# This investigation is looking at three water samples

This investigation is looking at three water samples A, B, and C, which have been associated with a gastrointestinal infection of children in a small village. In which three walls have been associated with the path of infection. The likely cause of outbreak has been identified by using a serial dilution of the samples, which have then been grown on nutrient and Mac Conkeys ager. The growth on Mac Conkeys ager will establish if the organism has come likely from contanated by faecal matter.

The growth of the culture was analysed, to try to determine the likely cause and type of organism responsible. The information collected show that the likely cause of infection is from the samples taken from A or B, but further test will have to be carried out to monitor if the organisms or pathogenic or not. Due to sample A and B both having lactose forming bacteria in them, further tests will have to be carried out to determine which is contains the pathogenic bacteria so the best form of treatment can be given, and further further infections can be prevented.

There has been an outbreak of a gastrointestinal infection of some young children in a small village, in which the primarily source of infection has been associated three wells on farms which are used for drinking water, which all the infected the children have been to. Samples have been taken to try to establish the like source of infection. This was done by the use of two different ager, one being nutrient ager and the other Mac Conkeys ager.

Nutrient ager is used to establish the amount of bacteria present in the samples of water. It is not possible to tell if the microorganisms detected on

nutrient ager or pathogenic, as the medium will grow all organisms in the water that can grow in the conditions provided in the petri dishes.

The other ager was Mac Conkeys, which is a nutrient ager, which contains bile salts and crystal violet. The main use of this ager is differentiating between gram negative and gram-positive bacteria. This is done by the inhibiting the growth of gram-positive bacteria due to the addition of the bile salts and crystal violet. The sugar source in Mac Conkeys ager is lactose, which goes red in acidic conditions, which will help in identifying bacteria, which can ferment lactose, them bacteria will appear pink. Bacteria, which do not ferment lactose, will often use amino acids as the nutrient source, realising ammonia, which results in alkaline conditions, so the colonies appearing colourless on the ager. The colour change is, due to the indicator natural red which is in the Mac Conkeys ager.

The infections are likely to be due to a water infection, which are most likely to affect the small intestine resulting in diarrhoea. This is because the cells in the small intestine help maintain the water flow in the body. This is done, by ‘ ion pumps,’ controlling the water potential, and flow across the cell membrane and mucosal cells. The loss of water results in dehydration, and in some cases death, this is a particular problem in this case as the people infected or children, which are more septable to the infections.

Entry is usually through the intestinal tract during in ingestion of contaminated food or water substances, and they leave the body by faeces. The fact that the organisms leave the body by faeces often means that the contamination may of come from a person who has been infected by the

pathogenic microbe, and if the course is not found then other waters supplies can become infected to so, the water supplies around people who have been infected should be monitored.

There are a number of possible micro organisms, which has caused this out break; they include Vibrio cholera, Eschericha coli, Giardiasis, Erytosporidiosis, Legininella pneumophillia, cytosporidosis and Salmonella typhi.

Vibrio cholera is the cause of cholera, it is a gram negative bacterial cell, and is passed in to people by the ingestion of contaminated water and food. It effects the small intestine, were it secretes and endotoxin which results in dehydration if treatment is not given.

Giardiasis, affects the intestinal tract and is another water bone infection, which is transmitted by contaminated water, food are sexual intercourse. It attaches to the intestinal cell wall, were it will form a cyst.

Crytosporidosis, grow in the mucosal epithelial cells of the stomach and intestine. It is passed on by faecal contaminated water.

Legininella pneumophillia causes legionellosis, it is due to a water bone infection, but is often transported by aerosol then drinking water, it is resistant to heating and chlorination, and is mostly found in water tanks.

Salmonella typhi causes typhoid fever and is a water bone infection, which is passed on by contaminated water or food. It can be destroyed by filtration of water and chlorination of the water.

Eschericha coli is a gram-negative exteracellur pathogen, which attaches to the intestinal epithelia cells, and causes diarrhoea and dehydration. It is often transmitted by food or water.

MATERIALS.

> Sterile water samples from well A, B and C.

> Sterile Nacl.

> Gilson pipette 1000??(variable)

> Gilson pipette 100 ? (fixed)

> Sterile automatic pipette tips.

> Nutrient ager plates.

> Mac Conkeys ager plates.

> Spreader and alcohol.

METHOD.

