EFFICIENCY OF SURFACE DISINFECTION BY NEBULIZATION USING CUBE ATOMIZERS IN A VETERINARY UNIT – PRELIMINARY STUDY

Catalina Valeria GARBACEA¹, Emoke PALL¹, Mihai CENARIU¹, Ioan Ștefan GROZA¹

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania

Corresponding author email: garbacea catalina@yahoo.com

Abstract

Maintaining proper asepsis and hygiene conditions in spaces intended for veterinary surgery remains a paramount for compliance with professional ethics. Moreover, environmental surfaces can contribute to the spread of cross-infections, and therefore constitute a likely transitory site for the accumulation of microorganisms. The aim of the current study was to evaluate, implement and measure the efficacy of a novel nebulization technique method for surface disinfection. The procedure was carried out in ten enclosed spaces used as surgery rooms within veterinary clinics. Disinfection was performed using Cube Atomizers, a nebulizer with a revolutionary spraying system, which transforms the biocide substance into microparticles that persist in the air for a long period of time, ensuring decontamination of the treated volume (air and all types of surfaces). Thus, using an approved biocide, the device ensured a successful disinfection of spaces, eliminating bacteria, and other biological pathogens. The microbiological tests were carried out before and after disinfection on different growth mediums (Agar for the total bacteria count, Chapmann for Staphylococcus, Holmes for Streptococcus, Levine for Gram-negative Cocci and Sabouraud for fungi). An increased efficiency of disinfection was observed, with a significant decrease in total bacteria count of almost 90-97% and the value of colony-forming units reaching 0 after nebulization in some cases; for Staphylococcus (Chapmann) there was a significant decrease, between 85-95%; for Streptococcus (Holmes) the decrease was almost 90-99%; for Gram-negative Cocci (Levine) the decrease was almost 92-99%; and for fungi (Sabouraud) the decrease was around 50%. The Cube Atomizers device is easy to use, can be fitted anywhere and guarantees safety for the user, environment, and all treated materials. Its revolutionary system ensures decomposition of the biocide into microparticles, leaving no residues.

Key words: disinfection, Cube Atomizer, veterinary unit, nebulization.

INTRODUCTION

Maintaining appropriate aseptic and hygienic conditions in spaces intended for veterinary surgery remains a paramount in the desire for success and respect for professional ethics. Animal surgery requires the same precautions regarding infections as human surgerv. (Andercou, 1993) In addition to the antisepsis used for disinfection of hands, instruments and materials. rigorous disinfection of the surgical/obstetrical area is also necessary. (Bogdan et al., 2009; Boitor et al., 1986; Bolte S., 1988; Cenariu M. et al., 2020; Groza I. et al., 1998) The occurrence of infection as a result of wound contamination is one of the greatest risks of surgery (Mates, 2001; Mitrănescu Elena, 2014; Oană L. et al., 2012; Răpuntean S. et al., 2017) Preventing this is done by the correct execution of asepsis, antisepsis and disinfection measures, by removing all possibilities of contamination of the surgical wound. (Leau et al., 2014) Although data related to nosocomial infections in veterinary medicine are limited. they are present and lately their frequency is increasing. (Ruple-Czerniak et. al, 2013; Ruple-Czerniak et al., 2014; Stull et al., 2015). In veterinary clinics, the fact that a large proportion of the pathogens involved in causing nosocomial infections are zoonotic is a big problem for both humans and animals, considering that many zoonoses can have a serious, sometimes fatal, course (Bîrțoiu A. et al., 2004; Gonciarov Magda, 2014; Igna C., 2001; Savu et al., 2000). Unlike human medicine, in veterinary medicine no hygiene protocols are developed for veterinary clinics. In a veterinary hospital, where faeces and different types of secretions are always present, the susceptibility of harbouring pathogens is much higher and represents an increased risk for contamination with nosocomial and zoonotic diseases, so that rigorous disinfection is crucial. Veterinarians and ancillary staff need to be educated in this regard and disinfection protocols need to be implemented in every veterinary medical unit. (Traverse et al., 2015) The aim of the current study was to evaluate, implement and measure the efficacy of a novel nebulization technique method for surface disinfection.

