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# IN VITRO LARVICIDAL EFFICACY OF SOME DISINFECTANTS AGAINST *AELUROSTRONGYLUS ABSTRUSUS* LARVAE

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**ABSTRACT**

The current study was performed to assess the efficiency of some commonly used disinfectants against first stage ($L_1$) *Aelurostrongylus abstrusus* larvae. A naturally infected cat was used as a donor of $L_1$. The larvae were collected from the cat faeces by the simplified Baermann's technique. A total of 12 disinfectants were tested, including acids, alkalis, aldehydes, quaternary ammonium compounds (QAC), phenols, chlorhexidine, chlorine-, iodine- and oxygen-releasing disinfectants. The activity of each disinfectant on $L_1$ was tested by the suspension method after 30 and 60 min exposure time. Key criteria in assessing the harmful effects of disifectants on the larvae were changes in motility and the larval morphology such as vesicular inclusions, fading and/or destruction of intestinal cells, wrinkling or folding.

The results revealed that sodium hydroxide, iodine- and oxygen-releasing disinfectants had the best efficacy and caused rapid 100% inactivation of $L_1$ after exposure of only 30 min. High efficiency after 30-min exposure was also observed for phenols (97.89%), aldehydes (96.84%) and the combination of QAC and chlorhexidine (96.84%). The efficacy of the other substances was between 39 and 90.53%.

The results obtained allowed selecting the appropriate chemical agent for disinfection of premises and disposal of faeces from animals infected with *A. abstrusus* or those at high risk – in shelters, foster homes, veterinary clinics and castration centres, in order to eliminate the risk of spreading the parasites.

**Key words:** *Aelurostrongylus abstrusus*, cat, lungworm, disinfectants, larvae.

## Introduction

It is acknowledged that areas with increased density of animals of various, often unclear origin (kennels, pet shops, shelters, veterinary clinics and hospitals, grooming salons) are associated with increased risk from emergence and spread of infectious and parasitic diseases (Greene *et al.*, 2012). This is due to the close contact between animals and potentially contaminated environment and equipment. A large part of diseases spread in these facilities are difficult to cure and incur long-term health problems, therefore the provision of a safe environment is of primary importance for animal protection (Greene *et al.*, 2012). Contrary to the relatively ample scientific information on disinfections targeted at different infectious pathogens, the lack of purposeful research studies aimed at decontamination of parasitic invasions is remarkable. Thus, one of leading manuals of disinfections of feline shelters „Disinfectant choices in veterinary practices, shelters and households: ABCD guidelines on safe and effective disinfection for feline environments" includes only several sentences about antiparasitic disinfection, which unequivocally justifies the need for research in this field (Addie, 2015).

*Aelurostrongylus abstrusus* is broadly spread parasitic pathogens among Bulgarian cat populations (Murad *et al.*, 2019; Borisov *et al.* 2018; Giannelli *et al.*, 2017). This fact poses a new challenge to the decontamination of the environment and polluted objects in veterinary practices. In an attempt to make a contribution to the scientific knowledge of these problems, the present studies

have evaluated the efficacy of some of the most commonly used disinfectants against *A. abstrusus* L1 in a laboratory setting.

## Materials and methods

The investigations were performed by the suspension test method with first-stage larval cultures of *A. abstrusus*. The larvae originated from a naturally infected male 10-year-old tomcat from the Stara Zagora region, referred for diagnostics and treatment to the University Veterinary Hospital of the Trakia University.

The Baermann technique was used for isolation of larvae (Zajac & Conboy, 2012), then isolated larvae were diluted in water to a concentration of 500 larvae/mL. The prepared suspensions contained 95% motile first-stage (L1) larvae with normal structure and preserved cuticle.

The study has tested 12 disinfectants often employed in disinfection practice (Table 1). Standard concentrations in line with recommendations of manufacturers were used.

