

Water Environmental Situation of Wells in Galda de Jos Village, Romania: Microbiological control

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Abstract: Quality water must be health providing, clean, devoided of microorganisms, parasites or substances. Homeowners with private water supplies may be exposed unknowingly to health related pollutants unless they voluntarily have their water tested for the correct water quality parameters, especially in water environmental situation. The present paper emphasizes that water microbiologically polluted, is a potential hazard for human and animal health. Due to heavy rains in 2006, in village Galda de Jos, from Alba County, 28 households were flooded. In a first stage, decontamination of water wells was achieved. After 4 days from water decontamination enteritis cases occurred in hens and cattle, except for pigs. Starting from the symptoms and the beginning of the problems, experimental studies were centred upon following in time the microbiological growth of water. Following the results of tests carried out (bacteria growing at 37°C - mesophilic, coliform bacteria, *E. coli* and enterococci) a new decontamination was initiated that led to the disappearance of diarrhea cases.

Keywords: water wells, flood, pollution, colony-forming unit, coliform bacteria, *E. Coli*, *Enterococcus*.

1. Introduction

Groundwater is in the earth's crust thickness. By rainwater (which can lead to flooding) and surface water infiltration into the earth shell (at a variable depth between 2 and 20 m), above the impermeable layers, groundwater is formed by accumulation [1].

Water in nature is never pure; given the interactions with the environment, it contains gases, mineral and organic substances, dissolved in suspension. Drinking water must be health providing, clean, without microorganisms, parasites or substances which, by number or concentration, can be a potential hazard for human and animal health [2-4].

Water is an indispensable factor for animal lives and the entire biological universe on the planet. "Clean" water is one of the most precious substances on earth, which ensure animal health and quality of their products.

Polluted water represents a source of infection and parasite infestation not only for animals, but also for humans [5], and water transmitted pathogenic bacteria lead to bacteriosis both in humans and in animals.

The role of water in the transmission of diseases to humans and animals is known even before some pathogens to be discovered. A good example is that of the Chicago 1885, when after a torrential rain, rain waters and sewage waters were poured into Michigan Lake. The town main source of water at the moment was Michigan Lake, the water being distributed to the network without

decontamination. Following this, it triggered an epidemic of dysentery, enteric fever and cholera.

Epidemics caused by water contamination occur explosively at any time of year and manifest in the territory fed by contaminated water source. They suddenly stop once the consumption of contaminated water is stopped [5, 6]. The main manifestations of the disease are the digestive illness (enteritis).

Conditions to trigger such an epidemic are: existence of a pollution source, germ's ability to survive in water for a sufficient time to cause the disease, existence of a population susceptible to illness.

Hydric diseases classification can be based on the number of illness cases, their occurrence and development mode. We can find *epidemics* (a large number of cases in a short time); *endemics* (small number of cases found continuously in an area), *sporadic progress diseases* (isolated). Curious is that cases of epidemic and epizootic diseases transmitted via water, are encountered occasionally even after water decontamination [7].

Primary biological water pollution is the consequence of the presence of viruses, bacteria and parasitic agents in water.

Enteritis is an inflammation of the intestinal mucosa caused by physico-chemical, biological, nutritional or toxic factors. Clinically it is manifested by poor general condition, loss of appetite, diarrhea and weight loss [8].

E.coli can be transmitted through water consumption and gives gastro-enteritis and sepsis in children and various species of young animals [9]. The incidence of *E.*

coli is about 17.5% in well water, surviving in drinkable water from wells for as long as six months, and in sewer water for four months. A severe epidemic of gastroenteritis transmitted by water appeared in England in 1980. Infection was possible due to a faulty sewage system, which allowed drinking water contamination [1, 10].

The purpose of the study is to determine associations between water contamination and gastro-enteritis in various species of animals, by using microbiological analysis as an indicator.

2. Experimental

Following heavy rains in the area of Galda de Jos, Alba County, in July 2006, there were floods. The most affected area was between the Tibru Valley and Galzii Valley. This area is located downstream of the local cemetery. In this area 28 households were inundated.

Firemen intervention was effective: water from basements and wells was discharge and decontamination was done.

Four days after decontamination, among animals from flooding houses cases of illness occurred: a large percentage of cattle and poultry and a smaller one of pigs. In cattle and pigs abdominal colic, diarrhea discharge occurred. Fever was present only in the early stages of disease. In poultry diarrhea was stated.

Therefore, water samples were collected and the following microbiological analysis were made:

Determination of total number of bacteria growing 37°C (mesophyll) [11,12]

The method consists in inoculation of 1-2 mL sample in 10-15 cm³ nutrient agar culture medium with yeast extract (melted and cooled at 45°C). The inoculation is made into a Petri plate. After the solidification, the plates are incubated at 37±2°C, for 44±4 h. The colony-forming unit (CFU)/mL are directed counting.

Detection and counting of *Escherichia coli* and coliform bacteria Part 1: Membrane filtration method [13]

100 mL water sample are filtered through a membrane, process in which microorganisms are retained on the membrane surface. Membrane filter is transferred on a selective culture medium (lactose TTC agar) in a Petri plate and incubated at 36±2°C, for 21±3 h.