MATERIALS AND METHODS

For this study, disinfection by nebulization was carried out in closed spaces intended for surgery rooms in ten veterinary clinics in Romania. Clui County. The device used was Cube Atomizers. a nebulizer with a revolutionary spraying system that transforms the biocide into microparticles that remain in the air for a long time, ensuring total decontamination of the treated volume (air and all types of surfaces). Thus, using an authorised biocide, the machine successfully disinfects premises, eliminating bacteria, fungi, viruses and other biological pathogens of any kind from all surfaces in just a few minutes. Its revolutionary system breaks down the biocide into micro-particles, leaving no residue. Compared to other means of disinfection used in current practice (e.g. steam generators or ultraviolet lamps) where more manpower is required, or the time of use is long, the Cube Atomizers disinfects the entire surface in just a few minutes, is easy to use, can be installed anywhere and requires only one person to handle it. Unlike the technique of disinfecting premises with ultraviolet lamps, the Cube nebuliser guarantees safety for the user, the environment and all treated materials. Ultraviolet lamps require a longer period of time to disinfect the space and can cause skin and eye damage, degrade treated surfaces over time, and cause respiratory tract damage through the generation of ozone (FDA, 2021). Sanitation samples were taken 2-3 hours before the start of the nebulization operation (BEFORE test), and 1-2 hours after the nebulization was performed (AFTER test).

A. Materials needed

The following materials were required (Figure 2): non sterile disposable gloves, protective equipment (disposable gown), protective goggles, utensils and measuring devices for

preparing the quantities of needed disinfectant product, cleaning materials (detergent and wipes), disinfectant, waste collector, a device for measuring room size and volume (laser telemeter), sterile swabs with culture medium, sterile test tubes specially prepared for sampling, Petri dishes and other utensils needed for sampling for bacteriological analysis, the Cube Atomizers nebulizer, a power supply (grounded socket) and a timer. Biosan Steridet disinfectant solution, 20 g/l water, was used for this study. Biosan Steridet (Figure 1) is a pink with strong disinfectant action. powder Pentapotassium containing Bis (peroxymonosulphate) Bis (sulphate) as active substance



Figure 1. Biosan Steridet solution (Original)



Figure 2. Materials needed: cube atomizers, Petri dishes, sterile swabs with culture medium, disinfectant solution, timer, graduated beakers, laser telemeter (Original)

B. Preparing the space for disinfection and actual nebulization

Mechanical cleaning of the space and surfaces was carried out prior to the commencement of nebulisation, according to general cleaning protocols. Coarse dirt was removed prior to disinfection and the floor was mopped and surfaces (tables, lamps, and equipment in the surgical rooms) were wiped with a washcloth dipped in cleaning solution (detergent). After preparing the space, the room volume was calculated using the laser telemeter to determine the time required to operate the machine and the amount of disinfectant material required for use. Finally, the space was closed as tightly as possible. Protective equipment was put on, and the machine was prepared and supplied with the required amount of disinfectant (Figure 3).



Figure 3. Positioning and preparing the machine (Original)

Then, using an earthed socket, the Cube Atomizers was placed in a corner of the room, as close as possible to the outlet and with the nozzle oriented diagonally across the room. In order to start the nebulisation process, the windows and doors of the room were closed, it was ensured that no other person was in the room, and access was prevented until the room was ventilated after the cycle (nebulisation/ activation/aeration) was completed. The button was placed in the ON position and the room was left, closing the door. The appliance was left to operate and waited until nebulisation was complete (completion of the process is identified by the cessation of the specific sound made by the appliance at the time of operation). The switch was set to the OFF position, the machine was removed from the room and the room was kept closed for the contact time and then the room was ventilated. After use, the machine is wiped with a cloth and stored in a place protected from the bad weather and out of direct sunlight.

C. Sample collection and analysis

For bacteriological analysis of air, the sedimentation method was used, thus collecting the microorganisms using Petri dishes. The sedimentation method consists of gravity deposition of airborne germs on the surface of solid culture media in Petri dishes. In the rooms where the determinations were carried out, the Petri dishes were placed horizontally in the middle of the room, their covers were removed and the exposure time was timed. Before misting for 2-3 hours, for sampling with Petri dishes the exposure time of the culture media was 15 minutes, and 1-2 hours after misting and venting, the exposure time of the Petri dishes was 30 minutes. Another method used for the determination of microorganisms was sampling by swabbing with sterile culture media swabs. Both before and after nebulisation, samples were collected with these swabs from various surfaces in the room (tables, floors, surgical lights, inhalers, cabinets and other appliances) (Figure 4). Samples collected in this way were seeded on solid culture media in Petri dishes. The plates were then incubated and after incubation the developed colonies were counted. Simple agarose (nutrient agar) known as agar is used to determine the total number of germs (NTG). By frequency in air and on all surfaces, as well as implications for pathology, in addition to the total number of germs, the following agents were determined in pathological particular: staphylococci Chapmann on medium, streptococci on blood agar, sodium azide and crystal violet (Holmes medium), gram-negative germs on Levine medium, and fungi on Sabouraud medium.