**Table 1: List of used disinfectants and their concentrations**

| Disinfectant group | Trade name (manufacturer, active substance) – concentration |
|---|---|
| 1. Oxidising: chlorine-releasing | Tabidez® (sodium dichloroisocyanurate) – 0.05% |
| 2. Alkalis | Caustic soda (sodium hydroxide) – 2% |
| 3. Oxidizing:oxygen-releasing | Ecocid S® [pentapotassium bis(peroxymono sulfate bis(sulfate)] – 1% |
| 4. Aldehydes, QAC | Cetridine RD® (glutaraldehyde, didecyldimethyl ammonium chloride) –1% |
| 5. Phenolics, acids | Interkokask® (chlorocresol, phosphoric acid) – 4% |
| 6. QAC, biguianides | Cetridine Forte CE® (N,N-didecyl-n, n- dimethyl ammonium chloride; chlorhexidine digluconate) – 1% |
| 7. Biguianides | Chlorhexidine digluconate BP88 (chlorhexidine digluconate) – 5% |
| 8. Oxidising: iodine-releasing | HMI Iodalin® (1.7% iodine, surfactants) – 2% |
| 9. Aldehydes, QAC | HMI Glikofin® (benzyl-C12-16-alkyldimethyl, chlorides; benzalkonium chloride; glutaraldehyde) – 1% |
| 10. QAC | HMI Roda® [benzyl-C12-16-alkyldimethyl, chlorides; C12-14-alkyl [(ethylphenyl)methyl] dimethyl, chlorides – 1% |
| 11. Alkalies, oxidising: chlorine-releasing | Trizon (sodium hypochlorite. sodium hydroxide) – 10% |
| 12. Acids | Kislol (hydrochloric acid) – 10% |

***Legend:*** *QAC – quaternary ammonium compounds.*

The evaluation of disinfection efficacy was done by means of suspension test. To this end, 1 mL aqueous solution of larval culture with density 500 larvae/mL was added to 9 mL disinfectant solution (correction of the concentrations to avoid further dilution was made). Each test was repeated six times, and average values with respective deviations were determined.

The microscopic examination of larval vitality was done after 30-minute and 60-minute exposure to the disinfectant. The tests were done at room temperature (21°C).

The antiparasitic efficacy of tested disinfectant solutions was evaluated in vitro using the method described by Daugschies *et al.*, 2002 and Guimarães *et al.*, 2007, through the proportion of motile larvae with intact structure to immotile larvae and/or larvae with damaged cuticle or damages internal structure using the equation:

Efficacy (%) = 100x[( C – T )/C], where C is the average number of vital larvae before the contact with the tested disinfectant and T – is the number after the exposure to the disinfectant (Daugschies *et al.*, 2002; Guimarães *et al.*, 2007).

Criteria for evaluation of disinfectant's effect on larvae comprised changes in motility and structural changes (vesicular inclusions, fading and/or destruction of intestinal cells, wrinkling or folding). The results were compared with larval vitality in negative control samples – diluted with water containing no disinfectant components.

The statistical analysis of data was done with the descriptive statistics function of Microsoft Excel (ToolPack) and the IBM® SPSS® Statistics software platform. Differences with $p < 0.05$ were assumed to be statistically significant.

## Results

The results revealed that sodium hydroxide, iodine- and oxygen-releasing disinfectants had the best efficacy and caused rapid 100% inactivation of L1 in exposure of only 30 min. High efficiency at 30 min exposure was also observed in phenols (97.89%), aldehydes (96.84%) and the combination of QAC and chlorhexidine (96.84%). The efficacy of chlorine containing compounds was 39% (sodium dichloroisocyanurate) and 47.37% (sodium hypochlorite with sodium hydroxide) after 30-minute as well 59% and 91.58% after 60-minute exposure respectively. Five percent solution of chlorhexidine digluconate possessed efficiency 58.94% and 65.26% at 30- and 60-minute control measurements. almost all larvae survived the simulated washing with tap water (Table 2).

