
FS	Regression	11	41140.5047	3740.0458	0.87
	Error	8	34456.0453	4307.0057	
Cl. perfringens	Regression	11	164.0451	14.9132	0.57
	Error	8	208.5049	26.0631	

a - Significant at 10% level

Table 19a. Regression lines

y	Constant (a)	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	R^2
SPC	-4.352	-0.022	0.455*	-0.172	0.122	-0.059	0.215	-0.137	0.013	0-321*	-0.002	-0.051	0.77
MPN	4.728	-0.225	-0.099	-0.962	0.127	-0.182*	0.558	-0.247	0.097	0.074	0.003	-0.178	0.80
E.coli	-787.545	13.731	26.557	5.732	-2.53	-10.005	13.12	-17.226	4.461	-13.721	2.454	46.238	0.45
FS	-259.365	22.259	11.827	-11.257	-1.969	- 3.864	36.305	-19.662	4.54	- 0.296	2.281	-3.225	0.54
Cl.per fringens	25.547	1.138	1.194	-0-359	0.269	0.05	-1.999	0.599	-0.386	1.556	-0.132	-1.379	0.44

X_1 - Type of well
X_2 - Depth of well
X_3 - Depth of lining
X_4 - Type of cattle shed
X_6 - Location of shed
X_7 - Number of animals
X_8 - Distance between manure pit and well
X_9 - Type of latrine
X_{10} - Distance between latrine and well
X_{11} - Location of latrine

Table 20. Influence of the characteristics of wells, cattle keeping and latrines on the bacterial counts during monsoon

Bacterial counts	Source	DF	SS	MS	F
SPC	Regression	11	28.4055	2.58232	1.75
	Error	8	11.8174	1.47717	
MPN	Regression	11	14.4289	1.31171	1.36
	Error	8	7.7107	0.96384	
E.coli	Regression	11	2993.479	272.13445	1.58
	Error	8	1379.321	172.41513	
FS	Regression	11	12704.7259	1154.97509	2.56[a]
	Error	8	3604.4741	450.55926	
Cl. perfringens	Regression	11	86.4613	7.86012	0.99
	Error	8	63.2887	7.91108	

a - Significant at 10% level.

Table 20a. Regression lines

Y	Constant (a)	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	R^2
SPC	-19.698	-0.502	0.096	0.333	-0.658	0.002	-0.564	-0.345	0.069	0.16	0.3	1.418	0.71
MPN	-2.598	-0.09	0.021	-0.652	-0.469	-0.233	-0.074	-0.251	0.189	0.128	-0.006	0.537	0.65
E. coli	133.408	4.307	6.554	-3.930	1.856	-0.782	-3.147	6.199	-0.104	7.685	-0.724	-10.992	0.68
FS	72.198	0.423	19.374*	-8.475	6.654	-5.389	20.897	0.529	1.749	6.384	-2.806*	-29.465	0.78
Cl. perfringens	-12.918	-0.18	1.381	0.58	-0.704	-0.405	-0.529	-0.24	0.201	0.085	-0.199	0.723	0.58

X_1 - Type of well
X_2 - Depth of well
X_3 - Depth of lining
X_4 - Type of cattle shed
X_5 - Distance between shed and well
X_6 - Location of shed
X_7 - Number of animals
X_8 - Distance between manure pit and well
X_9 - Type of latrine
X_{10} - Distance between latrine and well
X_{11} - Location of latrine

Table 21. Influence of the characteristics of wells and latrines on the bacterial counts in summer (households without cattle keeping)

Bacterial counts	Source	DF	SS	MS	F
SPC	Regression	6	2.0773	0.34622	0.99
	Error	13	4.5353	0.34887	
MPN	Regression	6	2.5148	0.41913	1.31
	Error	13	4.16	0.32	
E. coli	Regression	6	5059.0454	843.17431	1.26
	Error	13	8731.1541	671.62724	
FS	Regression	6	47450.0365	7908.33942	0.88
	Error	13	116223.71348	8940.28565	
Cl. perfringens	Regression	6	155.4008	25.90014	0.87
	Error	13	385.1492	29.62686	

Table 21a. Regression lines

y	Constant (a)	X_1	X_2	X_3	X_4	X_5	X_6	R^2
SPC	-0.181	-0.001	0.338	0.021	0.203	-0.043	-0.075	0.31
MPN	-2.531	0.424*	-0.063	0.446	-0.0004	0.032	-0.094	0.38
E. coli	229.2166	0.869	-0.82	16.241	9.191	-1.606	6.70	0.37
FS	637.792	9.386	26.851	-20.604	24.308	-0.18	-41.011	0.29
Cl. perfringens	43.558	1.599	- 0.053	3.412	0.759	0.154	0.991	0.29

X_1 - Type of well
X_2 - Depth of well
X_3 - Depth of lining
X_4 - Type of latrine
X_5 - Distance between latrine and well
X_6 - Location of latrine

Table 22. Influence of the characteristics of wells and latrines on the bacterial counts in monsoon (households without cattle keeping)

Bacterial counts	Source	DF	SS	MS	F
SPC	Regression	6	7.2	1.2	0.51
	Error	13	30.583	2.352	
MPN	Regression	6	6.534	1.089	3.00*
	Error	13	4.727	0.363	
E. coli	Regression	6	48.5205	8.087	0.83
	Error	13	126.4295	9.725	
FS	Regression	6	158.2441	26.374	0.45
	Error	13	266.706	58.977	
Cl. perfringens	Regression	6	5.884	0.981	0.74
	Error	13	17.316	1.332	

* Significant at 5% level

Table 22a. Regression lines

y	Constant (a)	X_1	X_2	X_3	X_4	X_5	X_6	R^2
SPC	4.93	-0.506	-0.102	-0.581	-0.381	0.052	0.032	0.19
MPN	-0.3034	0.058	-0.218	0.221	0.273	0.047	-0.103	0.58
E. coli	9.619	0.175	1.726	-1.047	0.89	-0.021	-0.448	0.28
FS	14.8695	-1.793	0.041	-1.904	2.418	-0.384	0 .043	0.17
Cl. perfringens	-4.2184	0.19	0.548	0.088	0.198	-0.062	0.143	0.25

