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Studies regarding the quality of water and food for utilitarian dogs from Alba County, Romania

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Abstract

The present paper intends to investigate the relevance of water and food quality for utilitarian dogs in several institutions in Alba County, as the main source of their digestive problems. Starting from the symptoms, the occurrence and history of the appearance of the problems, experimental studies centred upon following in time the microbiological growth, and the overfulfilment of physico-chemical parameters limits, as well as the estimation of enterobacteria presence in food. The analysis results (the presence of total coliforms, thermotolerants, and Streptococcus fecalis, respective acid pH of the water, added to the microbiological contamination of food), and the measures taken, have resulted in the disappearance of gastro enteritis in utilitarian dogs.Grape seed oil obtained by extraction with petroleum ether can be used for technical purposes only.

Keywords: water and food quality, physico-chemical and microbiological parameters, contamination, wolfhound

1. Introduction

Water in nature is never pure; given the interractions with the environment, it contains gases, mineral and organic substances, dissolved in suspension. Drinking water must be health providing, clean, without microorganisms, parasits or substances which, by number or concentration, can be a potential hazard for human and animal health [1].

Water contamination is usually because of the presence of bacteria that are indicators of fecal contamination: *E. coli*, *Clostridium perfringens* and enterococci. Pet food spoilage is inevitable, because the same nutrients in foods are also the same nutrients microbes need for their growth. Diarrhoea in dogs may be influenced by numerous factors, including stress, change in diet, water contamination, primary gastrointestinal pathogens, opportunistic infections, and predisposing disease conditions.

Primary and opportunistic gastrointestinal pathogens affecting domestic dogs include bacteria, viruses, protozoa, and helminths. Many gastrointestinal pathogens in dogs pose a zoonotic risk to humans, including *Campylobacter* spp., *Salmonella enterica*, *Trichuris vulpis (whipworm), Strongyloides stercoralis*, *Clostridium difficile*, *Cryptosporidium* spp., and *Escherichia coli* strain O157:H7 [2].

Routine monitoring for all suspected pathogens is not feasible. Therefore, methods of disease prevention are often non-specific, including prophylactic disinfection of the environment and isolation, treatment, or culling of apparently sick animals. The development of effective strategies for prevention of diarrhoea depends on identification of the most important causal and predisposing factors. However, results from any single or small number of diagnostic tests could be misleading because they may imply causation inaccurately.

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If sufficient information concerning diarrhoea were available, diagnostic profiles could be developed with diagnostic tests, treatments, and preventive measures designed to target the most important pathogens and could lead to cost-effective management and prevention programs [3].

Enteritis is an inflammation of the intestinal mucous membrane, and it is determined by physico-chemical, biological, food and toxicity factors, and it clinically manifests through a general discomfort, anorexia, diarrhoea, weakness. This disease is generally caused by bad feeding factors, such as abrupt changes in daily food ration, fat food, easily fermenting feeding stuff (milk, cream etc.), spoiled food; fatigue, overworking, parasitosis (ascaridis, dipilidiosis etc.) and some infectious diseases. Enteritis can be prevented just through a rigorous supervision of the food ration: the dog should not switch abruptly from a diet to another, food should not be spoiled, mouldy, cold or too hot, or served in dirty dishes. [4]. The standard of care for the treatment of acute diarrhoea is rehydration (required more or less intravenous fluid treatment) and the administration of antibiotics, motility modifiers, synthetic prostaglandins or anti-secretor agents [5].

The purpose of the study is to determine associations between water and food contamination and diarrhoea disease in dogs.

2. Materials and method

During 2003, three firefighters and genrarmery units, two from Alba Iulia, and one from Blaj, have discovered that all the dogs, german shepherds, used in interventions (guarding, protection and salvation activities) had digestive problems.

Samples have been collected from the wells in the three units, during May 2003 – January 2007, to cover both the intervals with frequent digestive problems in all the dogs, and the intervals with low incidence of disease.