**Table 2: Larvicidal efficacy of disinfectants against L1 stage larvae of *A. abstrusus* in suspension test**

| Disinfectant: type, concentration | Exposure | | | |
|---|---|---|---|---|
| | 30 minutes | | 60 minutes | |
| | Vital larvae (%) Mean (range) | Larvicidal efficacy (%) | Vital larvae (%) Mean (range) | Larvicidal efficacy (%) |
| Tabidez, 0.05 % | 58.33 (50÷75) | 39* | 39.17 (30÷45) | 59* |
| Caustic soda, 2% | 0 | 100* | 0 | 100* |
| Ecocid S, 2% | 0 | 100* | 0 | 100* |
| Cetridine RD, 1% | 11.67 (10÷15) | 87.37* | 0.83 (0÷5) | 98.95* |
| Interkokask, 4% | 1.67 (0÷10) | 97.89* | 0 | 100* |
| Cetridine Forte CE, 1% | 3.33 0÷10 | 96.84* | 0 | 100* |
| Chlorhexidine digluconate BP88, 5% | 39.17 (25÷50) | 58.94* | 33.33 (10÷50) | 65.26* |
| HMI Iodalin, 2% | 0 | 100* | 0 | 100* |
| HMI Glikofin, 1% | 3.33 (0÷10) | 96.84* | 0 | 100* |
| HMI Roda, 1% | 9.17 (0÷15) | 90.53* | 2.5 (0÷5) | 96.84* |
| Trizon, 10% | 50 (50) | 47.37* | 7.5 (5÷10) | 91.58* |
| Kislol, 10% | 13.33 (10÷25) | 86.32* | 10 (5÷20) | 89.47* |
| Water, 0% (negative control) | 90 (90) | 5.26 | 80 (80) | 15.79 |

***Legend:*** *The asterisk denotes statistically significant differences in the vitality of larvae compared to the control group ($p<0.05$).*

The disinfection treatments demonstrated statistically significant ($p<0.05$) reduction of the vitality of larvae for both exposure periods - 30 and 60 minutes (Table 2).

The main morphological alterations resulting from the exposure of larvae to disinfectant solutions are shown on Fig. 1; Fig. 2. They included cuticle vacuolation; abnormal granulation and staining; vesicular inclusions; fading and/or destruction of intestinal cells; wrinkling or folding.

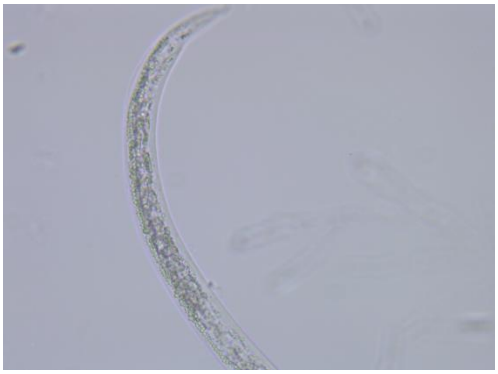

**Figure 1: Increased transparency, lack of morphological structures and granulation induced by 30-minute exposure to caustic soda**

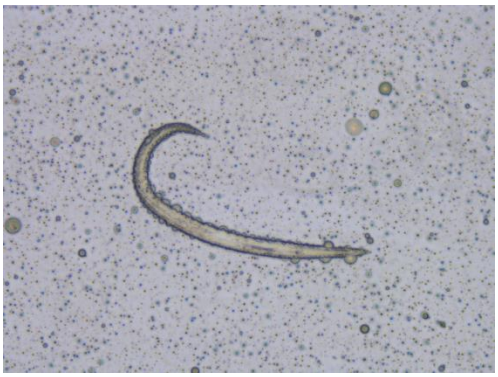

**Figure 2: Vacuolation of the cuticle induced by 30-minute exposure to 4% Interkokask**

## Discussion

The highest larvicidal efficacy was found out for exposure to 2% caustic soda (100%); 2% Ecocid S (100%) and 2% HMI Iodalin (100%), which attained a complete larvicidal effect as early as after 30 minutes. Very high larvicidal efficacy was demonstrated by 4% Interkokask (97.89%); 1% Cetridine Forte CE (96.84%), 1% HMI Glikofin (96.58%) and 1% Cetridine RD (87.37%). Both tested chlorine-releasing disinfectants - Tabidez and Trizon, had a significantly lower efficacy after 30-minute exposure: 39 and 47.37%, respectively. Similar effect from chlorine preparations on the development of *Toxascaris leonina* and *Toxocara canis* was reported by Morrondo *et al.* (2006) and El-Dakhly *et al.* (2017).

