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EFFECT OF SOME COMMERCIALLY AVAILABLE DISINFECTANTS AGAINST E.COLI O126 AND SALMONELLA KENTUCKY STRAINS ISOLATED FROM OSTRICH FARM ENVIRONMENT

BY

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ABSTRACT

The present work was directed to study the efficiency of some commercially available disinfectants against *E.coli* O126 and *Salmonella Kentucky* strains isolated from ostrich' environment. Selected disinfectants represent four different groups of active ingredients that most commonly used in poultry farms. The obtained results showed that after 1 minute contact time,three disinfectants Virkcon-S®, Zix Verox® and Synergize® achieved bactericidal effect as they showed significantly reduction in the growth of *E.coli* (O126) achieving the 6.38 , 5.21 and 5.38 log reduction respectively . While Ground Zero® failed to achieve 5-log reduction of bacterial population 4.82 after 1 min contact time. Although it can pass and achieve the log reduction at 5, 15 min. On the other hand, *S .Kentucky* resists the disinfectant at 1 min. contact time and need for increase the contact time or the disinfectant concentration as all the tested disinfectants showed a lower bactericidal effect against *S. Kentucky,* After 5, and 15 min contact time. All tested disinfectants were able to achieve significant reduction and inhibition of the bacterial growth.

Key words:

Ostrich-Environment, *E.coli O126, Salmonella Kentucky*, Disinfectants, Virkcon-S.

INTRODUCTION

Ostrich productions are rapidly expanding in Egypt to produce required products such as meat, hides, feathers, and eggs. Egypt has various strengths and opportunities to develop the ostrich sector. The high meat prices suggest that fresh ostrich meat is unaffordable to numerous locals. Each one ostrich gives five kilos of feathers that could be used in the manufacture of

quilts and hats. Moreover, feathers of ostrich are used in Egypt for women's headgear, decoration. Contaminated environmental sources (vegetation, soil and water) contribute to infection exposure of ostrich chicks, soon after birth. *E. coli* is commonly isolated from sick ostrich chicks **(Quinn *et al.,* 2011).** *Escherichia coli, Salmonella spp*. and other bacteria are involved in infectious enteritis of ostrich. **Doneley, 2006 and Foggin, 1992** reviewed that *E. coli* causing enteritis is mostly prevalent when management practices are inadequate with poor hygiene, existing overcrowding and some stress factors as improper temperature and excessive handling. **Michael and Larry (2007)** mentioned that widespread occurrence of *Salmonella* in natural environment and the intensive husbandry practice used for intensive animal production has been a significant problem in public health. **Sanaz Salehi *et al.* (2016)** cleared that, although *Salmonella Kentucky* serovar is not considered a major source of human disease, high incidence of *Salmonella Kentucky* and the emergence of its recent multi-drug resistant strain with high resistor level to ciprofloxacin, indicates this serovar could be a potential menace to public health. The sanitary condition of farming is essential, because the occurrence of different pathogens, including Enterobacteriaceae might produce commercial restrictions in meat, egg production and other trade products.Generally the Enterobacteriaceae represents a matter of concern to public health, being a widespread human food borne pathogen **Rasschaert *et al.,* 2008; Smith *et al.,* 2008).** The main role of cleaning and disinfection regime is to reduce the number of pathogens in animal environment for reducing potency of disease in flock and so we should make regular evaluation of the used disinfection programmed and the used disinfectants depending on microbial assessment.

Various deficiencies in the process of disinfection may induce chain of infections, which will not break from one stock to another **(Kasková *et al*., 2007**). Therefore, the present work aimed to study the effect of some commercially available disinfectants against *E.coli* O126 and *salmonella Kentucky* strains isolated from ostrich environment.

MATERIAL AND METHODS

The selected tested disinfectants represented four different groups of active ingredients that most commonly used in farms as showed in (Table 1).

Table (1): The groups of disinfectants, its active ingredients and recommended dilutions.

Disinfectant	Active ingredients	Recommended use dilution	Manufacture
VirKon-S®	Potassium peroxymonosulphate (20.4%) and NaCl (1.5%)	1:120	Antec, international, LTD ,Sudbury, Suffolk, England.
Synergize®	Glutraldhyde 1% and QACs 23%	5ml / 1 liter	Preserve international, U.S.A
ZixVirox®	H_2O_2 (25%), per-acetic acid (5%) Acetic acid (6.5%)	10 ml / 1 liter	Bbzix Company, Spain
Ground Zero®	Iodophore 3.5% glutraldhyde 3%	5 ml / liter	Preserve international, U.S.A

Hard water:

Water of standard hardness was prepared containing 375- 400 ppm. $CaCo_3$ for the dilution of the tested disinfectants according to **(BSI 2009) using $MgCl_2$ (Loba-chemie) and $CaCl_2$ (Chem.-lab).**

Tested Disinfectants:

The tested disinfectants were diluted in prepared hard water at the manufacture recommended use dilution.

Test organism and test suspension:

Two isolated strains were used, *E.coli O126* (16srRNA gene positive) and *Salmonella Kentucky* (stn gene positive).

