# ASSESSMENT OF BACTERIOLOGICAL CHARACTERISTICS OF WATER IN A FARM ANIMAL HEALTH

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**Abstract**

*The role of water in the transmission of infectious diseases is well known, even before the discovery of infectious agents of different diseases. Infectious diseases transmitted by water can take in terms of number of cases of disease and mode of occurrence and development of several forms. For this purpose, any farm animal health performing bacteriological analysis of water is required. The paper highlights the results of bacteriological analysis of water holding poultry SC Bio-Top-Ovo Ltd. Lipova, Vaslui.*

*Water samples were taken from the power supply, the collection basin and in the adăpătorilor and analysis were performed to determine Escherichia coli of faecal streptococci, coliforms, Clostridium perfringens.*

*The results were compared with values allowed by Law 458/2002 and 311/2004 on drinking water quality. For example, it has been determined:*

*E. coli in water source 17 colonies / 100 ml*
*Dock 33 colonies / 100 ml*
*In Adapters 17 colonies / 100 ml*

*for which the amount permitted under DIN EN ISO 9308-1/2004 LCCAP PS 14 is* ***absent.***

*Results require the application of measures for the treatment of water to change its microbiological characteristics. If these measures do not alter the biological value of water is recommended to change the source of water.*

**Key words**: bacteriological, germs, colonies, suspension, handling

## INTRODUCTION

In situations of poor hygiene in the water supply or power distribution system and water is a vector of transmission of various infectious diseases, which can take many forms in terms of number of cases of disease and mode of occurrence and development.

Drinkability of the water conditions are:

- Is colorless, transparent, odorless, relatively tasteless, do not contain organic chemicals and other than the maximum allowable of mandatory standards;
- To be free of pathogenic microorganisms and relatively pathogen;
- Saprophytic microflora is strictly limited to a few small;
- Be acceptable composition of calcium salts that print so - called water hardness.

Regarding measures to prevent the disease through water should be given first mode of supply of drinking water to the community, central installation of water control and distribution pipes, bacteriological and chemical control of drinkability water, maintenance of hygienic sources water, control of germ carrier status among staff serving water supply enterprises. It also requires water decontamination. For a disease due to water required three conditions, namely: the existence of a germ removed (sick or carrier), the viability of pathogens in water, a sufficient time to produce disease and the existence of susceptible populations.

## MATERIAL AND METHOD

The research was conducted in farm poultry SC Bio-Top-Ovo Ltd. Lipova,

Vaslui, has conducted a series of tests to determine the bacteriological characteristics of water used in watering the ground reared broiler.

For bacteriological examination of water samples were collected on April 28, May 12, 2009 respectively (in a growth cycle), so the source (a well located at about 30 meters away from the farm) and the basin collection of farm and watered. Analysis were performed at the microbiology laboratory of the Directorate of Public Health Vaslui. Bacteriological examinations consisted of determination *Escherichiacoli, enterococci, coliforms, bacteria Clostridium perfringens,* and the total number of number of colonies on 22 and 37°C. Between periods of sampling water samples not spoke with factors external modifiers on water (solution treatment, adjacent filters, etc.). Samples were collected in sterile glass vials, plugged with glass stoppers. The vials were filled up to 2-3 cm below the closures and were taken to conduct experiments in place a cooler to preserve the microbial content of water. Bacteriological analysis involves performing sowing the culture media, comment and microscopic comparison.

Determination of the probable number of total coliforms bacteria [1].

It was used multiple tube method.

Materials and culture media

- Sterile buffered water
- Average broth Lauryl Sulfate
- Average double-concentrated broth Lauryl Sulfate
- Medium-lactose-agar medium eosine-methylene blue
- Bottles of 250-300 $cm^3$ in Durham fermentation tube cross sterile
- Pipettes graded 1 $cm^3$ and 10 $cm^3$ sterile
- 16/160 mm tubes, sterile
- 12/120 mm tubes, sterile
- Petri dish with a diameter of 10 cm, sterile
- Thermostat to ensure temperature of 37ºC

Procedure:

Presumption test

To take this harvested water volume is 300 $cm^3$ water in 100 $cm^3$ water in the Sow every two bottles each containing 100 $cm^3$ Lauryl Sulfate and one double concentrated medium broth Lauryl Sulfate 10 $cm^3$ double concentrate.

Water harvested from other central facilities (reservoirs and distribution network) to analyze a volume of 100 $cm^3$ water in the Sow 50 $cm^3$ water in a vial containing 50 $cm^3$ medium concentrated broth Lauryl Sulfate and one double in 10 $cm^3$ water 5 tubes each containing 10 $cm^3$ double concentrated medium Lauryl Sulfate.