X_1 - Type of well
X_2 - Depth of well
X_3 - Depth of lining
X_4 - Type of latrine
X_5 - Distance between latrine and well
X_6 - Location of latrine

Table 23. Influence of the characteristics of wells and latrines alone (in households with cattle keeping) on the bacterial counts in summer

Bacterial counts	Source	DF	SS	MS	F
SPC	Regression	6	5.144	0.857	3.27[a]
	Error	13	3.404	0.262	
MPN	Regression	6	6.7288	1.1215	2.34[a]
	Error	13	6.2295	0.4792	
E. coli	Regression	6	40225.6761	6704.2793	1.23
	Error	13	71074.1239	5467.2403	
PS	Regression	6	20510.0539	3418.3423	0.81
	Error	13	54654.7461	4204.2112	
Cl. perfringens	Regression	6	85.771	14.2952	0.65
	Error	13	286.779	22.0599	

a Significant at 10 % level

Table 23a. Regression lines

y	Constant (a)	X_1	X_2	X_3	X_4	X_5	X_6	R^2
SPC	-1.465	-0.0893	0.3289*	-0.2326	0.1062	0.0038	0.1747	0.6
MPN	6.7695	-0.4109*	-0.255	-1.1183*	-0.28	-0.0022	0.2336	0.52
E.coli	-754.0434	3.8618	18.4659	-10.2701	-28.9145	1.9363	53.8427	0.36
FS	-294.8516	25.1085	2.889	21.3463	-15.7569	0.1305	8.3055	0.27
Cl. perfringens	29.8994	1.1696	-0.2454	0.5129	1.1892	0.0231	-2.2821	0.23

X_1 - Type of well
X_2 - Depth of well
X_3 - Depth of lining
X_4 - Type of latrine
X_5 - Distance between latrine and well
X_6 - Location of latrine

Table 24. Influence of the characteristics of wells and latrines alone (in households with cattle keeping) on the bacterial counts in monsoon

Bacterial counts	Source	DF	SS	MS	F
SPC	Regression	6	23.6536	3.9423	3.6*
	Error	13	16.5696	1.2746	
MPN	Regression	6	8.2862	1.3810	1.3
	Error	13	13.3533	1.0696	
E. coli	Regression	6	1973.4194	328.9032	1.78
	Error	13	2399.3806	184.5677	
FS	Regression	6	6790.013	1131.6688	1.55
	Error	13	9519.187	732.2452	
Cl. perfringens	Regression	6	54.2448	9.0408	1.23
	Error	13	95.5052	7.3466	

* Significant at 5% level

Table 24a. Regression line

y	Constant (a)	X_1	X_2	X_3	X_4	X_5	X_6	R^2
SPC	-11.5481	-0.5424	0.4038	0.1592	0.5697	0.018	0.5904	0.59
MPN	5.6077	-0.3437	0.108	-0.7101	0.2314	-0.0079	-0.002	0.37
E. coli	170.0956	3.9368	3.2639	2.3718	5.5352	-0.1132	-13.4461*	0.45
FS	88.704	-5.1358	11.8656	-2.6502	-4.3597	-2.4485*	-5.8209	0.41
Cl. perfringens	-3.3039	-0.6244	1.066	0.7213	0.1587	-0.1686	-0.2922	0.36

X_1 - Type of well
X_2 - Depth of well
X_3 - Depth of lining
X_4 - Type of latrine
X_5 - Distance between latrine and well
X_6 - Location of latrine

Table 25. FC/FS ratios in well water from households with and without cattle keeping

	No. of wells	FC/FS ratios	
		Summer	Monsoon
With cattle keeping	1	15.0	5.0
	2	5.5	7.0
	3	18.0	0.15
	4	1.08	7.66
	5	0.3125	1.368
	6	10.0	0.33
	7	1.0	4.5
	8	2.47	2.0
	9	0.00045	1.2
	10	4.615	6.0
	11	14.0	1.0
	12	..	33.5
Without cattle keeping	1	16.5	2.0
	2.	5.666	0.5
	3	5.75	2.0
	4	2.4	3.0
	5	13.0	1.0
	6	1.644	0.185
	7	1.4	0.33
	8	5.3	0.75
	9	0.045	3.0
	10	0.33	0.684
	11	1.0	2.5
	12	0.441	3.0

Discussion

DISCUSSION

Next to air, water is essential for the existence of life. But many diseases affecting man as well as animals are water-borne. Though wholesome water is ideal, it is not available in all places at all time. People in developing countries depend on well water in majority of cases. The wells in the villages in India are generally shallow type and improperly constructed and maintained. As observed, such wells are likely to be polluted from different sources such as animal sheds, manure piles, agricultural land manured with animal excreta, compost pits and latrines. The conditions in Kerala are not much different from others in this matter. Due to limitations of land the houses were built in limited holdings and the cattle were kept very close to human dwellings. The wells were also sunk not far from the human and animal habitations. Under the above circumstances it was appropriate to make a study of the extend of contamination of well water due to cattle keeping and factors related to that.

The classification of the wells into pucca, pucca-katcha and katcha was made to differentiate them on the basis of the type of construction. Cattle keeping as a probable source of contamination, the wells were further categorised

on the basis of the presence and absence of cattle keeping in the households.

The sanitary survey of the wells in forty households selected at random from the area of investigation revealed that none of them satisfy the requirements of a sanitary well, being shallow open wells. Poor sanitary quality of such wells were earlier reported by Wilson and Miles (1975). They were poorly designed from a sanitary point of view. Their constructions were imperfect and protection was inadequate. Therefore, there was every possibility of contamination of water in the wells from the surrounding sources of pollution. There were many sources of pollution for all wells such as want of cover at the top of wells, human activity such as bathing, washing of clothes, utensils and animals, proximity to latrines, animal sheds and manure pits, use of dirty buckets and ropes for drawing water and improper maintenance of the wells. Though there was no storm water runoff into the wells, water which stagnated around the shaft of the wells could seep through the lining stones into the wells. The average distance of the wells from cattle sheds, manure pits and latrines was lesser than that recommended by Park (1971), enhancing the chances of water pollution.

