CASE REPORT

ASSESSMENT OF THE HYGIENIC CORRECTNESS OF DRINKING WATER ON THE FARM OF SOUTH AMERICAN CHINCHILLA BEFORE AND AFTER "SHOCK" TREATMENT WITH STABLE LIQUID CHLORINE DIOXIDE: A CASE REPORT

Ališah Ajla[1*], Bešić Aida[2], Nenadović Katarina[3], Agić Hasan[4], Gagić Abdulah[5]

[1]Department of Animal Production and Biotechnology, University of Sarajevo Veterinary Faculty, Bosnia and Herzegovina
[2]Department of Clinical Sciences of Veterinary Medicine, University of Sarajevo Veterinary Faculty, Bosnia and Herzegovina
[3]Department, of Animal Hygiene, Faculty of Veterinary Medicine, University of Belgrade, Serbia
[4]Cantonal Administration of Inspection Affairs, Sarajevo Canton, Bosnia and Herzegovina
[5]University of Sarajevo Veterinary Faculty, Bosnia and Herzegovina

[*]**Corresponding author:**
Assistant professor Ajla Ališah
Address:
Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina
Phone: +38761691818
ORCID: 0009-0000-5951-0871
E-mail: ajla.alisah@vfs.unsa.ba

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ABSTRACT

Chinchillas consume low daily amounts of water, and due to their sensitivity and susceptibility to digestive tract diseases, they require extremely high-quality water. For the conventionally used disinfectants it is difficult or almost impossible to reach the standards of cleanliness of water distribution systems. Stable liquid chlorine dioxide is a disinfectant, which successfully and provenly removes most microorganisms and has no negative effect on the equipment and animal health.

The aim of the study was to investigate a desinfectant potential in establishing the microbiological purity of the water tank at critical points before and after treatment with chlorine dioxide. The results show that microbiological contamination at both critical points is higher before than after treatment.

In conclusion, more frequent control of water quality is needed as well as more frequent disinfection of the water distribution system on the farm. Also, stable liquid chlorine dioxide can be a desinfectant of choice in order to meet the criteria prescribed by the Rulebook on the Healthiness of Drinking Water.

Key words: Stable liquide chlorine dioxide (Cl_2O), disinfection, water hygiene

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INTRODUCTION

Chinchilla originates from South America, more specifically from central Chile, Bolivia and Peru. It is an animal from the order Rodentia (rodents) (Quesenberry, 2015), and there are several types of chinchilla: *Chinchila chinchilla* (large or royal chinchilla), *Chinchila brevi caudata* (small short-tailed chinchilla) and *Chinchila lanigera* (small long-tailed chinchilla). The last two mentioned chinchilla species exist in nature (wilderness of the Andes), but their population is rare today, because the local population, in addition to their fur, also hunts them for their meat (Katica and Crnkić, 1999; Spotorno et al., 2004).

Chinchila chinchilla (large or royal chinchilla) and *Chinchila lanigera* (small long-tailed chinchilla) are listed as critically endangered species by the International Union for Conservation of Nature due to drastic population decline (IUCN, 2013).

The beginning of chinchilla farming dates back to 1923, with the arrival of 13 individuals from Chile to the United States. In this way, the number of individuals on the first established farm multiplied (Katica and Crnkić, 1999; Richardson, 2003; Hsu et al., 2015). The trend of farming chinchillas was actualized in Europe in the middle of the last century. Thanks to farm breeding, this rodent species has been saved from extinction, since in South American countries it is exploited for human consumption and mass exploitation of its high-quality fur (Katica and Crnkić, 1999; Katica and Delibegović, 2019).

Access to quality, fresh hay must be provided at all times with fresh water to maintain dental and digestive health (Donnelly and Schaeffer, 1997). Daily water intake is 2-12ml/100g of body weight at room temperature. Chinchillas drink much more when fed exclusively with hay than with mixed or pelleted food (Katica and Delibegović, 2019; Wolf et al., 2020). Although drinking tap water is adequate, excessive chlorine can adversely affect general health (Hoefer and Crossley, 2001).

Also, in addition to this, for the successful intensive farming of chinchillas, adequate accommodation and necessary hygiene of equipment and inventory inside the cages with which the animals are in contact is important.