Water harvested from local sources (wells, springs) Sow a volume of 55.5 $cm^3$ of water is 10 $cm^3$ Sow 5 tubes each containing 1 $cm^3$ of water in 5 test tubes containing 10 $cm^3$ each medium broth Lauryl Sulfate and 1 $cm^3$ of each dilution of 1 / 10 water analysis in 5 tubes each containing 10 $cm^3$ medium broth Lauryl Sulfate.

Bottles and tubes are inserted thermostat sown and incubated at 37º C + 0,5° C for 48 hours. After 24 hours is a first reading and pass on environment-lactose-agar medium coesine-methylene blue test for confirmation of vials and tubes where there is disorder and gas. It is considered positive tubes that lactose fermentation is highlighted by the presence of gas in fermentation tubes no matter how small the quantity of gas would be discharged into that tube. Next tubes are kept in thermostat up to 48 hours. The 48 is the final reading and passage for confirmatory test of all vials and tubes in which microbial growth was obtained with or without gas, except those who were confirmed at 24 hours.

Confirmatory test

To specify whether the fermentation was produced by bacteria coliforms, in each bottle or tube considered presumptive positive test are sowing the chance, in advance Flambeau, the environment-lactose-agar medium eosine-methylene blue. Dispersions are in striae sectors to obtain isolated colonies on a Petri box, no more than 5 or 6 areas where crossings of tubes and 2 or 4 areas where crossings of bottles. Incubate plates thermostat at 37ºC + 0,5ºC for 24 hours.

The presence of coliforms is confirmed if the bacilli have developed typical colonies (colony flat dark blue-purple, with metallic

luster in the center, were pink with violet blue center).

The number of bacteria probably total coliforms

Determination of the probable number of total coliforms bacteria l $dm^3$ water is calculated using tables Mc Crady 1,2,3 (Table 1) according to the amount of water sample analyzed, taking into account the vials and tubes confirmed.

Table 1
Method of 3 tubes (Mac Crady)

| Characteristic cipher | Number of microorganisms | Characteristic cipher | Number of microorganisms |
|---|---|---|---|
| 000 | 0.0 | 222 | 3.5 |
| 001 | 0.3 | 223 | 4.0 |
| 010 | 0.3 | 230 | 3.0 |
| 011 | 0.6 | 231 | 3.5 |
| 020 | 0.6 | 232 | 4.0 |
| 100 | 0.4 | 300 | 2.5 |
| 101 | 0.7 | 301 | 4.0 |
| 102 | 1.1 | 302 | 6.5 |
| 110 | 0.7 | 310 | 4.5 |
| 111 | 1.1 | 311 | 7.5 |
| 120 | 1.1 | 312 | 11.5 |
| 121 | 1.5 | 313 | 16.0 |
| 130 | 1.6 | 320 | 9.5 |
| 200 | 0.9 | 321 | 15.0 |
| 201 | 1.4 | 322 | 20.0 |
| 202 | 2.0 | 323 | 30.0 |
| 210 | 1.5 | 330 | 25.0 |
| 211 | 2.0 | 331 | 45.0 |
| 212 | 3.0 | 332 | 110 |
| 220 | 2.0 | 333 | 140 |
| 221 | 3.0 | | |

Determination of the probable number of bacteria of faecal coliforms

Faecal coliforms bacilli to confirm all the same test tube considered positive presumption that chance was passing on one sector of the environment GEANÃ Petri plate to confirm total coliforms, are crossing the Pasteur pipette (few drops) in tubes with Lauryl sulfate broth medium and were inside Durham fermentation tube. Tubes are incubated at 44 $\pm$ 0,5ºC for 24 hours. Faecal coliforms bacteria are considered present if the tubes with liquid medium have lactose fermentation and gas production.

Test for identifying *E. coli*

All the passages are Pasteur pipette (few drops) of the same test tube considered positive presumption in tubes with peptone water environment and put in thermostat of 44,5 $\pm$ 0,5ºC for 24 hours. To add a few drops indole reaction Kovac's reagent Triptofanasis catabolis *E. coli* that the enzyme tryptophan leading to indole formation. Indole in the presence of Kovac's reagent leads to the formation of red nitrozoindol. Red ring appearance certifies that the presence of *E. coli* in the tube. There are strains of *E. coli* (10%) that do not ferment lactose and strains of E. coli (1%) are indole negative. The bacteriological analysis of water, interests only those strains of *E. coli* to ferment lactose at 44,5 $\pm$ 0,5ºC for 24 hours with acid and gas production and are indole positive.

