



EVALUATION OF THE EFFECTIVENESS OF THE DISINFECTANT SANDEZIA® ON DIFFERENT SURFACES

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ABSTRACT

Breeding and growing rabbits are kept to a large extent in intensive husbandry systems, mainly in cages with wire nets or slatted floor. The housing of rabbits is related to hygienic, behavioural, environmental and welfare aspects. Cleaning and disinfecting is imperative to maintain the wellbeing and health of animals. Thorough cleaning and adapted disinfection can help decrease the pathogen level and prevent or break the disease cycle. In this study we aimed on the efficacy of disinfection by using disinfectant Sandezia® in the form of powder applicated by scattering on the plastic and wooden floors of cages at a dose of 50g/m². Evaluation of the effectiveness of the disinfectant was perform by microbiological swabs from the area 5 x 2 cm² after

mechanical cleaning and after disinfection, from evaluated surfaces in the rabbits cages. Disinfectant was effective on evaluated surfaces where significant decrease of microorganisms, except molds was obtained. After disinfection we determined 6.3x10³ CFU of TCB (P<0.01); 0 CFU of CB (P<0.001); 1.6x10³ CFU of molds (P<0.05) on plastic floor. Results from wooden floor was 1.4x10³ CFU of TCB (P<0.05); 2.2x10¹ CFU of CB (P<0.05) and 3.6x10³ CFU of molds after disinfection. Presented results from the microbiological swabs represent decrease of microorganisms, in compare with numbers of colonies forming units of microorganisms before disinfection which shows that disinfectant Sandezia® was effective. Improved training in the correct application of hygiene measures is necessary due to ensuring correct zoohygienic conditions.

KEYWORDS: disinfection, microbiological swabs, disinfectant, rabbit cage.

INTRODUCTION

The biosecurity program of pet rabbits is an area of growing interest in Europe. Rabbits are common pets in many western European countries.^[1] They are kept in a variety of different housing types, the most common being a traditional cage. Some sanitary and environmental arrangements are very helpful and needful, including the application of biosecurity programs, the culling and removal of ill or dead animals, mechanical cleaning and disinfection of equipment, cages, instruments, insect traps on windows and ventilation intakes and avoiding wild rabbits entering the farm.^[2]

Good sanitation practices are especially important in stopping the spread of diseases.^[3] Sanitation means the maintenance of environmental conditions conducive to health and well-being and involve mechanical cleaning, and disinfection. The goal of any sanitation program is to maintain sufficiently clean and dry bedding, adequate air quality, and clean cage surfaces and accessories.^[4]

Mechanical cleaning can be defined as the removal of “soil” from surfaces and is important in all types of environments^[5], because it removes the majority of disease causing pathogens, but also removes excessive amounts of excrement, dirt, and debris. Subsequent disinfection reduces or eliminates unacceptable concentrations of microorganisms. All bedding material as hay, shavings, paper, straw, blankets, and litter soiled by urine and faeces should be removed and disposed of. Other potentially contaminated surfaces include cage or run floor, walls, ceiling, door, and door latch, door handles, hay racks, water bottle clips, and any other cage furniture which cannot be removed. Individual items that can be removed should be cleaned and then thoroughly wetted in the disinfectant. All surfaces of the enclosure, hutch or room should be mechanically cleaned first, washed with washing up liquid and water.^[6] After this step surfaces should be wetted thoroughly with a suitable disinfectant. Cages should be cleaned as often as necessary to prevent the unwanted microbiological contamination and formation of ammonia. Cages are generally sanitized on at least a weekly basis.^[7]

Disinfection is a process which reduces the number of organisms present to a level where they do not pose a threat to health. Disinfection should not be carried out in the same room as the rabbits. Most disinfectants may cause irritation to the eyes, skin and if inhaled or ingested

irritation to the respiratory or digestive system.^[8] General requirements for the housing of rabbits are summarized as follows:

- No avoidable suffering and no injuries caused by housing (floor, walls, equipment);
- Protection against ectoparasites and endoparasites;
- Provision of feed and water according to the needs of rabbits (*ad libitum*);
- Protection against adverse climatic conditions;
- Removal of gases, dust and pathogenic germs from the rabbit house;
- A good handling of animals (safe and quick catching – no frightening, no injuries);
- Separation of rabbits from their excrements by using perforated floors if possible (especially in the intensive housing);
- From time to time use of “all in – all out” with mechanical cleaning and disinfection;
- Enriched housing system – e.g. 2nd floor or elevated platform for the rabbits.^[9]

Swabbing in one form or another remains the oldest and probably the most widely used method for “surface microbial monitoring”.^[10]