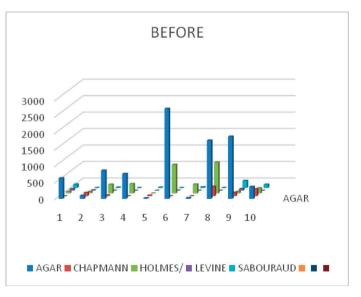
Figure 4. Sampling procedure (Original)

RESULTS AND DISCUSSIONS

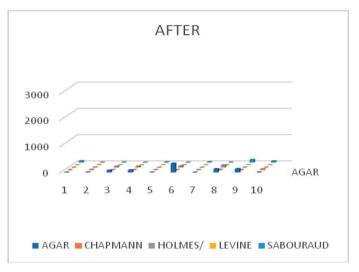
The results of the sanitation tests before the start of the disinfection procedure (Before test) and after mechanical cleaning and disinfection (After test) in the 10 premises used as surgery rooms in the veterinary clinics can be seen in the table (Table 1) and graphs (Graph 1 and Graph 2) below.

SAMPLE	Exposure time (minutes)/ METHOD	AGAR (Total bacteria count) (ufc/m ³)	CHAPMANN (Staphylococc us) (ufc/m ³)	HOLMES (Streptoco ccus) (ufc/m ³)	LEVINE (Gram-negative Cocci) (ufc/m ³)	SABOURAUD (fungi) (ufc/m ³)
1-Before disinfection	15'/Sedimentation	628	0	52	52	105
1-After disinfection	30'/Sedimentation	0	0	0	0	52
2-Before disinfection	15'/Sedimentation	98	100	47	12	2
2-After disinfection	30'/Sedimentation	8	11	4	1	1
3-Before disinfection	15'/Sedimentation	859	25	269	0	12
3-After disinfection	30'/Sedimentation	78	2	39	0	4
4-Before disinfection	15'/Sedimentation	759	0	287	0	0
4-After disinfection	30'/Sedimentation	87	0	38	0	0
5-Before disinfection	15'/Sedimentation	21	20	0	0	12
5-After disinfection	30'/Sedimentation	0	0	0	0	7
6-Before disinfection	-/Sterile swab	2740	0	871	3	2
6-After disinfection	-/Sterile swab	342	0	49	0	0
7-Before disinfection	-/Sterile swab	27	0	274	0	22
7-After disinfection	-/Sterile swab	0	0	0	0	12
8-Before disinfection	-/Sterile swab	1772	290	940	0	0
8-After disinfection	-/Sterile swab	131	12	82	0	0
9-Before disinfection	-/Sterile swab	1891	105	52	52	219
9-After disinfection	-/Sterile swab	135	0	0	0	98
10-Before disinfection	-/Sterile swab	367	210	157	0	104
10-After disinfection	-/Sterile swab	6	29	0	0	49

Table 1 - Results of sanitation tests



Graph 1. Sanitation sample results before nebulization (before test)



Graph 2. Sanitation sample results after nebulization (after test)

After keeping the samples in the thermostat at the appropriate temperature and conditions, the results were interpreted (Figure 5).



Figure 5. Sanitation samples - Petri dishes (Original)

Thus, in the samples cultivated on Agar culture medium, a significant decrease in the total number of germs was observed with an average of 94.26% after disinfection by the method chosen by us for this research, in some cases the success rate was 100%, reaching a value of 0 after disinfection. When using Chapmann medium in some cases we did not find the presence of staphylococci either at the beginning of the experiment or 30 minutes after the application of disinfection, but in the cases where we found the presence of staphylococci, the number of staphylococci was reduced to 93.84% after disinfection and in some cases the

value reached 0 (100%) after disinfection. In the case of Holmes culture media, the number of streptococci was observed to decrease by an average of 94.36%, and in some cases the number reached 0 (100%) following disinfecttion. The same was true for the Levine medium, specific for the isolation of gram negative bacteria, with a decrease of 97.9% on average and 100% efficiency in some cases. In the case of Sabouraud medium a decrease of 57.79% on average was observed. After processing the samples, we found a 30-40% higher efficacy of the machine used in this study than in the case of using the ultraviolet lamp or the steam generator machine as a disinfection method, where the same disinfectant was used. Due to the speed, dispersion and reflux of microparticles throughout the space, the use of Cube Atomizers ensures disinfection of all surfaces (appliances, furniture, ceiling, floor, windows, as well as hidden parts of furniture and appliances etc.) unlike the steam generator or UV lamp which achieve partial disinfection of surfaces.