The number of bacteria of faecal coliforms

Determination of the probable number of bacteria of faecal coliforms (E. coli) / $dm^3$ is like for coliforms utilizing the same calculation tables (1,2,3) according to the amount of water sample analyzed, taking into calculation vials and tubes that held the

fermentation of lactose with acid and gas production to 44,5ºC and positive indole reaction. Coliforms results for both total and faecal coliformsto be assessed with reference to the rules specified in standards.

Quantitative clostridium method of determining sulphite-reducing anaerobes and *Clostridium perfringens species*

Determining the number of sulphite-reducing clostridium is by multiple tube method, a method applicable to all categories of water and provides data for assessing the likely number of clostridium sulphite-reducing conditions using a liquid medium of anaerobiosis.

Collection of anaerobiosis is ensured by removing dissolved oxygen from the environment, physical process used as regeneration by keeping in boiling water or steam flow of bottles or tubes with the environment. As a result, the average mass vapor formed in the lead and dissolved oxygen in it. After regeneration vials or tubes are cooled suddenly in water. Pprotection of anaerobiosis is achieved in addition to the reduced surface / volume by:

-Cover surface environment with an insulating layer of sterile paraffin oil

-Placing the time of preparation of directly reducing substances in the environment as: glucose 0.2%, 0.05% cysteine hydrochloride, 0.5-1g agar possibly a volume of one liter broth solvirea limiting oxygen in broth regenerated.

Environment broth for sulphite-reducing clostridii contains an indicator light to show status oxidoreducere environmental resazurină or methylene blue. As long as the color of the surface environment to depth not exceeding ¼ of the height of the liquid column, the environment can be used as such, otherwise use as a prior regeneration at 100°C.

Materials and methods of work

- Balls of 200 $cm^3$ and 100 $cm^3$ with high neck and narrow, sterile
- 25x250 mm tubes, sterile
- 16x160 mm tubes and sterile 18x180mm
- 100 $cm^3$ sterile graduated cylinder
- Graduated pipette 2 $cm^3$, 10 $cm^3$ and 25 $cm^3$ sterile
- Water bath for heating samples to 80ºC
- Thermostat that provides temperature 37ºC
- Sterile diluents
- Medium broth for clostridium sulphite-reducing, simply concentrate
- Clostridii environment for sulphite-reducing broth, double concentrated
- Plain milk or sterile litmus milk
- Magler environment or environment-Willis Hobs
- Sachets with mixture achieve anaerobiosis IC
- 2% nutrient agar medium
- 1% neomycin solution

Procedure:

Probable number of sulphite-reducing paote clostridii be estimated using varying amounts of water. The presumption test for volumes of water sown more than 10 $cm^3$ or 10 $cm^3$ double concentrated broth is used for clostridii sulphite-reducing. Same environment simply concentrated volumes of water used for sowing of 1 $cm^3$.

To determine the number of spores of sulphite-reducing clostridium water samples are inactivated in advance: put in a water bath at a temperature of 60ºC, the temperature is raised gradually and maintained for 15 minutes at 180ºC to destroy all vegetative forms of bacteria in water. Then water samples as necessary as such and their decimal dilutions are sown in test tubes with medium bubbles and reclaimed water bath at a temperature of 100ºC, cooled suddenly, using pipettes of capacity greater than the amount to be sown. Pipettes will be introduced in depth leaving environment to fall freely without blowing the volume of water they sown.

A layer of 0.5-1 $cm^3$ sterile paraffin oil is added to the surface to keep the puree inoculated anaerobiosis conditions. Samples are then incubated at a temperature of 37ºC for 48 hours. Reading is the final 24 hours and 48 hours later. A positive reaction is indicated by smutch environment and record the number of bubbles and tubes with blackened environment. Based on these

tables are calculated using tables for calculating the STAS site effect.

To determine the probable number of spores of *Clostridium perfringens* inoculum of 1 cm3 is transferred from each balloon or tube with broth for sulphite-reducing clostridii positive tubes with sterile litmus milk or milk simple. Probable number of spores of *Clostridium perfringens* is calculated from the number of positive tubes, the alveolar rennet test, also used for calculations Mc Crady tables.

Determination of the total number of germ

Common methods for determining the bacterial count of water is based on solid nutrient medium water seeding, incubating them at a certain time thermostat at a certain temperature and counting the colonies developed, considering that each colony is growing at least 10 Ge.