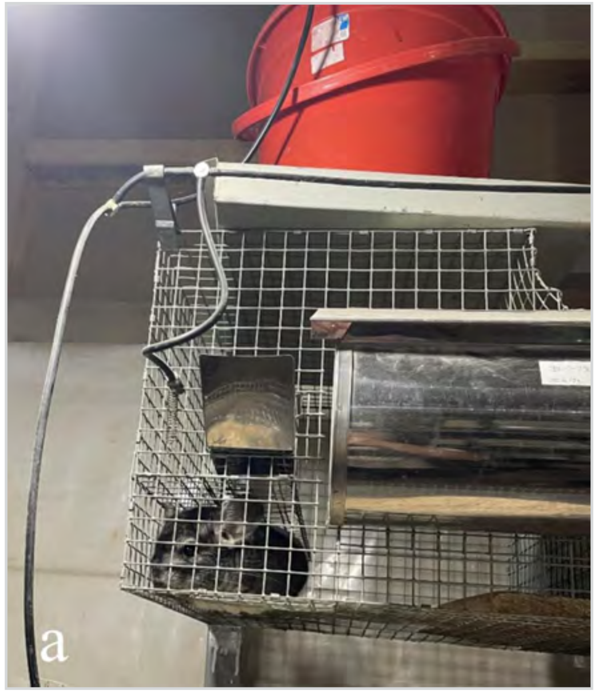
Opportunistic bacterial infections in chinchillas can cause disease, either localized to one organ or as septicemia. Animals that become ill are usually immunocompromised due to, for example, age, malnutrition or factors related to breeding (poor hygiene, poor ventilation, contaminated food and water). *Enterobacteriaceae* and *Pseudomonas aeruginosa* are associated with significant morbidity and mortality in chinchillas. However, these agents can also be isolated from clinically healthy animals, so most of these agents are not considered primary pathogens (MSD MANUAL, 2023). Stress or contaminated drinking water are predisposing factors for the development of infection with *Pseudomonas aeruginosa*.

In the context of maintaining the necessary inventory hygiene and drinking water sanitation inside the premises of the chinchilla farm, numerous disinfectants are used in practice. One of these is stable liquid chlorine dioxide, which is increasingly used for these purposes (Ališah, 2020). In a stable liquid form, chlorine dioxide is very easy to use in all types of water sanitation, both for drinking and for all other categories (swimming pools, waste water, etc.). It is characterized by stability at a wide range of temperatures (10 ^{0}C to 40 ^{0}C), as well as pH values (from 1.5 to 10) (Ališah, 2020).

Stabilized liquid chlorine dioxide is a yellow liquid with a chlorine smell. It does not contain aldehydes and does not foam, and works very efficiently even at low concentrations. It has a wide range of effects on all types of microorganisms, so it can be said that it is bactericidal, including sporogenous bacteria, virucidal, fungicidal and algicidal. After application on work surfaces, it is not necessary to rinse since, apart from negligible amounts of chloride, it leaves no residues or odors (Gagić et al., 2013).

We present a case report from one of the farms of South American chinchillas in the Canton of Sarajevo, that is, an assessment of the hygienic

Picture 1 Aspect of the chinchilla farm cage



Picture 2a Critical point I – water tank

Picture 2b Critical point II - nipple system

correctness of drinking water before and after the "shock" treatment with stable liquid chlorine dioxide in the water distribution system of the farm.

Case description

The farm of South American chinchillas is located on the outskirts of Sarajevo Canton. The farm premises where the mentioned animals (*Chinchilla lanigera* - small long-tailed chinchilla) are grown meet all the standards of good production practice and have built-in equipment for temperature and air humidity regulation (temp. 23.5°C, relative humidity about 60%), which follows daily.

The animals are housed individually in cages, and 60 cages belong to one so-called battery (Picture 1). The dimensions of the cage are: width 40 x length 50 x height 35 cm. Each cage has a feeder as well as drinking bowls that end in the form of a nipple in the cage.

Chinchillas are fed with conventional pelleted food, which consists of pressed grains with appropriate vitamin and mineral supplements. The interior of the cage is organized in such a way that the animals are kept on plastic trays lined with spruce and pine sawdust, and the mat is changed weekly and more often, if necessary. All animals in the cages appeared clinically healthy. We have identified critical points based on the results of our previous research, which prove that biofilm formations which are created in the interior of closed water distribution systems, are one of the leading both health and hygiene problems on animal farms (Ališah, 2020; Jović, 2020).

