Bactericidal, protistocidal and nematodicidal properties of mixtures of alkylidimethylbenzyl ammonium chloride, didecyldimethyl ammonium chloride, glutaraldehyde and formaldehyde

V. V. Zazharskyi*, P. Davydenko*, O. Kulishenko*, V. Chumak*, A. Kryvaya*, I. A. Biben*, N. M. Tishkina*, I. Borovik*, O. O. Boyko*, V. V. Brygadyrenko*,**

*Dnipro State Agrarian and Economic University, Dnipro, Ukraine
**Oles Honchar Dnipro National University, Dnipro, Ukraine

Keywords: disinfectant; bactericidal action; toxicity; Paramecium caudatum; Tetrahymena pyriformis; Haemonchus contortus

Introduction

An obligatory component in the system of veterinary-sanitary measures for the objects of livestock farming is performance of disinfection. Prevention of diseases of infectious etiology conditioned by conditions for the objects of livestock farming and healthcare of the population is played by the conducting of effective disinfection. Effective disinfection measures perform a leading role in providing stable veterinary well-being of livestock and healthcare of the population. Maximum toxicity during usage of the mixtures on P. caudatum was observed for the mixture of alkylbenzyldimethylammonium chloride and didecyldimethylammonium chloride, and also for formaldehyde and glutaraldehyde. The lowest toxicity for T. pyriformis was observed with use of the mixture of glutaraldehyde, sodium dodecylsulfate (SDS) and oleum terebinthini, and also the mixture of formaldehyde and glutaraldehyde, the highest – formaldehyde and alkylbenzyldimethylammonium chloride. Thus, the most promising mixtures for use in veterinary medicine were determined to the following: alkylbenzyldimethylammonium chloride, didecyldimethylammonium chloride and glutaraldehyde, and also formaldehyde, alkylbenzyldimethylammonium chloride and glutaraldehyde.

Keywords: disinfectant; bactericidal action; toxicity; Paramecium caudatum; Tetrahymena pyriformis; Haemonchus contortus

We conducted a comparative analysis of the impact of disinfecting preparations on the cryogenic stains of microorganisms, and also on Haemonchus contortus (Rudolphi 1803), invasive larvae of the ruminants. To test the preparations for disinfection, we used laboratory analyses with methods of biotesting, particularly with the use of Paramecium caudatum Her., Tetrahymena pyriformis Ehrenberg. We researched mixtures of substances: alkylbenzyldimethylammonium chloride (C24H42IN, BAK, mixture of homologues alkylbenzyldimethylammonium chloride and with n-C12H25, n-C15H31 and n-C16H33), didecyldimethylammonium Chloride (DDAC, C24H45N) and glutaraldehyde (C5H10O2); formaldehyde (CH2O), alkylbenzyldimethylammonium chloride and glutaraldehyde in 1% have bactericidal properties for the following cryogenic strains of microorganisms: Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Proteus vulgaris, Serratia marcescens, Pseudomonas aeruginosa, Enterococcus faecalis and Verronia enterococci. The Bacillus cereus were affected by the preparations bacteriostatically: we observed growth in the colonies in the medium with addition of 1% solution of mixture of alkylbenzyldimethylammonium chloride and didecyldimethylammonium chloride, and also 1%, 5% and 10% of solution of mixture of glutaraldehyde, formaldehyde and alkylbenzyldimethylammonium chloride. Also, these mixtures of substances have nematocidal properties. Death of 100% of L3 H. contortus after 24 hour exposure was observed with use of 1% solution of mixture of alkylbenzyldimethylammonium chloride and didecyldimethylammonium chloride, and also 5% – glutaraldehyde, formaldehyde and alkylbenzyldimethylammonium chloride. Effective disinfection measures perform a leading role in providing stable veterinary well-being of livestock and healthcare of the population. Maximum toxicity during usage of the mixtures on P. caudatum was observed for the mixture of alkylbenzyldimethylammonium chloride and didecyldimethylammonium chloride, and also for formaldehyde and glutaraldehyde. We conducted a comparative analysis of the impact of disinfecting preparations on the cryogenic stains of microorganisms, and also on Haemonchus contortus (Rudolphi 1803), invasive larvae of the ruminants. To test the preparations for disinfection, we used laboratory analyses with methods of biotesting, particularly with the use of Paramecium caudatum Her., Tetrahymena pyriformis Ehrenberg. We researched mixtures of substances: alkylbenzyldimethylammonium chloride (C24H42IN, BAK, mixture of homologues alkylbenzyldimethylammonium chloride and with n-C12H25, n-C15H31 and n-C16H33), didecyldimethylammonium Chloride (DDAC, C24H45N) and glutaraldehyde (C5H10O2); formaldehyde (CH2O), alkylbenzyldimethylammonium chloride and glutaraldehyde in 1% have bactericidal properties for the following cryogenic strains of microorganisms: Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Proteus vulgaris, Serratia marcescens, Pseudomonas aeruginosa, Enterococcus faecalis and Verronia enterococci. The Bacillus cereus were affected by the preparations bacteriostatically: we observed growth in the colonies in the medium with addition of 1% solution of mixture of alkylbenzyldimethylammonium chloride and didecyldimethylammonium chloride, and also 1%, 5% and 10% of solution of mixture of glutaraldehyde, formaldehyde and alkylbenzyldimethylammonium chloride. Also, these mixtures of substances have nematocidal properties. Death of 100% of L3 H. contortus after 24 hour exposure was observed with use of 1% solution of mixture of alkylbenzyldimethylammonium chloride and didecyldimethylammonium chloride, and also 5% – glutaraldehyde, formaldehyde and alkylbenzyldimethylammonium chloride. Effective disinfection measures perform a leading role in providing stable veterinary well-being of livestock and healthcare of the population. Maximum toxicity during usage of the mixtures on P. caudatum was observed for the mixture of alkylbenzyldimethylammonium chloride and didecyldimethylammonium chloride, and also for formaldehyde and glutaraldehyde. The lowest toxicity for T. pyriformis was observed with use of the mixture of glutaraldehyde, sodium dodecylsulfate (SDS) and oleum terebinthini, and also the mixture of formaldehyde and glutaraldehyde, the highest – formaldehyde and alkylbenzyldimethylammonium chloride. Thus, the most promising mixtures for use in veterinary medicine were determined to the following: alkylbenzyldimethylammonium chloride, didecyldimethylammonium chloride and glutaraldehyde, and also formaldehyde, alkylbenzyldimethylammonium chloride and glutaraldehyde.