From 2005 the food, both dried and wet, has been examined – the comercial products rich in nutrients: natural fibers, vitamin D3, proteins (beef/chicken meat), fat/meat and animal derivatives, extracts from vegetable proteins, vitamins (E and D3), copper, and copper sulfate.

There have been performed microbiological analysis – for water and food (the total number of bacteria developing at 37°C, the probable number of coliform bacteria and thermotolerant coliform bacteria, the probable number of *Streptococcus fecalis,* the presence of *Salmonella* spp. – for the water, and *Enterobacteriaceae* – for the food*),* as well as physico-chemical analysis for water (pH, NO^{3-}, NO^{2-}, NH^{4+}).

*1. Determination of total number of bacteria growing 37°C (mesophyll) [*6] A volume of 1 cm^3 from the sample and (10^{-1} and 10^{-2}) dilutions are introduced into a Petri plate and 10-15 cm^3 nutritive gellose (melted and cooled at 45°C) are added; after the solidification of the gellose the plates are incubated 37±0.5°C, for 48 h. The colonies are counted both those at the surface, and the ones within the gellose.

2. Determination of the probable number of coliform bacteria (total coliforms); multiple tube method [6] *Presumption test*: 5 volumes of 10 cm^3 from the sample is added in 5 sample tubes containing 10 cm^3 Lauryl- Sulphate Broth double concentrated; 5 volumes of 1 cm^3 of the sample and 1 cm^3 from the 10^{-1} dilution are added in 5 tubers containing 10 cm^3 Lauryl Sulfate Broth; they incubate at 37±0.5°C, 48 h; positives: the test-tubes with turbidity and lactose fermentation with gas presence in the fermentatin tubes. *Confirmation test*: from each positive test-tube inseminations take place on plates with gelose-lactose-eozyn-methyl blue agar, then they incubate at 37±0.5°C, for 24 h. The typical colonies: flat, dark violet blue, metalic shine; bulging. Going from the number of confirmed positive tubes, using tables, we calculate the probable number of coliform bacteria.

3. *Determination of probable number of thermotolerant coliforms (fecal coliforms)* going from positive vials in the presumption test for total coliforms through confirmation in selective liquid medium at 44±0.5°C in 24 h. Taking into consideration the number of positive tubes at 44±0.5°C (shifting of the medium to yellow/turbidity) it is calculate, using the tables, the probable number of thermotolerant coliform bacteria (fecal coliforms).

4. Determination of probable number of Streptococcus fecalis; multiple tubes method [6] *Presumption test*: 5 volumes of 10 cm^3 from the sample are added in 5 test-tubes with 10 cm^3 double concentrated sodium azide-broth; 5 volumes of 1cm^3 from the sample and 1cm^3 from the 10^{-1} dilution are

added in 5 test-tubes with 10 cm^3 of sodium azide-broth; they incubate at 37±0.5°C, for 48 hours. *Positives*: turning yellow/turbidity. *Confirmation test*: from the positive tubes we take 1-2 drops in sodium azide-broth and brom-cresol-purple; they incubate at 44±0.5°C, for 24 h. The positive reaction: turning yellow with or without sediment. Starting from the confirmed positive tubes it is calculate, using tables, the number of *Streptococcus fecalis*.

The interpretation according to STAS 1342 agrees to the following limits: CFU/ml<300; Total coliforms/100cm^3<10; Fecal coliforms/100cm^3<2; *Streptococcus fecalis*/100cm^3<2 [7].

From 2006 the analysis on water samples have been performed according to Water Law, no. 435/2006.

5. Counting of culture microorganisms. Colony counting through sowing in agar medium at 37°C [8]. The method consists in the innoculation, in a specific medium (agar with yeast) in Petri plates, of a quantity of 1-2 ml sample or decimal dilutions. The estimation of colonies forming units (CFU)/ml is performed by direct counting, after incubation at 37±2°C, for 44±4 h [9].