A ten fold serial dilution is produced from each of the three water samples, ranging from 10-1 to 10-6. A 100? measurement of each of the serial dilutions was put on the surface, of a nutrient ager plate, and spread across the ager. Repeat the transferral process of the serial dilutions to the Mac Conkeys ager. Incubate all the samples for forty-eight hours at 37oc. The plates should be inverted before incubation,

RESULTS

A table, to show the number of colony forming units per I ml of the sample (CFU ml-1) in the nutrient and Mac Conkeys ager.

Sample A

Sample B

Sample C

Nutrient ager

2. 10 x 108 CFU ml-1

5. 60 x 104 CFU ml-1

N/A

Mac Conkeys ager

2. 90 x 108 CFU ml-1

4. 20 x 104 CFU ml-1

N/A

Both sample A and B had colonies with fermented lactose, shown by the formation of pink colonies on the Mac Conkeys ager.

ANALYSIS

The results show that sample C is sterile water as there was no growth on the Mac Conkeys ager, and although there was growth on two of the nutrient ager plates, it is likely that this was due to contamination from outside sources. The reason for this is that the organism was only growing on two of the plates, and if the sample itself contained an organism, it would be present in the first serial dilution, and most likely through out the rest of the series of plates.

Both sample A and B had colonies on both the nutrient and Mac Conkeys ager which followed the serial dilutions. This means that both of the samples maybe a cause of the gasteralintestine outbreak. In the Mac Conkeys ager, both A and B formed pink colonies, so this means that both of them are lactose fermenting bacteria.

Because the bacteria in sample A and B can grow presence of bile salts, it suggests that the contamination of the water has been through faecal matter of mammals, birds or humans near the well. The reason for this is that only faecal bacteria are able to grow on this type of medium, as the evolution process has enabled then to grow in the presence of bile salts.

One of the most likely causes of the infection is Eschericha coli, as it is contained in faecal matter and is able to ferment lactose.

A further test can be carried out the see which of sample A and B or pathogenic strain of E. coli, is by growing the samples on a Mac Conkeys tet ager which contains a teracyline and also on a Mac Conkeys amp ager which contains ampicillin which is an antibiotic. The pathogenic microorganism will likely to grow on both types of ager, as it has antibiotic resistance. This idea

of the pathogenic organism being antibiotic resistant is supported by the article: Surveillance and epidemiology of antibiotic resistance in E. coli and salmonella, published by the food microbiological research group. It shows that pathogenic forms of microorganisms are antibiotic resistant, due to characterisation of mutations, which have occurred.

The spread of the organisms are mostly spread through food and water contamination. If one of the well samples A or B tests positive to having a pathogenic organism, the sample should be looked at under a electron microscope to see if they have the characteristics of any known pathogenic bacteria. A stool sample should be taken from the infected people and tested in the same manner, and compassion made. If the results are the same, it is likely that is the cause of the infection.

To rule out the possibility of the infection being Giardiasis, cryptoridiosis samonella typhi leginonella, is by exposing the samples to chlorine, if the sample is destroyed then it will be salmonella, as chlorine will destroy it. If the organism is still present, it is likely to be one of the other three as they are all restraint to chlorine. The samples may then be exposed to heat, and if the organism is still growing then it is likely to be Legininella as it has a high restance to heat. If not the characteristics of the sample can be looked to try to identify it.

As suggested the likely cause of the infection is Eschericha coli, the problem with this is that not all Eschericha coli or pathogenic, and some or found in the colon and in fact are protective of the host organism, as they prevent colonization of other virulent strains. The strains of Eschericha coli causing

the infections are likely to be Enterotoxignic Eschericha coli strains. They differed from the ones found in the colon, by the production of pili, which allows them to attach to the intestinalileal mucose. Once attached, they produce a protein toxin, resulting in loss of water from the cell.

The Enteropathogenic Eschericha coli strains, result in malabscrptive diarrhoea. They bind to the intestinal cells by a pili and damages the surface of the cell. They will then form a channel connecting the two cytoplasm in which toxic proteins can be injected. This helps prevent the antibodies produced from the host’s immune response from reaching them.

Enterohemorrhagic Eschericha coli strains cause malabscriptive diarrhoea. It is the most deadly strain, as they produce a toxin called shiga-like toxin, which will enter the blood stream and cause damage to the kidney, usually by haemorrhages.

The children if infected with Eschericha coli, the best form of treatment would be to give them intravenous fluids orally (containing different salts and sugars dissolved in water) to help replace the lost fluid, and reduce further loss.