Keywords: disinfectant; bactericidal action; toxicity; Paramecium caudatum; Tetrahymena pyriformis; Haemonchus contortus

We conducted a comparative analysis of the impact of disinfecting preparations on the cryogenic stains of microorganisms, and also on Haemonchus contortus (Rudolphi 1803), invasive larvae of the ruminants. To test the preparations for disinfection, we used laboratory analyses with methods of biotesting, particularly with the use of Paramecium caudatum Her., Tetrahymena pyriformis Ehrenberg. We researched mixtures of substances: alkylbenzyldimethylammonium chloride (C24H42IN, BAK, mixture of homologues alkylbenzyldimethylammonium chloride and with n-C12H25, n-C15H31 and n-C16H33), didecyldimethylammonium Chloride (DDAC, C24H45N) and glutaraldehyde (C5H10O2); formaldehyde (CH2O), alkylbenzyldimethylammonium chloride and glutaraldehyde in 1% have bactericidal properties for the following cryogenic strains of microorganisms: Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Proteus vulgaris, Serratia marcescens, Pseudomonas aeruginosa, Enterococcus faecalis and Verronia enterococci. The Bacillus cereus were affected by the preparations bacteriostatically: we observed growth in the colonies in the medium with addition of 1% solution of mixture of alkylbenzyldimethylammonium chloride and didecyldimethylammonium chloride, and also 1%, 5% and 10% of solution of mixture of glutaraldehyde, formaldehyde and alkylbenzyldimethylammonium chloride. Also, these mixtures of substances have nematocidal properties. Death of 100% of L3 H. contortus after 24 hour exposure was observed with use of 1% solution of mixture of alkylbenzyldimethylammonium chloride and didecyldimethylammonium chloride, and also 5% – glutaraldehyde, formaldehyde and alkylbenzyldimethylammonium chloride. Effective disinfection measures perform a leading role in providing stable veterinary well-being of livestock and healthcare of the population. Maximum toxicity during usage of the mixtures on P. caudatum was observed for the mixture of alkylbenzyldimethylammonium chloride and didecyldimethylammonium chloride, and also for formaldehyde and glutaraldehyde. The lowest toxicity for T. pyriformis was observed with use of the mixture of glutaraldehyde, sodium dodecylsulfate (SDS) and oleum terebinthini, and also the mixture of formaldehyde and glutaraldehyde, the highest – formaldehyde and alkylbenzyldimethylammonium chloride. Thus, the most promising mixtures for use in veterinary medicine were determined to the following: alkylbenzyldimethylammonium chloride, didecyldimethylammonium chloride and glutaraldehyde, and also formaldehyde, alkylbenzyldimethylammonium chloride and glutaraldehyde.
animals. At the impact (24h) of 1% solution of cinnamaldehyde, there was observed death of 100% of eggs of Ascaris lum (LD₅₀ = 2437 ± 864 mg/l) (Boyko & Brygadirenko, 2017a). Larvae of Strongyloids ransomi, nematodes of pigs, also died over 24 hours at the impact of 0.1% solution of benzaldehyde. LD₅₀ for benzaldehyde – 142 ± 64 mg/l (Boyko & Brygadirenko, 2017b). The literature contains a large amount of data on the impact of alkyl/benzylidemethylammonium chloride, didecylidemethylammonium chloride and glutaraldehyde; alkyl/benzylidemethylammonium chloride, formaldehyde and glutaraldehyde; sodium docetyl sulfate (SDS), oleum terebinthini and glutaraldehyde, and also formaldehyde and glutaraldehyde.

**Materials and methods**

The research was conducted in the laboratories of the departments of Epizootology and Infectious Diseases of Animals, Physiology and Biochemistry of Agricultural Animals, Parasitology and Veterinary-Sanitary Examination of the Faculty of Veterinary Medicine of Dniprop Regional Agrarian-Economic University, and also in the Bacteriological Department of Dniprop Regional National Laboratory of Veterinary Medicine in 2017–2018.