6. Determination and counting of Escherichia coli and coliform bacteria. Part 1: Membrane filtration method [10]. 100 ml from the water sample are filteres through a membrane settled on lactose TTC agar: they incubate at 36±2°C, 21±3 h. It is count as lactoso-positive bacteria the colonies developing on yellow medium. The confirmation: a) *Oxidase test*: 10 characteristic colonies are passed on nonselective agar (TSA) and incubate at 36±2°C, 21±3h; then, from each Petri plates it is take one colony and put it on filtering paper impregnated with oxidase reagent. Positive reaction: the occurence of purple blue coloration in 30 sec. b) *Indole test*: is make simultaneous inseminations in tryptophane broth which incubates at 44°C, 21±3h, then is added 0.2-0.3 ml of Kovac's reagent. Positive reaction: the occurrence of a purple red ring. It is count the colonies with negative reaction to oxidase as being coliform bacteria. It is count the colonies with negative reaction to the oxidase and positive reaction with the indol as being *E. coli* [9].

7. Identification and counting of intestinal enterococci Part 2 Membrane filtration method [11]. It is filter 100 ml or 10 ml from the sample and/or dilutions through the membrane placed on Slanetz Bartley medium and incubate at 36±2°C, 44±4h. The typical colonies are pink or brown, in the middle or totally. We transfer them on bile-esculin-azide agar, pre-heated at 44°C. They incubate at 44°C, for 2h. We count the characteristic colonies which are bronze-black, and apply the calculus method from SR EN ISO 8199: 2008. Note: The preparation of culture medium is performed according to *The Guide of Preparation and Obtaining the culture medium* [13,16].

8. The orizontal method for counting the enterobacteria; The method of enumeration of colonies [12, 13]. It is added 1g from the sample in 9 ml (10^{-1}) of peptone buffered water (PBW) and make 1 or 2 dilutions from 1 to 10 with PBW. In two Petri plates it is transfer 1 ml from the first dilution: we repeat with the other dilutions. It is added 10 ml of Violet Red Bile Glucose (VRBG) Agar cooled at 44°C, we stirr and, after cooling, is added 15 ml of medium. After hardening is incubate at 37°C, for 24h. It is choose the plates with less than 150 characteristic colonies: pink to purple red with/without precipitation halo. It is choose 5 colonies and inseminate on nutritive agar, and incubate at 37°C, for 24h. Biochemical confirmation: *the oxidase reaction* – described in „Detection and counting of *E. coli*" and *the fermentation test*: we transfer the same selected colonies on nutritive agar, which were oxidase-negatives, in test tubes with purple brom-cresol glucose agar. Positive reaction: yellow coloration within the whole test tube. Expressing the results: according SR EN ISO 7218/2002/2005 [14]. Note: The preparation of all the culture mediums followed *The Guide of Preparation and Obtaining the culture* [15,16].

9. The methods of rapid spectrophotometric determinations involve the use of the spectrophotometer Spectroquant NOVA 60 (SQ) and SQ specific kits (with reagents and reaction tubes). Following the work pattern from the kit, we read the SQ. The value appears on the screen in 2 seconds [17].

Ammonium: Kit SQ domain 0.010-2 mg/l NH_4-N or 0.01-2.58 mg/l. It is drop 0.5 ml from the sample in the reaction tube and homogenised. Is added a dose of NH_4-1K, closed, shake, and, after 15 min. readed. In high alkaline solutions, the nitrogen ammonia is present almost totally as ammonia, reacts with hypochlorite ions resulting in monochloramine,

which reacts with substitute fenol and forms a blue indocarbolitic derivative.

Nitrates: Kit SQ domain 1.0-50.0 mg/dm^3 NO_3-N or 2.2 – 79.7 mg/dm^3 NO^-_3. 0.5 ml of test is putted in the tube. Is added 1 ml NO_3-1K, shake for a minute, let aside for 10 minutes. In sulphuric acid solution nitrate ions react with a derivative of benzoic acid to form a red nitro compound.