In order to assess the bacteriological quality of well water, the SPC, MPN of coliforms, E. coli, FS and Cl. perfringens were determined. Their counts far exceeded the recommended standards indicating contamination with the faecal materials of warm-blooded animals. As the counts of indicator bacteria were not considerably high in any well any time of collection, direct contamination with the faecal materials could not be suspected. This was supported by the fat that during summer only 87.5 per cent, 60 per cent and 80 per cent of the wells did harbour E. coli, FS and Cl perfringens, respectively. Whereas in monsoon they were 95 per cent, 65 per cent and 67.5 per cent. The presence of E. coli and FS gave an idea of recent pollution of wells.

The absence of FS and presence of E. coli and Cl. perfringens in wells showed that they had been recently polluted prior to the day of collection of water. This was because of the rapid die-off rate of faecal streptococci out side its natural host. Some of the wells showed the presence of Cl. perfringens only which indicated a past pollution. Any how, all the wells were invariably contaminated with faecal matter, but indirectly as the counts of indicator bacteria were low.

The total bacterial count at 35°C suggested the possible presence of parasitic bacteria of warm-blooded animals rather than saprophytic bacteria, though they may also grow slowly even at 35°C as has been reported by Cruickshank et al. (1975). A hundred-fold increase in the SPC at 35°C revealed a sudden change in the sanitary conditions of the wells during monsoon. But this increase in SPC was not accompanied by a corresponding rise in the indicator bacteria. Though a slight increase in the presumptive coliform count was observed; it was not statistically significant. Therefore, it is presumed that the entry of soil bacteria into the well water was responsible for the enhanced SPC during monsoon. Gilereas (1975) suggested the sudden increase in the SPC as an indication of pollution with surface drainage or sewage. Unhygienic human activities and the use of dirty ropes and buckets for drawing water itself were sufficient to pollute water with the surface soil. The surface soil surrounding the wells were contaminated with the excreta of warm-blooded animals and birds, especially during monsoon owing to water logging.

Percolation of unsufficiently filtered water through the spaces in the lining of the wells could also be another

reason for the unilateral increase in the SPC during monsoon. This conclusion is supported by the observations of Steel (1961). Though in the present study a change in the SPC in association with the onset of rains was found, the report of Barrell and Rowland (1979) do not support this.

The statistical analysis of the SPC revealed that the counts were not significantly different in different types of wells and among wells with and without cattle keeping. This observation was in agreement with that of Sandhu et al. (1979). He found that the basic well design had little effect on the extent of bacteriological pollution of water. A significant positive correlation with the type of latrine was found. The counts increased in the households with pit latrines than R-C-A type and septic tanks. The pits were not lined or plastered. The depth of latrines were almost the same as that of wells in some households and it could permit cross contamination between wells and latrines during monsoon. When the water table rose up during monsoon, bacteria could move through the laterite soil into the wells. The movement of septic tank effluent laterally through crevices in the rock for great distances with little change in its bacteriological characteristics was reported by

Vogt (1961). But the water movement through laterite soil was not studied in this area.

Geldreich (1970) observed a positive correlation between a coliform count and warm-blooded animal's faecal contamination. The coliform count includes both faecal and non-faecal coliforms, which were differentiated by means of Eijkman test.

In all the wells the total coliform density was higher than the prescribed standards for shallow wells. Before the onset of rains, counts were relatively low. But higher counts were observed during the early weeks of the rains. This increase was statistically not significant. The slight variation in the count may be due to the nonfaecal coliforms of soil origin as evidenced by a statistically significant reduction in the E. coli count during monsoon. The MPN of coliforms in water were high even when E. coli were absent. This showed the presence of nonfaecal coliforms. The high increase in the nonfaecal coliforms in water indicate either contamination of the well water with soil or with faecal matter at a time sufficiently remote to allow faecal coliforms to die out. In tropical waters the nonfaecal coliforms appear in abundance. This might be the cause of rapid

increase in the SPC and the slight increase in MPN of coliforms, but reduction in indicator bacterial counts. Similar conclusion was reached by APHA (1971).

It was observed that the depth of lining of the well and the distance between the well and cattle shed influenced the MPN of coliforms. They were negatively correlated. As the depth of the lining increased the count decreased, so also farther the cattle shed, lower the presumptive coliform count. Minton (1975) and Barrell and Rowland (1979) have also observed that some degree of protection from pollution was afforded by lining the wells.

Though pH of the well waters was noted its correlation with the counts were not computed as they were all on alkaline side without much variation in both seasons. Sandhu et al. (1979) found a weak positive correlation between pH and total coliforms in drinking water.

The presence of indicator bacteria in water is accepted as a clear indication of its contamination with faecal materials. The faecal coliforms(E. coli) and FS grown at 35°C were the commonly accepted bacterial indicators of pollution (Moterman et al. 1974). Cl. perfringens, FS and E. coli were considered as common indicator bacteria and

pathogens by Cruickshank et al. (1975) and Harigan and McCance (1976). Faecal coliforms, FS and Cl. perfringens along with entroviruses and P. aeruginosa could be used as indicators of faecal pollution of water (Verstraete and Voets, 1975). Therefore, the organisms which have grown at 35°C in the respective selective media for E. coli, FS and Cl. perfringens could be considered as indicators of faecal pollution. All the wells had at least one or more of this bacteria showing that they had been polluted currently or in the past with the faecal matter of human and/or animal sources.

The wells from households without cattle keeping have also shown E. coli and other indicator bacteria. This could come from either the latrines or from the vegetations on the lining of the wells or insects which have part of their life cycle in cow dung, as suggested by Geldrich (1970). Unhygienic human activities could also contribute to the contamination of well water.

The E. coli isolates from all samples being positive to Mijkman test indicated them as E. coli type I. For confirmation indole production at 44.5°C was suggested by Barrel and Rowland (1979). But Sojka (1965) and Geldreich (1970)

have suggested Eijkman test for the detection of E. coli type I. But in tropical waters Klebsiella and Citrobacter groups also grow at 44°C as observed by Katugampola (1958), Mousse (1965) and Evison and James (1973).