CONCLUSIONS

Following the application of the new method of disinfection in veterinary medical premises dedicated to surgical interventions, the following were observed:

- In many cases, for most categories of germs, the value of colony-forming units reached 0 after

nebulisation (efficiency was 90-97% for total germ count, 85-95% for staphylococci, 90-99% for streptococci, 92-99% for Gram-negative bacteria and around 50% for fungi), which is of particular importance in view of the obligation of a high degree of hygiene in these premises;

- Efficiency is 30-40% higher for the method proposed for this study than for other disinfection methods used in current practice (e.g. steam generator or UV lamp);

- The Cube Atomizer is more efficient as it does not require a long time for use, does not leave residues, uses a small amount of disinfectant, is easy to handle and does not require changing the disinfectant used by the unit;

- Due to the short disinfection application time, the easy handling, its efficiency, on average 80-90%, compared to the other methods used, where the results were 30-40% lower;

- The small volume of disinfectant used, the short application time and the low labour involved lead to lower costs and a short payback of the initial investment;

- The method used for disinfecting veterinary medical premises has the advantage of being able to disinfect surfaces (floors, ceilings, appliances, windows, furniture, etc.) on all sides, including the back, areas underneath, edges, pipes, etc.), which would take much longer or be impossible to achieve with conventional decontamination methods, and would result in partial disinfection of surfaces compared to the above;

- Periodic disinfection with the Cube Atomizers in surgical wards on the basis of a planned schedule can ensure a high level of hygiene at all times and prevent nosocomial infections;

- In case of epizootiologic risk it should be carried out whenever necessary;

- It is necessary to educate veterinary staff on the obligation to maintain a high level of hygiene in veterinary hospitals.

REFERENCES

- Andercou A. (1993) *Surgical trauma ointments*, Dacia Publishing House, Cluj-Napoca.
- Bîrțoiu A., Seiciu F. (2004) Animal Reproduction Treatise, All Publishing House, Bucharest, Romania
- Bogdan L.M., Groza I. (2009) Veterinary obstetrics, Editura Academic Pres, Cluj-Napoca.
- Boitor I., Muntean M., Groza I., Moise D., Muscă M. (1986) - Veterinary obstetrics and gynaecology guide, Tipo Agronomia, Cluj-Napoca.

- Bolte S. (1988) *Practical guide to surgical propaedeutics*, Ceres Publishing House, Bucharest, Romania.
- Cenariu M., Pop R., Sonea A., coordinator Groza I. et al (2020) - *Physiology and pathology of the puerperal period in domestic animals*, Romanian Academy Publishing House.
- Coman I. (1988) Zooigiena şi protecção mediului ambienteurător, "Institutul Agronomic Ion Ionescu de la Brad" Publishing House, Iaşi.
- Gonciarov Magda (2014) Basics of Veterinary Epidemiology, Printech Publishing House, Bucharest, Romania.
- Groza I., Munteanu M. (1998) *Elements of Animal Reproductive Physiology*, Academic Press Publishing House, Cluj-Napoca.
- Groza I., Munteanu M. (1998) Obstetrics-Physiological and pathological endocrinology in dogs, Genesis-Tipo Publishing House, Cluj-Napoca.
- Hafez E.S.E., Hafez B. (2000) Reproduction in Farm-Animals, Seventh edition, Lippincott Williams & Wikins, A. Walters Jluwer Company, Philadelphia, Penmsylvania, USA.
- Igna C. (2001) Veterinary Surgical Techniques, Brumar Publishing House, Timişoara.
- Ivana Simona (2013) Manual of general microbiology, Asclepius Publishing House, Bucharest, Romania.
- Leau T., Leau F., Mihai Iuliana (2013) Practical guide to veterinary surgical techniques and propaedeutics, Printech Publishing House.
- Mateş N. (2001) Veterinary operative surgery, Risoprint Publishing House, Cluj-Napoca, 2001.
- Mitrănescu Elena (2014) *Hygiene*, Printech Publishing House, Bucharest, Romania.
- Oană L. I., Peştean C. P., Ober C. A., (2012) Veterinary anaesthesiology and surgical propaedeutics, Risoprint Publishing House, Cluj-Napoca.
- Răpuntean S., Răpuntean G. (2017) Veterinary medical bacteriology and bacterial zoonoses, AcademicPres Publishing House, Cluj-Napoca.
- Ruple-Czerniak A.A., Aceto H., Bender J.B. (2013) Nosocomial infection rates in small animal referral hospitals: using syndromic surveillance to establish baseline rates. *J Vet Intern Med.*; 27(6): 1392–1399.
- Ruple-Czerniak A.A., Aceto H., Bender J.B. (2014) Syndromic surveillance for evaluating the occurrence of healthcare-associated infections in equine hospitals. *Equine Vet J.*; 46(4): 435-440.
- Savu C., Petcu Carmen, Savu G. (2000) Zoonoses and common human and animal diseases with infectious etiology, Semne Publishing House, Bucharest.Stull JW, Weese JS. (2015) Hospital-associated infections in small animal practice. Vet Clin North Am Small Animal Pract., 45(2): 217-33, doi: 10.1016/j.cvsm.2014.11.009.
- Traverse M, Aceto H. (2015) Environmental cleaning and disinfection. Vet Clin North Am Small Animal Pract.; 45(2): 299-330, vi. doi: 10.1016/j.cvsm.2014.11.011..
- U.S. Food and Drug Administration (2021). Available online at:https://www.fda.gov/ Accessed March 26, 2023.