**Bacteria.** The cultures of microorganisms of standardized strains (Table 2), cultivated on a dense growth medium over 18–24 hours were washed out with sterile isotonic solution of sodium chloride at temperature of 37 ± 2 °C. The weighed microbial amounts were processed to 5 · 10⁸ CFU/ml of McFarland turbidity standard for optical standartisation of bacteria using a Dilushaker III Digital densitometer, France. The solutions of disinfectants in the working concentration (0.9 ml) were poured into sterile test tubes. To the test tubes with disinfectant solutions (1, 5, 10, 25%), 0.1 ml of weighed microbial amounts were added, mixed, and then the tubes were shaken for a few seconds (Table 1).

Then, 0.5 ml of solution of the neutralizer was added (Tvin-80 – 3%, saponin – 3%, histidine – 0.1%, cysteine – 0.1%) and the tubes were shaken. The inoculations were made on to a specific differential-diagnostic medium by 0.1 ml of the mixture, and the caps with inoculated cultures were put in a thermostatic bath for 24 hours. The methods are described in detail in the articles by Zazharskyi et al. (2018a, 2018b).

The incubation was performed in accordance with the passport for the growth media. After the time necessary for the cultivation of the studied microorganisms, we assessed the number of microorganisms that grew in the Petri dish. Distinctive typical colonies were described in detail in the articles by Zazharskyi et al. (2018a, 2018b).

The larvae of nematodes in feces of ruminants were found using the Baermann test (Zajac et al., 2011). Then, 1 ml of the studied mixtures of the substances in different concentrations (1%, 5%, 10%, 25%) was added to each culture of H. contortus and T. pyriformis larvae (in five times replication). The experimental exposure equalled 24 hours. We determined the number of vital and dead larvae. The methods are described in detail in articles by Boyko & Brygadirenko (2018a, 2018b).

**Statistical analysis.** The extrapolation of the data on acute toxicity of the studied substances, obtained for T. pyriformis, to animals was implemented in accordance with the recommended methods of express biotesting. For this purpose, effective dose of a certain substance, obtained in the experiment in determining acute toxicity, was expressed as probit which was placed in the graph of the lethality line of T. pyriformis. LC₅₀ value was calculated. The results are satisfactory if LC₅₀ value obtained using the method of express biotesting is within the confidence interval (error). Value of LC₅₀ for ciliates was determined using probit-analysis of lethality curves. Probit-analysis is recommend-
ed by OECD Guidelines for the Testing of Chemicals for assessment of harmful impact of different toxicants. The statistical analysis of the results with *H. contortus* was performed through a set of Statistica 8.0 (StatSoft Inc., USA), the figures show the median, 25% and 75% quartiles, minimum and maximum values. LD₅₀ (%) was calculated as mean ± standard deviation (x ± SD).

**Results**

The mixtures we studied – alkylbenzyldimethylammonium chloride, didecylmethyl ammonium chloride, glutaraldehyde, and also alkylbenzyldimethylammonium chloride, formaldehyde, and glutaraldehyde – demonstrated bactericidal properties even in 1% concentration against cryogenic strains of the following microorganisms: S. aureus, S. typhimurium, E. coli, L. monocytogenes, P. vulgaris, S. marcescens, P. aeruginosa, E. faecalis and Y. enterocolitica. The mixtures of these substances was used (Fig. 2).

**Table 2**

| Strains of microorganisms | No of medium | name | Growth medium, HiMedia Laboratories Pvt. Ltd (India) | reason for study |
|---------------------------|-------------|------|-------------------------------------------------------|-----------------|
| *Staphylococcus aureus* ATCC 25923 | M043-500G | Baird Parker agar base | Baird Parker agar base, 500 g (REF 2009/03709) ([ISO 6887:2003](https://www.iso.org/standard/6887.html)) | for selection and assessment of coagulase-positive *Staphylococcus* in food products and other examined material; FD046 egg yolk tellurite emulsion (100 ml/vial)/ yolk emulsion with tellurite; FD069 B P sulpha supplement/additive with sulfamethazine for Baird Parker medium |
| *Salmonella typhimurium* 144 | M031-500G | xylose lysine deoxycholate agar | xylose lysine deoxycholate agar (XLD agar), 500 g (REF 2009/03709) ([ISO 6887:2003](https://www.iso.org/standard/6887.html)) | for selection and assessment of *Salmonella* typhi and other *Salmonella* |
| *Bacillus cereus* ATCC 10702 | M833-500G | Bacillus cereus agar base | Bacillus cereus agar base, 500 g (REF 2009/03709) ([ISO 6887:2003](https://www.iso.org/standard/6887.html)) | FD003 polymyxin B selective supplement; FD045 egg yolk emulsion (100 ml/vial); for selection and count of colonies of anthracoid *Bacillus*; FD003 polymyxin B selective supplement; FD045 egg yolk emulsion (100 ml/vial) |
| *Escherichia coli* (F 50) ATCC 25922 | M065A | deoxycholate citrate agar (as per B.P.) | deoxycholate citrate agar (endogenous agar), 500 g (REF 2009/03709) | for selection of pathogens of intestinal infections |
| *Listeria monocytogenes* ATCC 19112 | M1064-500G | Listeria identification agar base (PALCAM) | Listeria identification agar base (PALCAM), 500 g (REF 2009/03709) ([ISO 6887:2003](https://www.iso.org/standard/6887.html)) | for identification and selection of coliform bacteria of the intestinal group |
| *Proteus vulgaris* HX 19 222 | M082-500G | MacConkey agar w/o CV, NaCl w/sodium taurocholate 0.5% | MacConkey agar without crystal violet, NaCl, with 0.5% taurocholic acid sodium, 500 g (REF 2009/03709) | this agar is prepared in accordance with the requirements for clinical microbiology; on this differential medium, swarming of most strains of *Proteus* is inhibited, which significantly facilitates the selection of intestinal bacteria; along with opportunistic gram-positive bacteria, a large number of *Proteus* can be maintained in it; enterococci in it form small reddish colonies |
| *Serratia marcescens* | M001-500G | nutrient agar | nutrient agar, 500 g (REF 2009/03709) ([ISO 6579:2002](https://www.iso.org/standard/6579.html)) | is used as the main medium for cultivating not very fastidious microorganisms or for preparing special media (after 10% of blood or other biological fluid) |
| *Pseudomonas aeruginosa* ATCC 28533(F) | M085-500G | Pseudomonas agar base | Pseudomonas agar base, 500 g (REF 2009/03709) | recommended by the International Committee (ISO); FD029 cetrinix supplement/cetrinix additive for *Pseudomonas* is recommended with additives for selection of *Pseudomonas*; |
| *Enterococcus faecalis* ATCC 19433 | M1075-500G | endo agar, modified | endo agar, modified, 500 g (REF 2009/03709) | for determining and selecting coliforms and other bacteria of the intestinal group |
| *Yersinia enterocolitica* | M001-500G | nutrient agar | nutrient agar, 500 g (REF 2009/03709) ([ISO 6579:2002](https://www.iso.org/standard/6579.html)) | is used as the main medium for cultivating not very fastidious microorganisms or for preparing special media (after 10% of blood or other biological fluid) |