There was no significant difference between the counts of E. coli, FS and Cl. perfringens in water collected from the wells in households with and without cattle keeping. But a seasonal reduction during monsoon was observed in these counts. This may be due to the dilution as a result of increase in the volume of well water or due to rapid die-off of the indicator bacteria as observed by McFeters et al. (1979). He observed that the die-off rates of coliforms were slightly more rapid that the FS. Steel (1960) found that coliforms die at logarithmic rate and few individuals existed for weeks or months. Similarly Cooke (1974) reported that E. coli did not multiply in natural waters but only survived for a limited time. Though E. coli type I die out in soil faster, as observed by Patterson et al. (1974), they could persist in surface soil underlying cow manure for 20 weeks (Thomas and Druce, 1955). As the temperature of water ranged 26 to 29°C during the study period, the E. coli could die off faster than nonfaecal coliforms. Yet another reason for the slump in the E. coli

count during monsoon might be due to its incompetency to survive with the natural bacterial flora of water.

The sources of indicator bacteria could be either from animals or from latrines. The survival of these bacteria in soil is limited but in cow dung it is longer. The depths of latrines were almost the same as that of wells in some house and it could help in the cross contamination between the wells and latrines during monsoon. The presence of FS in water indicated recent pollution. From the study it was observed that the Cl. perfringens counts in pucca wells during summer was significantly higher than in the other two types of wells, irrespective of cattle keeping. The count of Clostridium perfringens was significantly higher during summer than during monsoon.

From the results of the study it can be concluded that the type of well do not have any direct bearing on the bacteriological quality of well water. The sources of pollution could be different, such as soil, vegetables, animals and man. These well waters had shown clear indication of faecal pollution evidenced by the presence of indicator bacteria during all seasons, irrespective of the type of construction of wells. Though visibly there was

an increase in the bacterial load from those wells in the households having animal keeping than those without. This difference was not statistically significant. The absence of proportionate increase of coliform and indicator bacteria during monsoon corresponding to the SPC was probably because of t eir inability to survive and move through soil in adverse conditions. Coliforms and enterococci bacteria in animal wastes were removed by adsorption during soil percolation and by die-off because of their inability to compete with the established soil microflora. Several environmental variables, viz., the temperature, chemical composition and pH of wate not only affect the microbial population in aquatic environments but also determine the removal of bacteria by soil from percolating water.

The *E. coli* isolates could be considered as type I of faecal origin. The consideration of FC/FS ratio gave no indication of the relative im ortance of animal or human source of faecal contamination. Most results falling in the range 1.5 to 6.5 indicated both animal and/or human source of contamination or their admixture. As the exact time of faecal pollution of well water was no known the application of the FC/FS ratio to distinguish between the

animal and human source of pollution was not of much relevance. Geldreich and Kenner (1969) feels that FC/FS ratio is valid only during 24 h immediately following the discharge of bacteria into water and the use of ratio in the case of wells is not recommended.

Among the many factors which were responsible for the bacteriological quality of water, those included in the sanitary survey, were assessed for their role in the bacteriological quality of well water. It was found that the depth of well and type of latrine have a positive correlation with SPC and the depth of lining has a negative correlation with coliform count. The existence of the latrine also got a negative correlation with respect to MPN. The FS count has a positive correlation with depth of wells and a negative correlation with distance of latrines from the wells. The distance of the cattle shed and the type of construction and location did not show any significant difference in the bacterial counts in water. The significant difference in the Cl. perfringens count in water from pucca wells during summer cannot be explained. It is concluded that the cattle keeping has no significant influence on the bacte iological quality of domestic well water in Manmuthy area though all wells showed evidence of faecal contamination.

Summary

SUMMARY

Water in open shallow wells are known for its unsatisfactory quality from the sanitary point of view as it could be polluted from different sources. In the present study, an investigation was conducted to evaluate the bacteriological quality of domestic well water in Mannuthy area in the light of its total bacterial load, presumptive coliform count and the counts of common indicator bacteria of faecal origin such as E. coli, FS and Cl. perfringens.

Equal number of wells from households keeping cattle and otherwise were selected to find the influence of cattle keeping on the bacteriological quality of well water. Forty households having wells were randomly selected from the seventh and ninth wards of Ollukkara Panchayat, Trichur District, Kerala State for collection of water samples. The samples for bacteriological examination were collected for six months from March 1, 1981 to August 31, 1981, embracing both summer and south-west monsoon. A sanitary survey of the above wells was also conducted with the aid of a prepared proforma. The survey was conducted on August 23 and 24, 1981. The sanitary survey revealed that all the wells in the study area were shallow, open, dug wells which were not at all sanitary from the point of view of their construction and maintenance

All the wells were having proximity to different sources of pollution such as latrines, manure pits, cattle slurry, etc. They were located at an average distance of 14.25 m, 9.9 m and 10.2 m, respectively.

The water samples were collected aseptically in wide-mouthed reagent bottles and transported to the laboratory on ice in a thermocol chest. Water was drawn from wells using pails which were thoroughly cleansed and sterilized by means of a blow lamp. In the laboratory the water samples were stored at 5 to 10°C till they were subjected to analysis. Temperature and pH of the samples were also recorded. Standard methods agar, BGB broth, EMB, KF and TSN agar plates were used for the detection and enumeration of SPC, MPN of coliforms, E. coli, FS and Cl. perfringens, respectively. In order to identify the E. coli type I among the E. coli colonies on EMB agar plates, Eijkman test was conducted.

Water in all the wells had a very high bacterial load than prescribed for a sanitary well. The SPC in water from households with cattle keeping were higher than in those without cattle keeping. But statistically this difference was not significant. The SPC during monsoon increased

hundred-fold than in summer. But a statistically insignificant increase in the MPN of coliforms was noticed during monsoon. The indicator bacteria were found reduced during monsoon but significant reduction was observed only in the case of *E. coli* and *Cl. perfringens*.

The SPC and FS counts were found to have a positive correlation with the depth of wells. The SPC was also positively correlated with the type of latrine. The MPN of coliforms and depth of lining were negatively correlated. So also the distance between well and latrine is negatively correlated with the FS count. The analysis showed that the contribution from latrines to the bacteriological quality of water was more than from cattle keeping.

The bacteriological analysis revealed that all wells were contaminated with the faecal materials of warm-blooded animals either recently or remotely. By using FC/FS ratio in water the source of contamination could not be ascertained unequivocally.