Selecting the appropriate disinfectant for disinfection of rabbit cages can be helped by comparing different properties as dilution; contact time for surfaces; rinsing; what disease agent it is effective against of the disinfectants (e.g.: bleach = sodium hypochloride; Virkon S; acetic acid).^[11]

The type of surface, material to be disinfected have a great impact on effectiveness of a disinfection process. Porous, uneven, cracked, or pitted surfaces, especially wooden surfaces and earthen floors, can hide microorganisms and are difficult to disinfect.^[12]

Wooden housing is always difficult to clean effectively. This surface will always be a challenge to fully disinfect as there are many cracks and crevices that can collect contagious material and they are difficult to clean and disinfect completely. The wooden cage should be mechanically clean before using a suitable disinfectant. Steam cleaning may be useful after washing and before disinfection, to help get into the hard to reach areas. Rinsing with plenty of water after the correct contact time is useful to make the environment safe for rabbit again. The plastic surfaces inside rabbit’s cage must be also included in the cleaning and disinfection procedures and must be sanitizable.^[13]

The focus of the study was to analyze the effectiveness of disinfectant Sandezia[®] with active ingredient Tosylchloramide sodium in the form of powder by using microbiological swabs on monitored surfaces in the rabbit cages and on the basis of obtained results to assess the level of hygiene.

MATERIAL AND METHOD

Microbiological swabs were taken from Small animal breeding club where large (over 5 kg) and medium (3.5 - 5.0 kg) breeds of rabbits are bred. The club has 2 rabbits houses with 9 and 12 boxes. Large breeds of rabbits are housed in cages measuring 80 x 60 cm, small breeds of rabbits are housed in cages measuring 60 x 60 cm. In addition to rabbits, there are also 10 quails and 5 hens.

The hygienic level of different materials - plastic and wooden (Figure 1) was analyzed. Monitored surfaces were mechanically cleaned before disinfection. For disinfection of these surfaces was used disinfectant SANDEZIA[®] in powder form, without heating with exposure time 30 minutes, applicated by scattering on the floor at a dose of 50g / m². The disinfectant is composed of minerals, eucalyptus essential oil and the active ingredient is Tosylchloramide sodium 0.25g / 100g. Halogen-containing compounds, include chlorine, iodine, bromine and fluorine preparations are toxic to the cell and created by the action of oxygen in the initial phase. The optimum pH for the disinfection effect is from 5 to 8 and the presence of organic substances significantly reduces it. For practical disinfection, iodine, chlorine and its compounds are important.^[14] Disinfection was carried out in the presence of animals. It dries intensively wet bedding, preventing the conversion of urea into ammonia and the release of harmful gases. This product keeps the pH low, preventing the growth of pathogenic microorganisms. It reduces the number of diseases in a herd, the use of antibiotics and the costs of treatment. Moreover, in case of an infection, it supports the treatment. SANDEZIA[®] increases the content of nitrogen compounds in a stable or liquid manure that has a positive effect on crops increase when applying them as an organic fertiliser. Evaluated disinfectant prevents efficiently from *Brucella*, *Listeria*, *Escherichia coli*, *Salmonella Enteritidis*, *Staphylococcus aureus*, *Erysipelothrix rhusiopathiae*, *Streptococcus*, *Haemophilus influenzae*, *Botrytis cinerea*, *Coronavirus*, *Parvoviridae*, *Aspergillus*, *Chlamydiae*, *Clavibacter michiganensis*, *Pseudomonas*, *Pasteurella*, *Mycoplasma*.



Figure 1: Rabbits cages with plastic and wooden floor.

Sampling was performed with microbiological swabs after mechanical cleaning and after disinfection from 2 types of surfaces – plastic and wooden material. Each microbiological swab represented average value of 5 swabs taken from same place. The swabs were taken from area of 10 cm². ISO 18593 and ISO 21527 are standards for the method of sampling surfaces using swabs.^[15,16] Samples for microbiological analysis were taken with sterile cotton swabs using sterile templates, placed in a sterile tube containing 10 ml of sterile saline solution. From this mixture 0.1 ml was applied to the different agar plates. Endo agar (EA) was used for coliform bacteria (CB), Meat peptone agar (MPA) was used for total count of bacteria (TCB) and Sabouraud agar (SA) was used for molds. The results from the Endo agar and Meat peptone agar were obtained after 24 hours incubation at 37 °C and the results from the Sabouraud agar were obtained after 5 days incubation at room temperature. Results were expressed in colony forming units (CFU).