No negative impact on the mobility of *T. pyriformis* was demonstrated by the mixtures of sodium dodecyl sulfate (SDS), essential oil, glutaraldehyde, and also formaldehyde, glutaraldehyde with 0.01%, mixtures of alkylbenzyldimethylammonium chloride, didecylmethyl ammonium chloride, glutaraldehyde and also alkylbenzyldimethylammonium chloride, formaldehyde, glutaraldehyde with 0.0011% (Table 4). According to the results of our previous studies (Zazharskyi et al., 2018a, 2018b), the impact of 0.01 g/l mixture of alkylbenzyldimethylammonium chloride, didecylmethyl ammonium chloride, glutaraldehyde and formaldehyde, and glutaraldehyde caused the highest death rate of ciliates – 26% and 22% respectively (Table 5). LD₅₀ equalled 1.8 mg/l with use of the mixture of alkylbenzyldimethylammonium chloride, didecylmethyl ammonium chloride, glutaraldehyde, 8.4 mg/l – formaldehyde, glutaraldehyde, 27.2 mg/l – alkylbenzyldimethylammonium chloride, formaldehyde, glutaraldehyde, 53.4 mg/l – sodium dodecyl sulfate, essential oil, and glutaraldehyde. In the series of experiments on ciliates, the death of different numbers of them was observed in interval 0.001–10 mg/l with use of the mixture of alkylbenzyldimethylammonium chloride, didecylmethyl ammonium chloride, glutaraldehyde and formaldehyde, glutaraldehyde, 0.001–100 mg/l – alkylbenzyldimethylammonium chloride, formaldehyde, glutaraldehyde, 0.1–100 mg/l for sodium dodecyl sulfate, essential oil, glutaraldehyde.

The greatest impact on the vitality of nematode larvae in the environment was demonstrated by alkylbenzyldimethylammonium chloride, didecylmethyl ammonium chloride and glutaraldehyde. 100% death rate of *H. contortus*, nematode larvae of runnans was observed with use of 1% solution of this mixture. Nematocidal effect was exhibited by the mixture of alkylbenzyldimethylammonium chloride, formaldehyde and glutaraldehyde: nematode larvae of the studied species died at 5% concentration. Mixtures of sodium dodecyl sulfate, oleum terebinthini, glutaraldehyde, and also formaldehyde and glutaraldehyde were the least efficient against invasive larvae of *H. contortus*. 100% death rate of L₃ larvae was observed only when 25% solution of mixtures of these substances was used (Fig. 2).
Table 3 Influence of the studied mixtures on cryogenic strains of microorganisms (n = 5)

| Strains of microorganisms          | Alkylbenzyldimethylammonium chloride, didecyldimethyl ammonium chloride, glutaraldehyde, % | Alkylbenzyldimethyl-ammonium chloride, formaldehyde, glutaraldehyde, % | Sodium dodecyl sulfate, essential oil, glutaraldehyde, % | Formaldehyde, glutaraldehyde, % |
|-----------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------------|-------------------------------------------------|--------------------------------|
| S. aureus ATCC 25923              | 1                                                     | 5                                                   | 10                                                          | 25 |
| E. coli ATCC 10702                | 1                                                     | 5                                                   | 10                                                          | 25 |
| L. monocytogenes ATCC             | 1                                                     | 5                                                   | 10                                                          | 25 |
| P. vulgaris HX 19 222             | 1                                                     | 5                                                   | 10                                                          | 25 |
| S. marcescens                      | 1                                                     | 5                                                   | 10                                                          | 25 |
| E. coli ATCC 2053(F)              | 1                                                     | 5                                                   | 10                                                          | 25 |
| T. pyriformis                      | 1                                                     | 5                                                   | 10                                                          | 25 |
| T. naegleri                        | 1                                                     | 5                                                   | 10                                                          | 25 |

Note: “–” – no growth in colonies, “+” – one colony, “++” – two colonies, “+++” – three colonies.