After defining the critical points and marking them, we started with the sampling of the water consumed by the animals on the farm. The first critical point is the water tank from which the water is supplied to the chinchillas through the hoses (Picture 2a), while the second critical point is the end of the hose-nipple system in the cages (Picture 2b).

The water tank has a volume of 10 liters and is washed and disinfected daily. Water from the city's water supply is poured into it daily, and if necessary, more often, which is under the regular hygiene and sanitary supervision of the Institute for Public Health of the Canton of Sarajevo. From the water tank, water is distributed evenly through a rubber hose with a diameter of 0.5 cm, free-falling, to each cage (Picture 2a), which ends with a nipple (Picture 2b). Water samples were taken on two occasions. On the first, two samples were taken, one from the vessel marked "Inlet-before-treatment vessel", which is located on top of the battery (Picture 2a). We poured the water from the water tank directly into a sterile bottle. After that, another sample was taken from the water distribution system, that is, from the nipple from one of the cages at the bottom of the battery in such a way that the water was allowed to flow directly into the sterile bottle.

We chose the position of the bottom of the battery because we wanted to be sure that the sample we collected was water that had passed through the entire water distribution system. We labeled this sample "Last point of sampling - before treatment".

The samples taken were distributed to the laboratory for sanitary microbiology of the Public Institution - Institute of Public Health of the Sarajevo Canton for microbiological testing. The microbiological analysis of the water was performed according to the standard methodology, which is in accordance with the regulations of the Rulebook on Health Suitability of Drinking Water (Official Gazette of BiH, 2017).

Furthermore, we approached the so-called "shock" water treatment with a disinfectant based on stable liquid chlorine dioxide. Since it was a concentrated agent, it was necessary to make a solution. According to Ališah (2020), the appropriate dose of the mentioned disinfectant was determined as 40 ml/10L of water added to the water tank, previously emptied and refilled with tap water.

After that, we put the water tank back on top of the battery and connected it to the water distribution hose. To stop animals to drink this water, we separated all the hoses from the cage. In order to make sure that the solution entered the entire system evenly, we let the water from the pipe flow freely, until we smelled residual chlorine dioxide at all hose outlets. This was a sign that the entire system was filled with the used solution. Then, the entire system was closed and the solution was left to act for 60 minutes.

At the end of the scheduled time, the water with the solution was allowed to flow out of the entire system, after which we rinsed it with "new" water from the tap, in such a way that we filled the water tank and let the entire amount of new tap water pass through the system. We repeated the entire procedure of taking samples from the same defined critical points: "Inlet water tank - after treatment" and "Last point of sampling - after treatment".

Table 1 lists the results of the microbiological examination of the first critical point, the drinking water from the water tank before treatment and after it had previously been adequately treated with the "shock" treatment with stable liquid chlorine dioxide.

Table 1 Comparison of results before and after "shock" treatment of the critical point "water tank"

Test parameter	Unit of measure	Test result BEFORE	Test result AFTER TREATMENT	Reference value	Analytical method
Escherichia coli on 36°C±2°C;21h±3h	Cfu/100ml	<1 cfu	<1 cfu	0	MF BAS EN ISO 9308-1:205
Enterococcus spp. na 36°C±2°C;44h±4h.	**Cfu/100ml**	**50 cfu**	**<1 cfu**	**0**	**MF TM203:2021**
The number of coliform germs on 36°C±2°C;21h±3h	Cfu/100ml	<1 cfu	<1 cfu	0	MF BAS EN ISO 9308-1:205
Total number of live germs, 22°C±2°C;68h±4h	Cfu/ml	<1 cfu	<1 cfu	100	BAS EN ISO 6222:2003
Total number of live germs, 36°C±2°C;44h	**Cfu/ml**	**280 cfu**	**<1 cfu**	**20**	**BAS EN ISO 6222:2003**
Pseudomonas aeruginosa on 36°C±2°C;44h±4h.	**Cfu/100ml**	**80 cfu**	**<1 cfu**	**/**	**MF BAS EN ISO 16266:2009**
Sulfite-reducing anaerobes (Clostridia) on 37°C±1°C; 20h±4h ili 44h±4h.	Cfu/50ml	<1 cfu	<1 cfu	/	MF BAS EN 26461-2003
Staphylococcus aureus on 36h±2h; 21h±3h	Cfu/100ml	<1 cfu	<1 cfu	/	Membrane filtration method

Table 2 lists the results of the microbiological examination of the second critical point, the drinking water from the end of the hose-nipple before treatment and after it had previously been adequately treated with the "shock" treatment with stable liquid chlorine dioxide.