The bacteriological quality of water in households with and without cattle keeping were not significantly different. This shows that cattle keeping has got no influence on the bacteriological quality of well water in Mannuthy area.

The type of construction of wells has also got any influence on the bacteriological quality of well water in the light of its SPC, presumptive coliform count and counts of E. coli, FS and Cl. perfringens.

References

REFERENCES

American Public Health Association (1953). Standard Methods for Examination of Dairy Products. 10th ed. American Public Health Association, Inc., New York. p. 323.

American Public Health Association (1971). Standard Methods for the Examination of Water and Waste Water. 13th ed. American Public Health Association, Inc., New York.

*Barker, J.C. and Sewell, J.I. (1972). Effects of Spreading Manure on ground Water and Surface Runoff. Paper presented at 1972 Annual Meeting ASAE, Hot Springs, Ark., June 27-30. Cited by Jansen, J.J., Sodine, A.B. and Lusscz, L.J. (1974)

Barrell, R.A.E. and Rowland, M.G.M. (1979). The relationship Between Rainfall and Well Water Pollution in a West African (Gambian) Village. J. Hyg. 83: 143-150.

Bartley, C.H., Clara, H. and Slanetz, L.W. (1960). Types and Sanitary Significance of Faecal Streptococci Isolated from Faeces, Sewage and Water. Am. J. Public Health. 50 : 1545-1552.

*Bhatta, M.V.P. (1966). A study of Coli-aerogenes Contamination of Drinking Water From Human Hands. Indian J. Pub. Hlth. 10 : 129-132.

Bissonnette, G.K., Jezeski, J.J., McFeters, G.A. and Stuart, D.G. (1975). Influence of Environmental Stress on Enumeration of Indicator Bacteria from Natural Waters. Appl. Microbiol. 29 : 186-194.

Bolton, . (1961). Water Source Contamination. J. Am. Water Works Assoc. 53 : 1243-1250.

*Bonde, G. J. (1966). Bacteriological Methods for Estimation of Water Pollution. Health Lab. Sci. 3 : 124.

Brodsky, M.H. and Schiemann D.A. (1976). Evaluation of PSR and KF Media for Recovery of Faecal Streptococci from Water by Membrane Filter. Appl. Environ. Microbiol. 31 : 695-699.

Bureau of Economics and Statistics (1978). Kerala in Maps. Bureau of Economics and Statistics, Government of Kerala, Trivandrum. Map No. 9, 11.

Burrows, W. (1968). Text Book of Microbiology, 19th ed. W.B. Saunder's Co., Philadelphia. pp. 310-317.

Buttiaux, R. (1959). The Value of the Association of Escherichia, Group D Streptococci in the Diagnosis of Contamination of Foods. J. Appl. Bacteriol. 22: 153-158.

Buttiaux, R. and Mossel, D.A.A. (1961). The significance of Various Organisms of Faecal Origin in Foods and Drinking Water. J. Appl. Bacteriol. 24 : 353-364.

Campbell, R. (1979). Microbiology of Soil, Air and Water. In Micro-organisms. Function, form and Environment. 2nd ed. Hawker, L.E. and Linton, A.H. (Eds). Edward Arnold, London. pp. 263-273.

Christie, A.E. and Christie, M.C. (1971). Food Hygiene and Food Hazards. Faber and Faber, London. pp. 83-84.

*Ciravolo, T.G., Martens, D.C., Hallock, D.L., Collins, E.R. Jr., Kornegay, E.T. and Thomas, H.R. (1979). Pollutant Movement to Shallow Ground Water Tables from Anaerobic Swine Waste Lagoons. J. Environ. Qual. 8 : 126-130.

Clark, H.F. and Kabler, P.W. (1964). Re-evaluation of the Significance of the Coliform Bacteria. J. Am. Water Works Assoc. 56 : 931-936.

Cooke, E.M. (1974). Escherichia coli in Man. Churchill Livingston, Edinburg. pp. 13-16.

Cooper, K.E. and Ramadan, F.M. (1955). Studies in the Differentiation Between Human and Animal Pollution by means of Faecal Streptococci. J. gen. Microbiol. 12 : 190-190

*Crane, S.R., Westerman, P.W. and Overcash, M.R. (1980). Die-off of Faecal Indicator Organisms Following Land Application of Poultry Manure. J. Environ. Qual. 9 : 531-537.

Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. (1975). Medical Microbiology, Vol. II, 12th ed. Churchill Livingstone, Edinburgh. pp. 273-280.

Deaner, D.G. and Kerri, K.D. (1969). Regrowth of Faecal Coliform. J. Am. Water Works Assoc. 61 : 465-468.

*Diebel, R.H. (1964). The Group D Streptococci. Bacteriol. Rev. 28 : 330-366.

*Diesch, S.L. and McCulloch, W.F. (1966). Isolation of Pathogenic Leptospires from Water used for Recreation. Pub. Health Rep. 81 : 299.

Doran, J.W. and Linn, D.M. (1979). Bacteriological Quality of Runoff Water from Pasture Land. Appl. Environ. Microbiol. 37 : 985-991.

*Dutka, B.J. (1973). Coliforms are an Inadequate Index of Water Quality. J. Environ. Health. 36 : 39-46.

*Escherish, T. (1885). Die Darmbakterien des Neugeborenen und Saeuglinge. Fortschr. Med. (Ger.). 3 : 515-547.

*Evison, L.M. and James, A. (1973). A Comparison of Distribution of Intestinal Bacteria in British and E. African Water Sources. J. Appl. Bacteriol. 36 : 109-118.

*Evison, L.M. and James, A.(1974). Bifidobacterium as Indicator of Faecal Pollution in Water. Paper presented at 7th international conference on water pollution research, Paris, September . Cited by Evison, L.M. and James, A. (1978).

Evison, L.M. and James, A. (1978). Microbiological Criteria for Tropical Water Quality. In, Water, Wastes and Health in Hot Climates. Feachem, R., McGarry, M. and Mara, D. (Eds.). ELBS and John Wiley and Sons, Chichester. pp. 30-51.

*Fair, J.R. and Morrison, S.M. (1967). Recovery of Bacterial Pathogens from High Quality Surface Water. Water Resour. Res. 3 : 799-803.