Table 4 Influence of studied substances on T. pyriformis (n = 5)

| Concentration, % | Exposure hour | alkylbenzyldimethylammonium chloride, didecyldimethyl ammonium chloride, glutaraldehyde | Types of mixtures | sodium dodecyl sulfate, essential oil, glutaraldehyde | formaldehyde, glutaraldehyde |
|------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------|-----------------|-------------------------------------------------|--------------------------------|
| 0.1              | 24            | +                                                                                                                                | +               | +                                               | +                             |
| 1                | 24            | +                                                                                                                                | +               | +                                               | +                             |
| 1.0 x 10⁻⁷       | 24            | +                                                                                                                                | +               | +                                               | +                             |
| 1.0 x 10⁻⁵       | 24            | +                                                                                                                                | +               | +                                               | +                             |
| 1.0 x 10⁻⁶       | 24            | +                                                                                                                                | +               | +                                               | +                             |
| 1.0 x 10⁻⁷       | 24            | +                                                                                                                                | +               | +                                               | +                             |

Note: “–” – no growth (death), “+” – movement slowed, “++” – active movement; 1 – after the addition, movement intensifies, the direction changes, after 60 minutes – single moving individuals, movement slowed; 2 – restoration of movement, decrease in density of the culture, movement slowed; 3 – restoration of movement, decrease in density of the culture, movement slowed; 4 – insignificant decrease in density; 5 – slowed movement; 6 – rotation, slowed movement, decrease in density; 7 – slow movement, insignificant decrease in density (Zhmin’ko et al., 2006).

Table 5 Influence of the studied substances on P. caudatum (% dead ciliates; n = 5)

| Mixture compound                  | Concentration of mixtures in the sample, mg/l |
|-----------------------------------|-----------------------------------------------|
|                                  | 0.01  | 0.1   | 1     | 10    | 100   | 100   |
|                                  | control | experiment | control | experiment | control | experiment | control | experiment | control | experiment | control | experiment |
| Alkylbenzyldimethylammonium chloride, didecyldimethyl ammonium chloride, glutaraldehyde | 0260   | 420   | 660   | 0100   | 0100   |
| Alkylbenzyldimethylammonium chloride, formaldehyde, glutaraldehyde                      | 0120   | 230   | 0330  | 0650   | 0100   |
| Sodium dodecyl sulfate, oleum terebinthini, glutaraldehyde                               | 0000   | 120   | 0120  | 070    | 0100   |
| Formaldehyde, glutaraldehyde                                                             | 0220   | 0210  | 0400  | 0100   | 0100   |

During usage of mixture of sodium dodecyl sulfate, oleum terebinthini and glutaraldehyde in 5% concentration, the vitality of nematode larvae was observed on average to be 25%, in 10% concentration it was 8% of individuals. When the mixture of formaldehyde, glutaraldehyde was used in 1%, 5% and 10% concentration, on average 1–10% of individuals survived. LD₅₀ for mixture of sodium dodecyl sulfate, essential oil and glutaraldehyde equals 2.3 ± 0.8%, for formaldehyde, glutaraldehyde it was 0.45 ± 0.16%.

Discussion

The series of studies Takashi & Kei-Ichiro (2007) proved the bactericidal effect of didecyldimethyl ammonium chloride in minimum inhibiting concentration 1.3 mg/l against E. coli. The studies by Shirron et al. (2009) allow us to state that didecyldimethyl ammonium chloride causes a bactericidal effect against S. typhimurium. Walsh et al. (2003) reported that didecyldimethyl ammonium chloride has a bactericidal effect on E. coli, S. aureus, P. aeruginosa and L. monocytogenes. According to Ioannou et al. (2007), alkylbenzyldimethylammonium chloride and didecyldimethyl ammonium chloride are at the same time membrane-active agents with subtly different mechanisms of action, which reflect the previous interaction with S. aureus.

The studies by Lasemt et al. (2017) demonstrated the impact of 2% solution of glutaraldehyde on the spores of B. subtilis. The results showed that 102 colonies were present on the 10th minute, 18.6 ± 3.4 on the 15th minute, 6.2 ± 1.4 on the 20th minute, 21.1 ± 0.8 on the 25th minute and no colonies after 30 minutes. Over the first 10 minutes, more colonies were observed, after 15–20 minutes this number significantly reduced. After 30 minutes, growth of the colonies completely stopped. 2% density of glutaraldehyde over 30 minutes was sufficient for eliminating the spores of B. subtilis. The data by Simrøes et al. (2008) indicate that sodium dodecyl sulfate has an anti-microbial effect on the biomembranes of P. fluorescens. In their studies, Chen et al. (2016) mention that pathogenic strains of E. coli, P. aeruginosa and K. pneumoniae have a FrmRAB regulator, and can be used for eliminating endogenous and exogenous Formaldehyde.