Table 2 Comparison of the obtained test results of water samples from the hose before and after the "shock" treatment

Test parameter	Unit of measure	Test res ult BEFORE TREATMENT	Test result AFTER TREATMENT	Reference value	Analytical method
Escherichia coli on 36°C±2°C;21h±3h	**Cfu/100ml**	**90 cfu**	**<1 cfu**	**0**	**MF BAS EN ISO 9308-1:205**
Enterococcus spp. on 36°C±2°C;44h±4h.	**Cfu/100ml**	**60 cfu**	**<1 cfu**	**0**	**MF TM203:2021**
The number of coliform germs on 36°C±2°C;21h±3h	**Cfu/100ml**	**160 cfu**	**<1 cfu**	**0**	**MF BAS EN ISO 9308-1:205**

Test parameter	Unit of measure	Test res ult BEFORE TREATMENT	Test result AFTER TREATMENT	Reference value	Analytical method
Total number of live germs, 22°C±2°C;68h±4h	Cfu/ml	<1 cfu	<1 cfu	100	BAS EN ISO 6222:2003
Total number of live germs, 36°C±2°C;44h	**Cfu/ml**	**160 cfu**	**<1 cfu**	**20**	**BAS EN ISO 6222:2003**
Pseudomonas aeruginosa on 36°C±2°C;44h±4h.	**Cfu/100ml**	**120 cfu**	**<1 cfu**	**/**	**MF BAS EN ISO 16266:2009**
Sulfite-reducing anaerobes (Clostridia) on 37°C±1°C;20h±4h or 44h±4h.	Cfu/50ml	<1 cfu	<1 cfu	/	MF BAS EN 26461-2003
Staphylococcus aureus on 36h±2h; 21h±3h	Cfu/100ml	<1 cfu	<1 cfu	/	Membrane filtration method

DISCUSSION AND CONCLUSIONS

The fact is that without ensuring a minimum of basic zoohygienic and ethological conditions, there is no successful breeding of South American chinchillas in farm conditions. We started the research at the personal request of the owner of the chinchilla farm, for whom the well-being of the animals is important, in addition to economic profit. Although in the literature and good production practice the emphasis is primarily on housing conditions, microenvironment, qualitative-quantitative food, regular veterinary supervision and preventive therapy, very often, if not always, the importance of drinking water is forgotten, which is evident when trying to find literature data on this topic.

In most farm animals, water represents two-thirds of the daily ration, and in addition to quantity, it must also meet strict quality standards. This means that no matter what is given to animals, it must have the same microbiological composition as for human consumption. Looking back at the rights of animals, which were proposed as early as 1965 and revised and adopted in 1979, it is clearly visible that food and water were put first, among other things, in the context of "freedom from hunger and thirst" (Vučinić et al., 2023).

When talking about the quality of water for chinchillas, there is a deficit in the available literature, so that bottled water is preferred for chinchillas as pets (Anonymous, 2022), and for farmed chinchillas, water from urban water distribution is used (Hagen et al., 2014).

Based on the successful application of the disinfectant based on stable liquid chlorine dioxide in poultry farms of broiler chickens (Ališah, 2020) in various aspects of disinfection, we decided to use this preparation in our study.

Our findings prove the presence of conditionally pathogenic bacteria *Escherichia coli, Enterococcus spp*, the number of coliform germs, the total number of live germs, which, according to the Rulebook on microbiological testing of drinking water: with the addition of the parameter *Pseudomonas aeruginosa*, should not be found in drinking water (Bach, 2018).