Cultures used for the disinfection assays were prepared from subculture on nutrient agar and incubated for 24 - 48 hours at 37˚C then stored in refrigerator at 5˚C until required.

-From these subcultures on nutrient agar, test suspensions were prepared and diluted using Tryptone -NaCl diluent solution and No. of colonies were adjusted as possible to ($1\text{-}5 \times 10^8$ CFU ml^{-1}) by surface spread viable counting method using tenfold serial dilution.

-The tested suspension was maintained at 20˚c ± 1˚C and used within two hours.

-Colony count was performed to the tested suspension through preparing further dilutions, plating and then incubated at 37˚C for 24 hours.

Interfering substance (Organic load):

Bovine albumin-yeast extract mixture was prepared by mixing an equal volume of 10% solution of bovine albumin (BA) and yeast extract to reach a final concentration of 5% (BA) and 5%yeast extract.

Neutralization media:

Neutralization media was prepared according to, **Payne *et al* (1999).**

Lecithin	**3 gm**
L-histidine	**1 gm**
Sodium thiosulphate	**5 gm**
Tween-80 (polysorbate)	**30 m**
Distilled water to	**990 ml**
The 0.25 N phosphate buffer saline	**10 ml**

Forall disinfectants, preliminary tests were performed to confirm the efficacy of neutralization media.

Contact time:

The European Committee for Standardization (CEN) method identifies 5 min as the disinfectant contact time. However, additional 1,5,15 min contact time were included for comparative purposes between the tested disinfectants **(BSI 2009).**

Disinfectant test method (phase2 /step 1):

Tests were performed according to **PrEN 1276, 2004,** Quantitative suspension test for the evaluation of chemical disinfectant and antiseptics used in food, and industrial, domestic and institutional area.

Test method and requirement, phase 2, step 1. **(BSI, 2009).**

-Before starting the test, all reagents were adjusted to 20˚c in a water bath.

-8 ml of the diluted disinfectant test solution (In hard water) at the manufacture recommended use dilution was added to 1 ml albumin / yeast mixture(organic load /interfering substance) **(Taylor *et al.*, 1999).** Mixed by vortexing and left for about 30 min to give chance of the disinfectant to react with the organic substance.

Then, 1 ml of the bacterial suspension was added and starting the stopwatch.

After each determined contact time 1,5,15 min, 1 ml was added to 8 ml neutralization medium

to which 1 ml sterile distilled water had been added, mixed by vortex and left at 20˚C for 5 min neutralization.

-Further decimal dilutions were made in diluents as appropriate and 100 µl were inoculated and spread onto each two plates, inverted and incubated at 37 ˚C for 24 hrs.

-viability reduction was calculated for each strain microorganism and tested disinfectant using the following formula:

$$R = \frac{N \times 10^{-1}}{Na}$$

Where:

R = Reduction of viability.

N = the initial test suspension bacterial count.

Na = the test mixture bacterial count at the end of the contact time.

Statistical analysis:

After designing and arranging the data using SPSS (Statistical package of social science), means were obtained and compared by one way analysis of variation (ANOVA) followed by least significant difference multiple comparisons test (LSD) and Duncan's new multiple range tests. These tests used to assess significance difference between disinfectants at different contact time.

RESULTS AND DISCUSSION

Table (2): The Mean Viable colony count (CFU/ml) of *E.coli (O126*) after contact time with the tested disinfectants.

Disinfectants / contact time	Initial count	1 min	5 min	15 min
Virkcon-S®	2.4×10^{8}	1×10^{2} a	0 a	`
Ground Zero®	2.4×10^{8}	3.7×10^{3} b	5.5×10^{2} a	0a
Zix Verox®	2.4×10^{8}	1.5×10^{3} a	6×10^{2} a	0a
Synergize®	2.4×10^{8}	1×10^{3} a	6.2×10^{2} a	0a

(a-b) different superscripts indicate the statistical significant difference $P \leq 0.05$.

Table (3): Microbial Effect (ME) (log reduction) of the tested-disinfectants against *E.coli (O126.)*

Disinfectants/ contact time	Initial Log count	ME.(log reduction) after contact time		
		1min	5min	15min
Virkcon-S®	8.38	6.38*	8.38*	8.38*
Ground Zero®	8.38	4.82**	5.64*	8.38*
Zix Verox®	8.38	5.21*	5.61*	8.38*
Synergize®	8.38	5.38*	5.59*	8.38*

*** Disinfectants passed of ME (log reduction) ≥ 5.**

****Disinfectants failed of log reduction< 5.**

Table (4) : The Mean Viable colony count (CFU/ml) of *S. Kentucky* after contact time with the tested disinfectants.