Vaerewijck et al. (2012) determined that alkylbenzyldimethylammonium chloride and sodium hypochlorite in concentration of active...
chlorine of 50 mg/l can inactivate Acanthamoeba and two species of Tetrahymena spp. in 15 minutes. The series of studies by Ivanovic et al. (2013) proved the lethal effect of alkylbenzyldimethylammonium chloride on Paramecium caudatum. The studies by Blondeau et al. (2007) allow us to state that alkylbenzyldimethylammonium chloride in a combination with gatifloxacin and moxifloxacin in concentration of 0.008–0.125 mg/l exhibited bactericidal effect against polyresistant S. aureus. In their studies, Carmen Velázquez et al. (2009) also mentioned that alkylbenzyldimethylammonium chloride has a bactericidal effect against E. coli. The studies by Bridier et al. (2011) allow us to state that alkylbenzyldimethylammonium chloride has a bactericidal effect on E. faecalis.

Fig. 2. Influence of the studied mixtures on vitality of nematode larvae of H. contortus: a – sodium dodecyl sulfate, oleum terebinthini, glutaraldehyde, b – formaldehyde, glutaraldehyde

Ibusquiza P. Suá et al. (2011) found resistance of Listeria monocytogenes to alkylbenzyldimethylammonium chloride. Hattori et al. (2003) observed resistance to this substance by P. vulgaris. Tiwari et al. (2003) also proved the resistance of Serratia marcescens to this substance. By contrast, Paul et al. (2010) allow us to state that alkylbenzyldimethylammonium chloride shows no bactericidal effect against P. aeruginosa.

There are data on using mixtures of formaldehyde and glutaraldehyde as a disinvasive preparation. The study by Palij et al. (2018) describes the effect of FAG aldehyde preparation on the eggs of nematodes of livestock contaminated with invasive helminths. The highest bactericidal, protistocidal, and also nematocidal effect were observed for use of mixtures of alkylbenzyldimethylammonium chloride, didecyldimethyl ammonium chloride and glutaraldehyde, and also alkylbenzyldimethylammonium chloride, formaldehyde and glutaraldehyde.

Mixtures of alkylbenzyldimethylammonium chloride, formaldehyde, glutaraldehyde, and also alkylbenzyldimethylammonium chloride, didecyldimethyl ammonium chloride, and glutaraldehyde demonstrated bactericidal properties on cryogenic strains of S. aureus, S. typhimurium, E. coli, L. monocytogenes, P. vulgaris, S. marcescens, P. aeruginosa, E. faecalis, and Y. enterocolitica, and also nematocidal properties against H. contortus, nematode larvae of ruminants. Maximum toxicity during use of the studied substances against P. caudatum was demonstrated by alkylbenzyldimethylammonium chloride, didecyldimethyl ammonium chloride, glutaraldehyde, and also formaldehyde and glutaraldehyde. The least toxic were mixtures of sodium dodecyl sulfate, oleum terebinthini, and glutaraldehyde (14–15 times safer). The mixture alkylbenzyldimethylammonium chloride, formaldehyde and glutaraldehyde showed a moderate level of toxicity. The least toxicity for T. pyriformis was observed for the mixture of sodium dodecyl sulfate, essential oil, glutaraldehyde, and also formaldehyde and glutaraldehyde, the highest for alkylbenzyldimethylammonium chloride, formaldehyde, and glutaraldehyde. The strongest effect on the viability of nematode larvae in the environment was shown by alkylbenzyldimethylammonium chloride, didecyldimethyl ammonium chloride, and glutaraldehyde. 100% death rate of H. contortus, nematode larvae of ruminants, was recorded already at using 1% solution of this mixture. Nematocidal effect was observed for mixture of alkylbenzyldimethylammonium chloride, formaldehyde, and glutaraldehyde: nematode larvae of the studied species died at 5% concentration. Thus, our observations can be useful for practicing doctors of human and veterinary medicine during preparation of antiseptics, disinfecting and disinvasive predictions with predicted biocidal effect of four ammonium compounds.

References

Blondeau, J. M., Borsos, S., & Hesje, C. K. (2007). Antimicrobial efficacy of gatifloxacin and moxifloxacin with and without benzalkonium chloride compared with ciprofloxacin and levofloxacin against methicillin-resistant Staphylococcus aureus. Journal of Chemotherapy, 19(2), 146–151.

Boyko, A. A., & Brygydaryenko, V. V. (2017a). Changes in the viability of the eggs of Ascaris suum under the influence of flavourings and source materials approved for use in and on foods. Biosystems Diversity, 25(2), 162–166.

Boyko, A. A., & Brygydaryenko, V. V. (2017b). Changes in the viability of Strongyloides ransomi larvae (Nematoda, Rhabditida) under the influence of synthetic flavourings. Regulatory Mechanisms in Biosystems, 3(1), 36–40.

Boyko, O. O., & Brygydaryenko, V. V. (2018a). The impact of certain flavourings and preservatives on the survivability of larvae of nematodes of Ruminantia. Regulatory Mechanisms in Biosystems, 9(1), 118–123.