Geldreich, E.E. (1970). Applying Bacteriological parameters to Recreational Water Quality. J. Am. Water Works Assoc. 62 : 113-120.

*Geldreich, E.E. (1976). Faecal Coliform and Faecal Streptococcus Density Relationships in Waste Discharges and Receiving Waters. Crit. Rev. Environ. Control. 6: 349-369.

*Geldreich, E.E., Best, L.C., Kenner, B.A. and Van Donsel, D. J. (1968). The Bacteriological Aspects of Storm Water Pollution. J. Water Pollut. Contr. Fed. 40 : 1861-1872.

*Geldreich, E.E., Clark, H.F. and Huff, C.B. (1964). A Study of Pollution Indicators in a Waste Stabilization Pond. J. Water Pollut. Contr. Fed. 36 : 1372-1379.

Geldreich, E.E., Huff, C.B., Bordner, R.H., Kabler, P.W. and Clark, H.F. (1962). The Faecal Coli-aerogenes Flora of Soil from Various Geographic areas. J. Appl. Bacteriol. 25 : 87.

*Geldreich, E.E. and Kenner, B.A. (1969). Concepts of Faecal Streptococci in Stream Pollution. J. Water Pollut. Contr. Fed. 41 : R 335- R 352.

Geldreich, E.E., Nash, H.D., Reasoner, D.J. and Taylor, R.H. (1975). The Necessity for Controlling Bacterial Population in Potable Waters, Bottled Water and Emerging Water Supplies. J. Am. Water Works Assoc. 67 : 117-124.

Ghosh, B.N. (1959). A Treatise on Hygiene and Public Health. 4th ed. Scientific Publishing Company, Calcutta. p. 39.

Gibbs, B.M. and Freame, B. (1965). Methods for the Recovery of Clostridia from Foods - Symposium on Clostridia paer XI. J. Appl. Bacteriol. 28 : 95-111.

Gilcreas, F.W. (1975). Water - Bacteriological Examination. In Standard Methods of Chemical Analysis, Vol. II., 6th ed. Welcher, F.J. (Ed.). Robert. E. Krieger Publishing Co., New York. p. 2509

Gupta, P., Ahuja, S., Saran, G. and Thomas, A.K. (1978). Bacteriological Survey of Drinking Water from Natural Sources around Kasauli, H.P. Indian J. Pathol. Microbiol. 21 : 213-217.

*Guy, E.M. and Small, J.A. (1977). Survival of Streptococci and Coliforms in Bovine Faecal Origin in Drainage Water and Soil Stored at Different Temperatures. New Zealand J. Agricultural Research. 20 : 13-18.

*Hagedon, C., Hansen, D.T. and Dimonson, G.H. (1978). Survival and Movement of Faecal Indicator Bacteria in Soil under Conditions of Saturated Flow. J. Environ. Qual. 7 : 55-58.

Hall, H.E., Brown, D.F. and Lewis, K.H. (1967). Examination of Market Food for Coliform Organisms. J. Appl. Microbiol. 15 : 1062-1069.

Hammer, M.J. (1977). Water and Waste Water Technology. John Wiley and Sons. Inc., New York. p. 68.

Hanes, N.B., Sarles, W.B. and Rohlich, G.A. (1964). Dissolved Oxygen and Survival of Coliform Organisms and Enterococci. J. Am. Water Works Assoc. 56 : 441-446.

Harmon, S.M., Kautter, D.A. and Peeler, J.T. (1971). Comparison of Media for the Enumeration of Clostridium perfringens. Appl. Microbiol. 21 : 922-927.

Harrigan, W.F. and McCance, M.E. (1976). Laboratory Methods in Food and Dairy Microbiology. Academic Press - London. pp. 27, 139, 142-144, 155, 162.

*Hartman, P.A., Reinbold, G.W. and Saraswat, D.S. (1966). Indicator Organisms - a Review. I. Taxonomy of the Fecal Streptococci. Int. J. Syst. Bacteriol. 16 : 197-221.

Hodges, L. (1977). Environmental pollution. 2nd ed. Holt Rinehart and Winston, New York. p. 243.

Janzen, J.J., Bodine, A.B. and Lunsoz, L.J. (1974). A Survey of Effects of Animal Wastes on Stream Pollution from Selected Dairy Farms. J. Dairy Science. 57 : 260-263.

Jayne and Williams, D.J. (1973). A Medium for Overcoming the in vitro Inhibition of Clostridium perfringens by Streptococcus faecalis var zymogenes and a note on the in vivo Interaction of the Two Organisms. J. Appl. Bacteriol. 36 : 575-583.

*John, R.C. (1970). The Movement of Bacteria and Viruses through Porous Media. Ground Water. 8 : 37-39.

Jones, P.H. (1980). Health Hazard Associated with Handling of Animal Wastes. Vet. Rec. 106 : 4-6.

Kabler, P.W. (1968). Microbiological Consideration in Drinking Water. J. Am. Water Works Assoc. 60 : 1173-1180.

*Katugampola, D.S. and Assim, T.H. (1958). Coliform Organisms in Domestic Water Supplies in Ceylon. Ceylon J. Med. Science. 9 : 95-101. Cited by Evison, L.M. and James, A. (1978).

Kaushik, N.K. and Bewtra, J.K. (1965). Incidence of Coliforms and Enterococci in Natural Waters. Environ. Hlth. 7 : 32-38.

Kenner, B.A., Clark, H.F. and Kabler, P.W. (1960). Faecal Streptococci. II. Quantification of Streptococci in Faeces. Am. J. Public Health. 50 : 1553-1559.

Kenner, B.A., Clark, H.F. and Kabler, P.W. (1961). Faecal Streptococci. I. Cultivation and Enumeration of Streptococci in Surface Waters. Appl. Microbiol. 9 : 15.

*Kjellander, J. (1960). Enteric Streptococci as Indicators of Faecal Contamination of Water. Acta Pathol. Microbiol. Scand. Suppl. 136. 48 : 9-124.

*Kunkle, S.H. and Meiman, J.R. (1967). Water Quality of Mountain Water Sheds. Hydrology papers, No. 21. Colorado State University, Fort Collins. (July 1967).