Despite the high efficacy of some disinfectants, their use in areas for felids housing should be done with increased caution. It is acknowledged that a distinctive feature of Felidae family is the deficiency of phenol UDP-glucuronosyltransferase (UGT), associated with increased sensitivity to the toxic effect of phenolic derivatives. That is why phenolic disinfectants e.g. Interkokask should be applied with caution and always in absence of animals due to released toxic phenol vapours. Measures to prevent direct and indirect contact with animals, by profuse washing with water to remove traces of disinfectant solutions, especially from feeders, drinkers and areas in direct contact with animals should be taken (Addie, 2015). The same is valid for disinfectants with marked caustic effect (2% caustic soda solutions). They may be applied for disinfection of corrosion-resistant materials – plastic food and water bowls, cages for transportation etc. as well as for decontamination of faeces and manure, cat litter (sand, bentonite etc.) due to the property of sodium hydroxide to preserve its antibacterial activity even at high levels of organic and inorganic contaminants.

Iodine-releasing disinfectants (iodophors) are appropriate for decontamination of facilities housing felids – they organic compounds of iodine and surfactants and are outlined with a broad spectrum of activity (Greene *et al.*, 2012; Moulay, 2013), result from short exposure time and very low toxicity. Unlike ethanol solutions of inorganic iodine, iodophors are mild skin irritants and very well tolerated (Punyani *et al.*, 2006). Some of disadvantages are the dark brown colour of solutions associated with spots and discoloration of some materials, as well as specific taste and odour. The long-term effect of iodine preparations on cats of various ages is still insufficiently investigated (Punyani *et al.*, 2006).

Oxidising agents such as pentapotassium bis (peroxymono sulfate bis(sulfate) are usually combined with surfactants and inorganic buffer system (Greene *et al.*, 2012). These disinfectants possess high antimicrobial activity, and excellent bactericidal and virucidal properties, are characterised with low toxicity and lack of toxic substances, and are completely biodegradable and therefore, safe for animals, humans and the environment (Greene *et al.*, 2012; Addie *et al.*, 2015). The high efficacy of these disinfectants observed in our studies makes them a very good alternative for disinfection in case of *A. abstrusus* invasions. It should be remembered that they may cause some surfaces and soft metals to corrode (Addie *et al.*, 2015).

Similarly to many other diseases, the primary source of infection are affected cats and their faeces. This makes cat litter and beds important factors for the spread of infection. Contaminated cells and especially food and water bowls are also associated with high epidemiological risk. It should be kept in mind that apart *A. abstrusus,* cats may also carry and shed numerous and often deadly pathogens as *FPV, FeCov, FHV, FCV, FeLV, FIV, Toxoplasma gondii, Cystisospora spp., Toxocara cati, T. leonina, Microsporum canis, Giardia duodenalis* (Greene *et al.*, 2012; Murad *et al.*, 2019; Addie *et al.*, 2000). Greene *et al.*, 2012 proposed using new food and water bowls or bowls cleaned with 10% bleach solution for at least 10 minutes, although our results demonstrated that this practice was not at all sufficient to destroy L1 of lung strongylids and creates prerequisites for infection of animals and contamination of the environment.

The results of these studies provide a clear evidence for the need of purposeful research on the species-specific sensitivity of microbial and parasitic pathogens to disinfectants. In our opinion, obtained data are the most appropriate criterion for disinfectant selection and finally, efficient disinfection to warrant both animal and public health.

## Conclusion

The different tested disinfectants at concentrations recommended by the manufacturers had a different effect on L1 of *A. abstrusus*, after exposure times of 30 and 60 minutes.

The most rapid and efficient inactivation of *A. abstrusus* larvae was achieved with the use of 2% caustic soda, 2% Ecocid S and 2% HMI Iodalin after 30-minute exposure.

In the presence of members of the *Felidae* family, phenol derivatives and caustic agents should be used with caution and with additional safety measures.

Oxygen-releasing disinfectants containing peroxides are safe and efficient disinfectants, even in the presence of cats.

Chlorine-containing disinfectants (Tabidez and Trizon) are insufficiently efficient for L1 inactivation and are not recommended for use alone for disinfection of feces in case of confirmed or probable infection with *A. abstrusus*.

The presented results allowed informed decision-making about a chemical agent for disinfection of facilities and decontamination of faeces from animals infected with *A. abstrusus* or animals at high risk – shelters, foster homes, veterinary clinics and castration centres for elimination of risk from spreading the parasites.

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