Boyko, O. O., Gavrilina, O. G., Gavrilin, P. N., Gugosyan, Yu. A., & Brygydaryenko, V. V. (2018b). Influence of formaldehyde on the viability of Strongyloides papillosus. Regulatory Mechanisms in Biosystems, 9(3), 435–439.

Braoudaki, M., & Hilton, A. C. (2005). Mechanisms of resistance in Salmonella enterica adapted to ethyleneglycol, benzalkonium chloride and triclosan. International Journal of Antimicrobial Agents, 25(1), 31–37.

Braoudaki, M., & Hilton, A. C. (2005). Mechanisms of resistance in Salmonella enterica adapted to ethyleneglycol, benzalkonium chloride and triclosan. International Journal of Antimicrobial Agents, 25(1), 31–37.

Boyko, A., Bryndret, R., Thomas, V., & Dubois-Brissonnet, F. (2011). Comparative biocidal activity of paraformaldehyde, benzalkonium chloride and orthophthalaldehyde on 77 bacterial strains. Journal of Hospital Infection, 78(3), 208–213.

Chen, N. H., Djoko, K. Y., Veyrier, F. J., & McEwan, A. G. (2016). Formaldehyde stress responses in bacterial pathogens. Frontiers in Microbiology, 7, 257.

del Carmen Velázquez, L., Barbini, B. N., Escudero, M. E., & Estrada, C. L., de del Carmen Velázquez, L., Barbini, B. N., Escudero, M. E., & Estrada, C. L. (2003). Enhanced microbial biomass assay using mutant luciferase resistant Yersinia enterocolitica on fresh vegetables. Food Control, 20(3), 262–268.

Fazlara, A., & Ekhtelat, M. (2012). The disinfectant effects of benzalkonium chloride, peracetic acid and nisin during formation of mature biofilms by Staphylococcus aureus. International Journal of Antimicrobial Agents, 25(1), 31–37.

Fig. 2. Influence of the studied mixtures on vitality of nematode larvae of H. contortus: a – sodium dodecyl sulfate, oleum terebinthini, glutaraldehyde, b – formaldehyde, glutaraldehyde

Ibusquiza P. Suá et al. (2011) found resistance of Listeria monocytogenes to alkylbenzyldimethylammonium chloride. Hattori et al. (2003) observed resistance to this substance by P. vulgaris. Tiwari et al. (2003) also proved the resistance of Serratia marcescens to this substance. By contrast, Paul et al. (2010) allow us to state that alkylbenzyldimethylammonium chloride shows no bactericidal effect against P. aeruginosa.

There are data on using mixtures of formaldehyde and glutaraldehyde as a disinvasive preparation. The study by Palij et al. (2018) describes the effect of FAG aldehyde preparation on the eggs of nematodes of agricultural animals. It was determined that the preparation in 6.0% concentration at 24 hours exposition demonstrates a disinvasive effect against eggs of Ascaris suum, Ascaridia galli and Toxocara canis. Mixture of these aldehydes is an efficient preparation for disinfecting the premises of livestock contaminated with invasive helminths.

The highest bactericidal, protistocidal, and also nematocidal effect were observed for use of mixtures of alkylbenzyldimethylammonium chloride, didecyldimethyl ammonium chloride and glutaraldehyde, and also alkylbenzyldimethylammonium chloride, formaldehyde and glutaraldehyde.
environmental conditions. The Journal of Bioadhesion and Biofilm Research, 29(6), 641–649.

Kotumabes, I. Y., Malýk, O. G., & Paterega, I. P. (2006). Doklinnich doslidhennya veterinarhnykh liakarskh zasobiv [Preclinical studies of veterinary medicinal products]. Triada Plus, Lviv (in Ukrainian).

Kovalenko, V. L., Grinenko, A. V., & Ponomarenko, G. V. (2012). Porivnialne vyznatchennya toksichnosti bacteritsydnyh zasobiv za pokaznykami gostroyi toksychnosti ta alternatyvnyh metodiv [Comparative definition of toxicity of bactericidal agents on indicators of acute toxicity and alternative methods]. Problems of Zoinengineering and Veterinary Medicine, 25(2), 169–173 (in Ukrainian).

Kovalenko, V. L., Lysavta, V. P., & Balats’kij, Y. O. (2014). Viznachennya toksichnosti bacteritsydnyh zasobiv [Determination of toxicity of bactericides]. Triada Plus, Lviv (in Ukrainian).

Lasemi, E., Kalantar, M. H., Navi, M. F., Rezae, M., Nikfar, N. H., Danial, Z., & Kovalenko, V. L., Gnatenko, A. V., & Ponomarenko, G. V. (2012). Porivnialne vyznatchennya toksichnosti bacteritsydnyh zasobiv za pokaznykami gostroyi toksychnosti ta alternatyvnyh metodiv [Comparative definition of toxicity of bactericidal agents on indicators of acute toxicity and alternative methods]. Problems of Zoinengineering and Veterinary Medicine, 29(2), 262–265 (in Ukrainian).

Lasernt, E., Kalarant, M. H., Navi, M. F., Rezae, M., Nikfar, N. H., Danial, Z., & Azizpour, R. (2017). Effects of different times of glutaraldehyde 2% on Bacillus subtilis spores (in vitro). Hospital Practices and Research, 2(4), 118–121.