RESULTS AND DISCUSSION

Different types of floor are used for both reproducing does and growing rabbits. The most common is wire mesh, frequently paired with a plastic footrest pad. Plastic slatted floors may also be used. Some niche systems also use a concrete floor covered by litter, combinations of solid and slatted floor, or animals may be kept directly on the ground as in outdoor movable cages (wire floor) or in open-air enclosures.^[17] Flooring material represents a critical point of sanitation, and it is a key factor for rabbit comfort and cage hygiene.^[18]

We determined higher number of CFU of coliform bacteria from wooden floor in compare with plastic floor after mechanical cleaning, which agree with facts regards differences in mechanical cleaning according using material. In our study, after disinfection of wooden floor the counts of coliform bacteria did not exceed 10 % of their original counts (2.2×10^1 CFU/10 cm²), which can be considered a very good result of the disinfection efficiency. This result agree with result of study perform by Ondrašovičová *et al.* who stated that the

effectiveness of preventive disinfection is satisfactory if the number of indicator bacteria is up to 10 % of the original number.^[19] The materials used should be smoothly surfaced, inert and resistant to dirt absorption, surfaces require construction materials that are cleaned and maintained easily.^[18] Wooden floors will always be a challenge to fully disinfect as there are many cracks and crevices that can collect contagious material and they are difficult to clean and disinfect completely.

In the present study, after disinfection of plastic floor we detected 0 CFU/10 cm² of CB, 6.3x10³ CFU/10 cm² of TCB and 1.6x10³ CFU/10 cm² of molds, which represent statistically significant decrease of bacteria from their original counts on the level which can be considered a very good result of the disinfection. The raised floors consisting of plastic slats which replace the solid wooden floors represent changes which are made to improve hygiene. The plastic pad must not obstruct the falling through of faecal drops and must be included in the cleaning and disinfection procedures.^[20]

Disinfectant SANDEZIA[®] was effective on monitored surfaces where significant decrease of microorganisms was obtained. After disinfection we determined 6.3x10³ CFU of TCB (P<0.01); 0 CFU of CB (P<0.001); 1.6x10³ CFU of molds (P<0.05) on plastic floor (Table 1). Count of colony forming units from wooden floor was: 1.4x10³ CFU of TCB (P<0.05); 2.2x10¹CFU of CB (P<0.05); 0 CFU of yeasts (P<0.001) and 3.6x10³ CFU of molds after disinfection (Table 2). Depending on the material of surface, mechanical cleaning gives a 3 log reduction of total bacterial count on the surface and disinfection another 3 log reduction. This means that under practical conditions generally 10³ CFU of bacteria remain per 1 cm² of surface.^[21] The choice of disinfectant depends on the purpose of disinfection. In the case of notifiable diseases, it must be active against a defined pathogen. In the case of prophylactic disinfection, it must be active against a broad spectrum of microorganisms.

Table 1: The Effectiveness of disinfectant SANDEZIA[®] on the plastic floor after mechanical cleaning and after disinfection.

Plastic surface of floor		
Log CFU/10 cm ²	After mechanical cleaning	After disinfection
TCB	4.7x10 ⁶	6.3x10 ³
P < 0.01		
CB	1.0x10 ⁴	0
P < 0.001		
M	5.2x10 ⁵	1.6x10 ³
P < 0.05		

Note: CFU - colony forming units, M – molds, TCB – total count of bacteria, CB – coliform bacteria

Table 2: The Effectiveness of disinfectant SANDEZIA® on the wooden floor after mechanical cleaning and after disinfection.

Wooden surface of floor		
Log CFU/10 cm ²	after mechanical cleaning	after disinfection
TCB	1.0x10 ⁷	1.4x10 ³
P<0.05		
CB	9.2x10 ⁵	2.2x10 ¹
P<0.05		
M	5.5x10 ⁵	3.6x10 ³
P = 0.06		

Note: CFU - colony forming units, M – molds, TCB – total count of bacteria, CB – coliform bacteria

Disinfection of cages means always a combination between mechanical cleaning and disinfection because the high amount of present organic material present will neutralize rapidly each disinfectant used to the surface if no cleaning step had been done before. Disinfectants used for veterinary hygiene may specifically be designed for the disinfection of floors, walls and ceilings of stables and vehicles, containers and cages for animal transport and animal housing and these preparations are an important tool to control animal pathogens in animal housing, animal diseases prevention, production increase and improvement of the quality of animal products.^[22]

CONCLUSION

Disinfection of animal houses is difficult to achieve. Rough surfaces, high organic material, low temperatures and many other circumstances are strongly limiting factors in this connection and only mechanical cleansing and disinfection together will ever lead to a sufficient reduction of the microorganisms count on the surfaces, but in certain situations even if the procedure is done correctly the result may not be satisfactory. The results of our study suggest that wooden surface of floor represent a greater potential risk for cross contamination than plastic surface of floor. Based on our results, disinfectant Sandezia® caused significant reduction of monitored microorganisms on the level, which doesn't represent hygienic risk of microbial contamination for animals.

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