Nitrites: Kit SQ 0.02-1.00 mg/dm^3 NO_2-N or 0.07-3.28 NO_2^- NO_2 10 mm tube. 5 ml of sample are drop in the tube. Is added a micro pallete knife of NO_2 -1 reagent and shake until total dissolution. Reaction time: 10 minutes. In acid solution, nitrite ions react with the sulphanilic acid resulting diazonium compound; which then reacts with N-1-naftiletilendiamine dihydro-chloride resulting in a violet red nitro compound.

3. Results and Discussion

Utilitarian dogs had digestive problems, shown by anorexia, cyfose posture, with their tails between their back legs, or curled position with the abdomen against cold places. Palpated, they presented abdominal defensing reaction, and the characteristic rumour of their intestinal accelerated tract. They also had abdominal colics, diarrhoeic discharges, wattery yellow fecies, sometimes hemorrhagic, with extremly heavy smell. Due to fluid loss through diarrhoea and dedydration, they had visible rib cages, sometimes deepened orbites, flabby skin, dull hair. Fever has been present just at the beginning of the illness (and not to all the dogs) all their vaccinations and vermin treatments being already performed. The digestive disorders appeared mostly in Spring and Winter. During the Summer and the Autumn, just some of them still presented the described symptoms. The problems came back in 2007. Our attention was drawn first towards the water analysis, assuming that their food was of good quality and they had no abrupt diet changes.

A number of 51 water samples have been analysed (4 water samples/year between 2003-2006 and 1 sample in January 2007) from each location; 27 samples have been analysed according STAS 3001/1991 Water – bacteriologic analysis, and the rest of the tests were performed accordin the present regulations (Figure 1-7 representative positive reaction).

1. The analysis performed in the Summer showed values over the maximum limits. The highest values were:

- the samples analyzed from Blaj: CFU/ml=340;Total coliforms/100cm³=546; Thermotolerant coliforms/100cm³=49 and *Streptococcus fecalis*/100cm³= 49 (2004), respective CFU/ml= 140; Total coliforms bacteria/100 ml = 150; *E.coli*/100ml=35; *Enterococcus*/100ml=27 (2006).
- the samples analyzed from Alba Iulia: CFU/ml=320;Total coliforms/100cm³=346; Thermotolerant coliforms/100cm³=18 and *Streptococcus fecalis*/100cm³= 49 (2003), respective CFU/ml= 240; Total coliforms bacteria/100 ml = 150; *E.coli*/100ml=29; *Enterococcus*/100ml=21 (2005); but the digestive problems appeared randomly in this period.

Water microbiological contamination kept a high rate in Winter and Spring, the values being as follows:

- the samples analyzed from Blaj: CFU/ml=340;Total coliforms/100cm³=346; Thermotolerant coliforms/100cm³=49 and *Streptococcus fecalis*/100cm³=18, CFU/ml= 180; Total coliforms bacteria/100 ml = 96; *E.coli*/100ml=15; *Enterococcus*/100ml=17.
- the samples analyzed from Alba Iulia: CFU/ml=300;Total coliforms/100cm³=346; Thermotolerant coliforms/100cm³=18 and *Streptococcus fecalis*/100cm³=18, CFU/ml= 210; Total coliforms bacteria/100 ml = 120; *E.coli*/100ml=10; *Enterococcus*/100ml=15; the digestive problems had maximum intensity in this period.

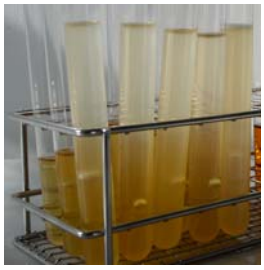

Figure 1. Multiple tube method-positive reaction for coliform bacteria (turbidity/gas production in Durham tubes)

Microbiological analysis for *Salmonella* spp. (STAS 3001/1991) show the absence of this in water.