Labbe, R.G. and Duncan, C.L. (1975). Influence of Carbohydrates on the Growth and Sporulation of Clostridium perfringens- type A. Appl. Microbiol. 29 : 345-351.

Linton, E.C. (1965). Veterinary Hygiene, 4th ed. Scientific Book Company, Calcutta. p. 46.

Loehr, R.C. (1977). Pollution Control for Agriculture. Academic Press, New York. p. 39.

Malaney, G.W., Weiser, H.H., Gerhold, R.M. and Garver, F.A. (1961). Evaluation of Methods for Coliform Counts in Farm Pond Water. J. Am. Water Works Assoc. 53 : 43-48.

Marshall, R.S., Steenbergen, J.F. and McClung, L.S. (1965). Rapid Technique for the Enumeration of Clostridium perfringens. Appl. Microbiol. 13 : 559-563.

McFeters, G.A., Bissonnette, G.K., Jezeski, J.J., Thomson, C.A. and Stuart, D.G. (1974). Comparative survival of Indicator Bacteria and Enteric Pathogens in Well Water. Appl. Microbiol. 27 : 823-829.

Moterman, W.F., Adams, J.C. and Reichard, P.A. (1974). Comparison of Methods for Enumerating Fluorescent Bacteria. Appl. Microbiol. 27 : 290-291.

Medrek, T.F. and Barnes (1962). The Distribution of Group D Streptococci in Cattle and Sheep. J. Appl. Bacteriol. 25 : 159-168.

Medrek, T.F. and Litsky, W. (1959). Comparative Incidence of Coliform Bacteria and Enterococci in Undisturbed Soil. Appl. Microbiol. 8 : 60.

*Milne, C.M. (1976). Effect of a Livestock Wintering Operation on a Western Mountain Stream. Trans. Am. Soc. Agric. Engs. 19 : 749-752.

Moore, B.W. (1973). Sanitary Analysis of Water. In Preventive Medicine and Public Health. 10th ed. Sartwell, P.E. (Ed.). Appleton - Century-Crofts, New York. pp. 1073-1074, 1077, 1091-1094.

*Morrison, S.M. and Fair, J.E. (1966). Influence of Environment on Stream Microbial Dynamics. Colorado State University (Microbiology), Hydrology Papers No. 13.

Mousea, R.S. (1965). Type Distribution of Coliforms isolated from Faecal and Non-faecal Habitats. Ind. J. Med. Res. 53 : 629-637.

*Muneto, M., Nishitai, Y. and Tanaka, A. (1973). The Distribution of Coliforms in Fresh Water. Bull. Fac. Agric. Shimane. Univ. 7 : 140-145.

Oblinger, J.L. (1975). Recovery of Streptococci from Variety of Foods. A Comparison of Media. J. Milk Food Technol. 38 : 323-326.

Oblinger, J.L. and Coburger, J.A. (1975). Understanding and Teaching the Most-Probable-technique. J. Milk. Food Technol. 38 : 540-545.

Park, J.E. (1971). Text Book of Preventive and Social Medicine, 2nd ed. Banarsidas Bhanot, Jabalpur, India. pp. 172-174, 188-190.

*Patterson, J.T., Cornforth, I.S. and McAllister, J.S.V. (1974). A Field and Laboratory Study of the Effect of Slurry Application to Soil on the Bacterial Contamination of Drainage Waters. North Irel. Dep. Agri. Res. 22 : 1-6.

*Pavlova, M.T., Bresenski, F.T. and Litsky, W. (1972). Evaluation of Various Media for Isolation, Enumeration and Identification of Faecal Streptococci from Natural Sources. Health Lab. Sci. 9 : 289-298.

Purdom, P.W. (Ed.) (1980). Environmental Health, 2nd ed. Academic Press, New York. pp. 16, 160, 190-191.

Ramadan, F.M. and Sabir, M.S. (1963). Differentiation Studies of Faecal Streptococci from Farm Animals. Can. J. Microbiol. 9 : 449-450.

Stuart, S.A., McPeters, C.A., Schillinger, J.E. and Stuart, D.G. (1976). Aquatic Indicator Bacteria in the High Alpine Zone. Appl. Environ. Microbiol. 31 : 163-167.

Sundstrom, D.W. and Klei, H.E. (1979). Waste Water Treatment. Prentice Hall. Inc., New Jersey. pp. 6-7.

*Thomas, S.B. and Druce, R.G. (1955). Cited by Cooke, E.M. (1974)

Tilton, R.C. and Litsky, W. (1967). The Characterisation of Faecal Streptococci. An Attempt to Differentiate Between Animal and Human Sources of Contamination. J. Milk Food Technol. 30 : 1-6.

Van Donsel, D.J., Geldreich, E.E. and Clark, N.A. (1967). Seasonal Variations in Survival of Indicator Bacteria in Soil and their Contribution to Storm-Water Pollution. Appl. Microbiol. 15: 1362-1370.

*Verstraete, W. and Voets, J.P. (1975). Microorganisms as Indicators of Environmental Hygine : Ecology, Taxonomy and Enumeration. Natuurwet. Tijdschr. 57 : 41-84.

Viraraghavan, T. and Warnock, R.G. (1976). Ground Water Quality Adjacent to Septic Tank System. J. Am. Water Works Assoc. 68 : 611-614.

Voelker, R.A., Heukelekian, H. and Oxford, E.E. (1960). Seasonal Coliform Variations in Well Waters. Am. J. Pub. Health. 50 : 1873-1881.

Vogt, J.B. (1961). Infectious Hepatitis at Posen, Michigan. J. Am. Water Works Assoc. 53 : 1238-1248.

*WHO (1973). Community Water Supply and Sewage Disposal in Developing Countries (end of 1970). World Health Statistics Report. 26 : 720-783.

Willis, A.T. (1956). Anaerobes as an Index of Faecal Pollution in Water. J. Appl. Bacteriol. 19 : 105.

Wilson, G.S. and Miles, A.A. (1975). Topley and Wilson's Principles of Bacteriology, Virology and Immunity. Vol. II. 6th ed. Edward Arnold (Pub). Ltd., London. pp. 2648-2656.