From the submitted test results, it is clearly visible that the water taken from the sites defined as critical points and before the "shock" treatment does not meet the criteria prescribed by the Rulebook on the Healthiness of Drinking Water (Official Gazette of BIH, 2017). The water from the position marked - "Water tank-before treatment" does not meet the criteria regarding the detection of enterococcal bacteria and the total number of live germs in the submitted sample. Water from the position marked “Last point of sampling - before treatment” by far exceeds the number of allowed tested bacteria, and as such is extremely health-hazardous for consumption.

The results of analysis of the samples from Table 1 and Table 2 clearly and unequivocally indicate the effectiveness of the “shock” treatment with the solution of stable liquid chlorine dioxide and its successful elimination of the mentioned bacteria in drinking water.

Since there are evident large gaps in the literature related to analysis of the hygienic correctness of drinking water as well as the use of disinfectants for the disinfection of water distribution systems on farms, we could not adequately compare the results of our study.

When taking anamnestic data from the owner of the farm as well as through our inspection, we found that the animals looked clinically healthy. However, they consumed water that was obviously hygienically incorrect. Looking for a rational explanation of a possibility for the animals to be clinically healthy despite it, the possible answer is that they are not immunocompromised, and that they consumed a sufficient amount of quality, balanced food with satisfactory other zoohygiene aspects. Furthermore, the chinchillas on the examined farm are young adults and their immune system successfully coped with conditionally pathogenic bacteria, *Escherichia coli, Enterococcus spp,* coliform germs, and live germs, as well as *Pseudomonas aeruginosa* (MSD MANUAL, 2023).

It is a wrong assumption that if the water is clean at the source, it is of the same quality at the place where the animal drinks from, which was also proven by this assessment of the hygienic correctness of drinking water. Our experiences with stable liquid chlorine dioxide have proven to be effective. It is significant to scientifically present the importance of hygiene in water distribution systems, because they are the transporters of drinking water.

The obtained results are alarming and indicate the necessity of periodic analysis of the hygienic correctness of drinking water as well as the necessity of disinfection of the water distribution system on the farm.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

CONTRIBUTIONS

AA: 1,2, 5, 6, 7, 8, 9; BA: 3, 6, 7, 8, 9; NK: 7, 8, 9, 10; AH: 4, 5, 6, 10; GA: 3, 5, 6, 8, 10

(1) Conception; **(2)** Design; **(3)** Supervision; **(4)** Fundings; **(5)** Materials; **(6)** Data Collection and/or Processing; **(7)** Analysis and/or Interpretation of the Data; **(8)** Literature Review; **(9)** Writing; **(10)** Critical Review

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PROCJENA HIGIJENSKE ISPRAVNOSTI VODE ZA PIĆE NA FARMI JUŽNOAMERIČKE ČINČILE PRIJE I POSLIJE "ŠOK" TERAPIJE STABILNIM TEČNIM HLORDIOKSIDOM: PRIKAZ SLUČAJA

SAŽETAK

Činčile konzumiraju male dnevne količine vode, ali zbog osjetljivosti i podložnosti obolijevanja digestivnog trakta, zahtjevaju izuzetno kvalitetnu vodu. Konvencionalna dezinfekciona sredstva teško ili skoro nikako ne mogu da zadovolje standarde čistoće vododistributivnih sistema. Stabilni tečni hlor dioksid je dezinfekciono sredstvo, koje uspješno i dokazano odstranjuje mikroorganizme i nema negativno djelovanje na opremu niti na zdravlje životinja.

Cilj rada je bio istražiti dezinfekcioni potencijal u utvrđivanju mikrobiološke čistoće spremnika za vodu, na određenim kritičnim tačkama, prije i nakon tretmana sa stabilnim tečnim hlor dioksidom. Rezultati su pokazali da mikrobiološka kontaminacija, na obje kritične tačke je veća prije, u odnosu na rezultate nakon tretmana.

Kao zaključak se izvodi da je neophodna mnogo češća kvalitativna kontrola vode, kao i redovnija dezinfekcija vododistributivnih sistema farme, a stabilni tečni hlor dioksid je dezinfekciono sredstvo koje, u pogledu efikasnosti, zadovoljava kriterije Pravilnika o zdravstvenoj ispravnosti vode za piće.

Ključne riječi: Stabilni tečni hlordioksid (Cl_2O), dezinfekcija, higijenska ispravnost vode