The colonies developing yellow colour on medium are counted as lactoso-positive bacteria. Confirmation: a) *Oxidase test*: 10 characteristic colonies are passed on nonselective agar (TSA) and are incubated at 36±2°C for 21±3h. Then, one colony is taken from each Petri plates and put it on filtering paper impregnated with oxidase reagent. Positive reaction: occurrence of purple blue coloration in about 30 sec. b) *Indole test*: simultaneous inseminations in tryptophan broth are made. After incubation at 44°C for 21±3h, 0.2-0.3 mL of Kovac's reagent is added. Positive reaction: occurrence of a purple ring. The colonies with negative reaction to oxidase are

counted as being *coliform bacteria*. The colonies with negative reaction to the oxidase and positive reaction with the indol are counted as being *E. coli*.

Identification and counting of intestinal enterococci Part 2: Membrane filtration method [14-16]

100 mL or 10 mL sample and/or dilutions are filtered through the membrane placed on Slanetz Bartley medium. The incubation is made at 36±2°C for 44±4h. The typical colonies are pink or brown, in the middle or totally. They are then transferred on bile-esculin-azide agar, pre-heated at 44°C and incubated at 44°C, for 2h. The bronze-black characteristic colonies are counted and the calculus method from SR EN ISO 8199: 2008 is applied. Note: The preparation of culture medium is performed according to the *Guide of culture medium Preparation and Obtaining* [17].

3. Results and Discussion

After water draining from the affected households and flooded wells, respectively after water decontamination with Javel (sodium diclorozocianurat) and lime chloride, theoretically it should be potable. However, after four days cases of illness have been announced - diarrhea, fever - both in cattle and poultry (most of the cases) and in pigs.

In this case, illness source was the direct consumption of polluted water, and the food that was prepared by adding slightly heated or unboiled water.

At the beginning, after four days from the decontamination process, when the first cases of illness occurred, water samples from all 28 wells were collected in order to carry out microbiological analysis (table 1). All analyzed samples had levels that exceeded the maximum limits (according to Law no. 311/2004 in addition to Law no. 458/2002 regarding water quality) [18, 19].

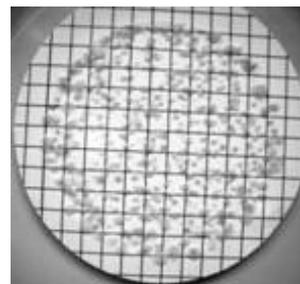


Figure 1. Membrane filtration method: *E. coli* and coliform bacteria presence

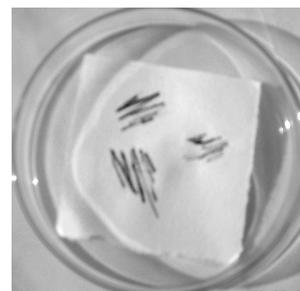


Figure 2. Positive oxidase reaction: in 30 min. occurrence of purple-blue coloration



Figure 3. Positive Escherichia coli

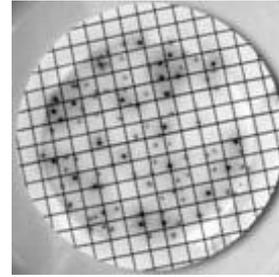


Figure 4. Enterococcus – positive reaction

TABLE 1. Microbiological results of water after first decontamination

| | Colony-forming unit, CFU/mL | Total coliform bacteria /100mL | E. coli/100mL | Enterococci/100mL |
|--|-----------------------------|--------------------------------|---------------|-------------------|
| Max. admitted limits according to Law 458/2002 amended with Law 311/2004 | 20 | 0 | 0 | 0 |
| Results after first decontamination | 315 - 380 | 340 - 5200 | 252 - 2600 | 20 - 147 |

TABLE 2. Microbiological results of water after second decontamination

| | Colony-forming unit, CFU/mL | Total coliform bacteria /100mL | E. coli/100mL | Enterococci/100mL |
|--|-----------------------------|--------------------------------|---------------|-------------------|
| Max. admitted limits according to Law 458/2002 amended with Law 311/2004 | 20 | 0 | 0 | 0 |
| Results after second decontamination | 150 – 180 (at 4 farms) | 12 – 20 (at 3 farms) | 0 | 0 |

The decontamination process was repeated using this time, lime and Quatersan. After another four days water samples from 20 households were taken. In these households, mortality occurred at birds (three farms) and signs of enteritis have been identified in animals and birds (9 of 11 cattle and two pigs of 20). These samples were subjected to microbiological analysis (table 2).

Table 2. Microbiological results of water after second decontamination

Tests conducted have shown that in some households' water remain polluted after the second decontamination process, although the values measured on CFU/mL and coliform bacteria/100mL were lower. In the three farms where birds have died, coliform bacteria were present even after the second decontamination. These results suggest that groundwater was polluted, which causes water pollution even after several decontamination processes.

Owners have been notified to use boiled water for animals and birds. All diseased animals and birds were rehydrated and treated with antibiotics.

Decontamination efficiency was examined further (repeated) until the microbiological parameters had values according to Law no. 311/2004.

4. Conclusions

After first decontamination, the water was considered drinkable and was administrated to animals without a preliminary analysis of its qualities. Therefore animals had digestive problems, which suggested that water was not drinkable.

After the occurrence of diseases, following the analysis of water samples, it was demonstrated that they were microbiologically contaminated.

After the second decontamination, in four of the twenty households in which samples were collected, water was still polluted.

Mortality was recorded in those households where microbial charge was the greatest. It was maintained even after decontamination.

The presence of polluted water after the first and second decontaminations, suggested that groundwater was also polluted.

By using boiled water (for drinking and food) and antibiotics, cases of diarrhea at bovines, swine and poultry have been rectified.

Cases like this require a control of decontamination efficiency by microbiological analysis in maximum 24 hours after decontamination.

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