Wilson, M.E., Weisburd, M.H., Miser, H.E. and Morello, J.A. (1979). Laboratory Manual and Work Book in Microbiology, 2nd ed. MacMillan Publishing Co., Inc., New York. p. 235.

Wistreich, G.A. and Lechtman (1980). Microbiology, 3rd ed. Glencoe Pub. Co., Inc., California. p. 777.

* Original not consulted.

Appendix

APPENDIX

1. Proforma of the sanitary survey

SANITARY SURVEY OF THE DOMESTIC WELLS IN OLUKKARA VILLAGE

PROFORMA

A

1. House No. :
2. Ward No. :
3. Panchayat :
4. Well
   a) Type of well : Lined-not plastered/Lined and plastered/Not lined
   b) Parapet : Present/Absent. Height :
   c) Platform : Present/Absent. Width :
   d) Drain : Present/Absent
   e) Covering : Present/Absent
   f) Method of drawing water : With pump/Without pump
   g) Depth of water :
   h) Depth of well :
   i) Depth of water table :
   j) Depth of lining :
5. Growth of vegetation inside the well : Present/Absent

6. Branches of trees over the well : Present/Absent

7. Surface runoff into the well : Present/Absent

8. Nature of the soil : Sand/Clay/Laterite/Rock

B

1. Distance between well and kitchen :

2. Cattle keeping : Present/Absent

3. No. of cattle : Young: Adult:

4. Distance between well and animal shed :

5. Distance between well and the neighbour's animal shed :

6. Location of animal in relation to well : Upstream/Same level/Downstream

7. Method of disposal of animal excreta : Adjacent to the shed/Manure pit/Land disposal

8. Distance between well and the place of disposal of animal excreta :

9. Location of the place of animal excreta disposal in relation to well : Upstream/Same level/Downstream

10. Type of latrine : Pit latrine/B-C-A type/ Septic tank

11. Distance between latrine and well :

12. Location of latrine in relation to well : Upstream/Same level/ Downstream

13. Other relevant data, if any :

Place:

Date :

2. Phosphate buffer (APHA, 1971)

Dissolved 34 g potassium dihydrogen phosphate, KH_2PO_4, in 500 ml distilled water. Adjusted the pH to 7.2 with 1 N NaOH, and made up the volume to one litre. Added 1.25 ml of the above stock solution to one litre distilled water for use in making dilution blanks. The buffer solution was sterilised in autoclave at 121°C for 15 minutes.

3. Tryptone sulphite neomycin agar (Marshall et al. 1965)

Composition

Peptone from casein	15.0	g/litre
Yeast extract	10.0	"

Sodium sulphate	1.0	g/l
Iron (III) citrate	0.5	"
Polymyxin B sulphate	0.02	"
Neomycin sulphate	0.05	"
Agar-agar	13.5	"

Preparation

Dissolved all the ingredients except agar in one litre of freshly distilled water. After adjusting the pH to 7.2 ± 0.2, agar was also added and allowed to stand for 10 minutes. Then boiled to dissolve completely, with frequent swirling. Filled in flasks and sterilized in autoclave at 121°C for 15 minutes.

One litre of the liquefied medium cooled to about 47°C was mixed with 25 ml of a buffered thioglycollate solution sterilized by filtration.

Thioglycollate buffer

Weighed 5.70 g dipotassium hydrogen phosphate, K_2HPO_4, and 2.80 g sodium hydrogen carbonate, $NaHCO_3$ and dissolved in 100 ml distilled water. Prepared 13.3 per cent sodium thioglycollate solution. Mixed 35 ml of the first solution with 15 ml of the second solution to prepare thioglycollate buffer and sterilized by filtration.

STUDY ON
THE INFLUENCE OF CATTLE KEEPING ON THE BACTERIOLOGICAL QUALITY OF DOMESTIC WELL WATER

By
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ABSTRACT OF A THESIS

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ABSTRACT

Wells are the main sources of water supply in Indian villages and towns as elsewhere in any of the rural areas of the developing tropical countries. The shallow, open, dug wells are liable to contamination from the surrounding sources by various means. Faecal contamination of water is considered as one of the major causes of health hazards resulting in innumerable diseases among people throughout the world. Therefore, the bacteriological analysis of water is aimed at the detection and enumeration of indicators of faecal pollution. The movement of animal excreta into surface and ground water is considered a major factor contributing to the bacteriological pollution of available water in many regions.

In order to assess the influence of cattle keeping on the bacteriological quality of domestic well water in Manuthy area, the present study was undertaken. Forty wells from households randomly selected, with and without cattle keeping, for the study spreading over for a period of six months covering the summer and south-west monsoon during 1981. Water samples were collected aseptically, once in each season. They were bacteriologically analysed for SPC, MPN of coliforms and the detection and enumeration of *E. coli*, FS and *Cl. perfringens*.

Bacterial counts in water from all wells far exceeded the standards recommended for open, shallow wells. Although an apparently higher SPC in water from households with cattle keeping than from those without was observed, it was not statistically significant. The seasonal variations in the counts showed a hundred-fold increase in the case of SPC during monsoon than summer. But the similar increase in the MPN was negligible. The indicator bacteria, especially E. coli and Cl. perfringens showed a significant reduction during monsoon.

The sanitary survey of the wells conducted with the help of a ready made performa revealed that none of the wells could be considered satisfactory either in construction or maintenance. The SPC and FS counts were found to have a positive correlation with the depth of wells. The SPC was positively correlated with the type of latrine also. The MPN of coliforms and the depth of lining and distance of cattle shed from well were negatively correlated; so also a negative correlation was observed in the case of FS count and the distance between latrine and well.

The various characteristics of wells, cattle keeping and latrines were found to have a significant correlation with the SPC and MPN of coliforms. When these of wells and latrines

were alone considered, they showed significant correlation only with FS count. But it was observed that the characteristics of wells and latrines alone could explain for the considerable variations in the SPC and MPN than those of cattle keeping, as a source of pollution.

It is concluded that the type of construction of well do not have any direct bearing on the bacteriological quality of well water except in the case of Cl. perfringens counts. Cattle keeping has also no significant influence on the bacteriological quality of domestic well water in Kannuthy area though all wells under study showed evidence of recent or past faecal contamination.