Technique to determine: As a culture medium using 2% nutrient agar medium. Before sowing for mixing, shake the bottle with water sample (about 20 times). The mouth of the bottle and are be on fire decimal dilutions of water sought (1 / 10, 1 / 100, 1 / 1000 etc..). Dilutions made by introducing 1 ml of water investigated in 9 ml of sterile water (1 / 10) and further, by introducing 1 ml of dilution lower in 9 ml sterile water to obtain a higher dilution. By 1 $cm^3$ sterile pipette is inserted 1 ml of undiluted water and desired decimal dilutions in sterile Petri boxes, over which pour about 10 ml of melted and cooled nutrient agar medium at 45°C, printing box rotary motion horizontally for mix the contents. After solidification of jealousy, Petri boxes are placed in thermostat cover down, where incubated 24 hours. Number Petri boxes sown, and sown the total amount of water varies with water quality sought. Each box Petri can not sow more than 1 ml of water. For each sample will sow at least two boxes of raw water Petri, if water is pure, and at least two packs of 3-4 Petri decimal dilutions, if water is contaminated. These multiple insemination is necessary because the Petri dish with a diameter of 10 cm can grow well and include more than 300 colonies properly so that it is necessary to obtain at least one of these plates less than 300 colonies / l ml Liquid seed.

After removal of the thermostat, the colonies are kept naked eye (if their density allows), dividing the plate into quarters, or by counting the colonies. Be taken into account only cards that were developed more than 300 colonies. The calculation is done by applying the formula: $\frac{n \times d}{N}$

here: n - number of colonies grown on each plate;

d - degree of dilution of the material sown;

N - number of plates making the calculation

For calculation and monograms can be calculated using the same principles. The results are compared with the rules given in the drinking water standard. They are more than 20 bacteria / ml for water supplied to urban and rural power plants with disinfected water, more than 100 organisms/ml for water supplied by water power plants undisinfected, more than 300 organisms/ml for water supplied from local sources (wells, springs, etc.).

Determining the number of seeds incubated at 20°C.

This test runs after the technique used to determine the total number of seeds incubated at 37°C, the only different incubation temperature. In natural conditions the flora incubated at 37°C and incubated at 20°C there is a 3 to 1 in favor of the last group.

## RESULTS AND DISCUSSION

Results of tests carried out are presented in Table 2.

The suspension of the examinations of water samples were found in microscopic deposits of organic matter (ciliații), sediment and invertebrates from family *Tardigrada* (Fig. 1).

Table 2
Result of bacteriological samples of water taken

| Determination name | P1- Borehole * | P2- Swimming * | P3- Adapters* | Values allowed [2] | Reference document |
|---|---|---|---|---|---|
| Determination Escherichia Coli (number/100ml) | a.17<br>b.33 | a.33<br>b.17 | a.17<br>b.11 | ABSENT | SR EN ISO 9308 -1/2004 LCCAP PS 14 |
| Determination Enterococus (Faecal streptococus/100m) | a.0<br>a.0 | a.17<br>b.11 | a.33<br>b.17 | ABSENT | SR EN ISO 7899 - 2/2002 LCCAP PS 15 |
| Determination coliforms (number/100ml) | a.17<br>b.17 | a.33<br>b.17 | a.17<br>b.17 | ABSENT | SR EN ISO 9308 - 1/2004 LCCAP PS 16 |
| Determination Clostridium Perfringens (number /100ml) | a.ABSENT<br>b.ABSENT | a.ABSENT<br>b.ABSENT | a.ABSENT<br>b.ABSENT | ABSENT | Method described by Law 458/2002,suppl emented by Law 311/2004 LCCAP PS 17 |
| Determining the number of colonies at 22 and 37º C (number / ml) | a.9 b.4 colonies from 22º<br>a.9 b.5 colonies from 37º | a.8 b.13 colonies from 22º<br>a.10 b.10 colonies from 37º | a.4 b.7 colonies from 22º<br>a.0 b.3 colonies from 37º | 22º - 100<br>37º - 20 | SR EN ISO 6222/2004 LCCAP PS 18 |

* a.28.04.2009 b.12.05.2009

1 microorganisms with cils-P3 Adapters 2 Tardigrada -P2 -basin 3 Sediments -P1- fountain

Fig. 1 Deposits of organic matter, sediment and invertebrates from family *Tardigrada* suspended in water samples analyzed

## CONCLUSIONS

Following bacteriological analysis to see that the water qualities of poultry farm SC Bio-Top-Ovo Ltd. Lipova, Vaslui not meet the required properties of water under the maximum admissible concentration (L311-L458 -2002 and 2004) of bacteriological characteristics.

The water samples analyzed were identified pathogen for which the law does not allow the presence in water: *Escherichia coli, faecal streptococcus, coliforms, Clostridium perfringens*. The suspension of water were identified deposits of organic matter, sediment and invertebrates family *Tardigrada*

It recommends filtering water, check and change any waste pipes through which water, clean pool accumulation and eventually replace the basin floor (pelvic wall is concrete), and water treatment for microbiological characteristics change of water used.

If these changes do not alter the biological value of water is recommended to change the source of water.

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