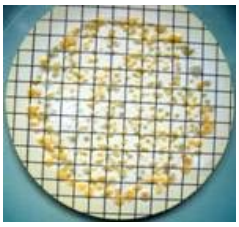

Figure 2. *Membrane filtration method*: *E.coli* and coliform bacteria presence

Figure 3. Positive oxidase reaction: in 30 min. occurence of purple-blue coloration

Figure 4. Positive *Escherichia coli*

2. From the begining of 2006, a number of physico-chemical analysis have been performed on water. All the parameters tested, except NH_4^+ and pH, have had normal values. In Alba Iulia, the highest value of NH_4^+ was 0.8 mg/dm^3 (December 2006); the value of pH was under the accepted limit, the lowest value was 5.67 in Blaj, in January 2006. The accepted limits for drinkable water, according to Law 311 (completing Law 458) [18,19] regarding the quality of the water are as follows: UFC/ml= 20; *E coli*/100ml = 0; *Enterococcus*/100ml=0; NH_4^+=0.5 mg/dm^3.

The microbiological water pollution reached a maximum in: May, August and October, but the digestive simptoms occured in Winter and Spring. The food given to dogs has been analized. 15 cans has been submitted to the test of the thermostat, but the results were negative. The source for dry food was the same for all the dogs, being kept in sacks.

Figure 5. Multiple tube method-positive reaction *Streptococcus fecalis* (shifting of the medium to yellow/turbidity)

Figure 6. *Enterococcus* – positive reaction

The samples of dry food have been analysed for the presence of enterobacteria, and they have been sampled even from the food dishes where the dogs ate from. The values found being small, the presence of enterobacteria (being higher for the tests sampled from the dogs' dishes) could not generate such an episode but in association with other favouring factors which could be: the low temperature of the environment, unwholesome and cold water.

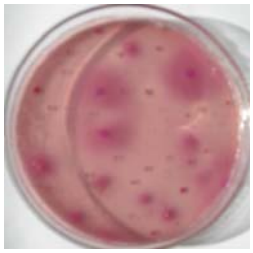

Figure 7. *Enterobacteriaceaee* positive reaction – pink colonies with/without precipitation halo

The fast treatment, in the first stage: urgent re-hydration and 3-4 pills of Eridiarom, and for those with severe affections, specific treatment for gastro-enteritis.The conclusion drawn was that the water should be decontaminated and Quatersan was used. Because of the occurrence of the cases in cold months, before administration, water was heated. The dog-dishes were cleaned after every meal, and the way of the dogs' feeding war supervised for all the winter period (the exclusion of kitchen or fat, or cold food); salt is added in the food.

After each enteritis episode, the place where the dogs lived was decontaminated with Virkon. In 2007 no enteritis episodes appeared in dogs.

4. Conclusion

During the Winter and Spring months the water contamination was constant; and although the analysis indicated smaller values than in Summer, we registered now the most of the enteritis cases.

Enterobacteria were present in the dry food, but a low charge of microorganisms in food, this copuld not be entirely responsible for the digestive problems, without any other favouring factors involved. The physico-chemical analysis showed an acid pH and the presence of NH_4^+.

The contaminated water, with physico-chemical modified characteristics (cold, slightly acid pH, cometimes the presence of ammonium) and cold food with a slight charge on enterobacteria have been all together responsible for the occurence of the enteritis.

The measures taken in Blaj in December 2006 and then in January 2007 in Alba Iulia – the decontamination of water sources with Quatersan, corection of the pH, warming water before administration, changing the food lot, decontamination of the area where the dogs were living – have lead to the dissapearence of enteritis cases.

The water sources must be kept under control, by decontamination and monitoring the physico-chemical and microbiological parameters in order to prevent hydric diseases occurence in animals in the processed units.

The food for the dogs must be kept in humidity and temperature conditions according to the producer's specifications to prevent the development of enterobacteria.

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