McCay, P. H., Ocampo-Sosa, A. A., & Fleming, G. T. (2010). Effect of subinhibitory concentrations of benzalkonium chloride on the competitiveness of Pseudomonas aeruginosa grown in continuous culture. Microbiology, 156(1), 30–38.

Myoshua, N., Kawano, T., & Tanaka, M. (2003). Use of Paramecium species in bioassays for environmental risk management: Determination of LC_{50} values for water pollutants. Journal of Health Science, 49(6), 429–435.

Oussa, A., Eldrisi, B, Ghamali, M., Chitra, S., Aoudia, A., Bouachrine, M., & Lakhlifi, T. (2018). Quantitative structure-toxicity relationship studies of aromatic aldehydes to Tetrahymena pyriformis based on electronic and topological descriptors. Journal of Materials and Environmental Science, 9(1), 256–266.

Pali, A. P., & Samkova, Z. G. (2018). Viznachennya dezinfikajnykh vlastivostey dezaasobu “FAG” [Determination of disinfective properties of disinfection “FAG”]. Veterinary Biotechnology, 32(2), 405–412.

Shirron, N., Kiskak, G., Zeilikovich, Y., Ervin, I., Shimoni, E., & Yaron, S. (2009). A comparative study assessing commonly used sanitizers for antimicrobial activity against indicator bacteria and a Salmo nephi strain on fresh produce. Journal of Food Protection, 72(11), 2413–2417.

Simões, M., Simões, L. C., Pereira, M. O., & Vieira, M. J. (2008). Sodium dodecyl sulfate allows the persistence and recovery of biofilms of Pseudomonas fluorescens formed under different hydrodynamic conditions. Biofouling, 24(1), 35–44.

Tiwari, T. S., Ray, P. B., Jost, K. C., Rathod, M. K., Zhang, Y., Brown-Elliott, B. A., Hendricks, K., & Wallace, R. J. (2003). Forty years of disinfectant failure: Outbreak of postinjection Mycobacterium abscessus infection caused by contamination of benzalkonium chloride. Clinical Infectious Diseases, 36(4), 954–962.

Vaerewijck, M., Sabbe, K., Jane, J., Spengler, H.-F., Faveone, H., & Houb, K. (2012). Assessment of the efficacy of benzalkonium chloride and sodium hypochlorite against Acinetobacter spp. and Pseudomonas spp. Journal of Food Protection, 75(3), 541–546.

Venkateswara Rao, J., Gunda, V., Srikanth, K., & Arepalli, S. K. (2007). Acute toxicity bioassay using Paramecium caudatum, a key member to study the effects of monocrotrophos on swimming behaviour, morphology and reproduction. Toxicological and Environmental Chemistry, 89(2), 307–317.

Walsh, S. E., Maillard, J.-Y., Catrenich, C. E., Charbonneau, D. L., & Bartolo, R. G. (2003). Activity and mechanisms of action of selected biocidal agents on Gram-positive and negative bacteria. Journal of Applied Microbiology, 94(2), 240–247.

Yoshimatsu, T., & Hiyarna, K. (2007). Mechanism of the action of didecyldimethylammonium chloride (DDAC) against Escherichia coli and morphological changes of the cells. Biocontrol Science, 12(3), 93–99.

Zasokin, D. A., Dimko, R. O., & Kovalenko, V. L. (2016). Efektyvnist’ dezinfektantu na osnovi organichnih kislot ta nanochastink na mukroorganizmiv [Efficiency of disinfectant based on organic acids and nanoparticles of metals in relation to test cultures of microorganisms]. Problems of Zoinengineering and Veterinary Medicine, 30(2), 358–360 (in Ukrainian).

Zazharskyi, V. V., Davydenko, P., Kulishenko, O., Chumak, V., Kryvaya, A., Baburuk, A., & Borovik, I. (2018b). Porivnialne doslidzhennya toksitchnosti liakarskih zasobiv na kriogennі shtami mіkroorganіzmіv [Influence of disinfectants on cryogenic strains of microorganisms]. Problems of Zoinengineering and Veterinary Medicine, 30(2), 358–360 (in Ukrainian).

Zazharskyi, V. V., Fotina, T. I., Berezovsky, A. V., Davydenko, P., Kulishenko, O., Chumak, V., Kryvaya, A., & Borovik, I. (2018a). Vpliv dezinfektsiyh zasobiv na kriogenni shtami mukroorganizmiv [Influence of disinfectants on cryogenic strains of microorganisms]. The Journal of the Donpetrovske State Agrarian and Economic University, 42(1), 273–276 (in Ukrainian).

Zazharskyi, V. V., Fotina, T. I., Berezovsky, A. V., Davydenko, P., Kulishenko, O., Chumak, V., Kryvaya, A., & Borovik, I. (2018a). Vpliv dezinfektsiyh zasobiv na kriogenni shtami mukroorganizmiv [Influence of disinfectants on cryogenic strains of microorganisms]. The Journal of the Donpetrovske State Agrarian and Economic University, 42(1), 273–276 (in Ukrainian).

Zhang, L., Koo, P. G., Kokolariova, N. V., & Dymtretno, M. P. (2006). Dosid v skrytynivuih doslidhennya toxichnostі liakarskh zasobiv [Experience of using different test systems in screening studies on drug toxicity]. Bulletin of Pharmacology and Pharmacy, 4, 21–27 (in Ukrainian).