Disinfectants/ contact time	Initial count	1 min	5 min	15 min
Virkcon-S®	1.9×10^{8}	$1.7\times10^{3\ a}$	$1.1\times10^{2\ aA}$	0^{a}
Ground Zero®	1.9×10^{8}	$2.5\times10^{3\ b}$	$1.5\times10^{3\ aB}$	$4.6\times10^{2\ a}$
Zix Verox®	1.9×10^{8}	$3\times10^{4\ b}$	$1\times10^{2\ c\ A}$	$0^{\ c}$
Synergize®	1.9×10^{8}	$3.1\times10^{5\ b}$	$0^{\ aA}$	$0^{\ a}$

(a-b) different superscripts indicate the statistical significant difference when P ≤ 0.05

(A-B) different superscripts indicate the statistical significant difference when P ≤ 0.05

Table(5): Microbial Effect (ME) (log reduction) of the tested disinfectants against *S .Kentucky.*

Disinfectants/ contact time	Initial Log count	ME.(log reduction) after contact time		
		1min	5min	15min
Virkcon-S®	8.27	5.04*	6.23*	8.27*
Ground Zero®	8.27	4.88**	5.1*	5.61*
Zix Verox®	8.27	3.8**	6.27*	8.27*
Synergize®	8.27	2.78**	8.27*	8.27*

*** Disinfectants passed of ME (log reduction) ≥ 5**

****Disinfectants failed of log reduction < 5**

Disinfectants are vital tools for effective farm biosecurity, there are three main stages in disinfectant testing; primary/screening testing begins with suspension tests to determine activity against indicator organisms.Laboratory tests determine whether the disinfectant can be active against tested organisms, interfering substances, temperature and contact time and Field tests. **Chima *et al.*, (2012)** declared the importance of considering the type of microorganisms isolated from the practical field instead of reference strain cultures in process of disinfection evaluation. To pass the test, disinfectants must achieve a five-log reduction (ME) in viable counts after a defined contact time **Sheraba *et al. (*2014).** Data obtained in (Tables 2, 3) clarified that, after 1 min contact time the 3 disinfectants Virkcon-S®, Zix Verox®, Synergize® considered bactericides as they showed significantly reduction in the growth of *E.coli* (O126) achieving the 6.38, 5.21 and 5.38 log reduction respectively. While Ground Zero® failed to achieve 5-log reduction of bacterial population 4.82 after 1 min contact time. Although it can pass and achieve the log reduction at 5, 15 min. After 5, 15 min contact time all tested disinfectants was enabled to achieve significant reduction and inhibition of the bacterial growth. Statistical analysis of data showed significant difference when $P \leq 0.05$. Which mean that all tested disinfectants except Ground Zero® increasing contact time not greatly affect the bactericide effect of disinfectant as they are considered as bactericide from 1 min contact time, but in case of Ground Zero® increasing contact time enabled it to achieve the microbial reduction and it considered bactericide after passing 5 min contact time the obtained results agreed with those obtained by **Gasparini *et al.*, (1995).**

They found that virkon-s is effective against *E.coli* at aforementioned recommended concentration. **Metawea and El-Shibiny (2013)** used Germicidan Iodes® and found that, releasing agents where their antimicrobial activities were greatly reduced in the presence of organic matter (Dust, chick fluffs, hatchery wastes and salts in tap water used for the dilution of disinfectant). Iodine containing disinfectant when applied to the poultry houses, it is required to remove the organic matter from the surfaces to prevent the retardation in action, as the organic matter prevent the Iodine to reach the target organism. Glutaldehyde alone is less effective in the sanitizing agents for presence of such organic matter, so the most disinfectants have a combination between glutraldhyde and QACs. It looks like Synergize®, which had higher antimicrobial activity even in the presence of organic matter. **McDonnell and Russell (2001)** stated that glutaradehyde and QACs have a great activity against bacteria and their

spores, fungi and viruses and **Youseif *et al*., (2001)** found that Aldekol®(glutaradehyde and QACs) was very effective on *Salmonella spp.* Zix Verox® had satisfactory antimicrobial effect in the presence of organic load. **Rodgers *et al*.,(2001) and Thamlikitkul *et al*.,(2001)** recommended the use of H_2O_2, peracetic acid and acetic acid containing disinfectant as a disinfectant in poultry farms and facilities. Data present in (Tables 4, 5) revealed that, after 1 min contact time, all the tested disinfectants showed a lower bactericidal effect against *S. Kentucky* as they were not able to achieve significant inhibition of the bacterial count, producing ME < 5 except Virkon- S® achieved 5.04 ME log reduction of the bacterial growth. It was declared that *S. Kentucky* resists the disinfectant at 1 min. contact time, as it needs either increasing the contact time or increasing the disinfectant concentration.

After 5, 15 min contact time all tested disinfectants was enabled to achieve significant reduction and inhibition of the bacterial growth.

-Statistical analysis of data showed significant difference when $P \leq 0.05$. Which mean that in all tested disinfectants except Verkon-S ® increasing contact time gave ability for disinfectants to achieve the bactericide effect as they fail in 1 min contact time.in case of Zix Virox ® increasing contact time from1 min. to5&15 min enable it to achieve more microbial reduction.

- Ground Zero® is the last choice of disinfectant at 5 min contact time as it has the lowest bactericide effect between other disinfectants. Rossoni and Gaylarde, 2000 mentioned that Zix Verox® could not recommend as the sanitizing agent of choice for chicken processing equipment as it has little inhibitory effect on *Salmonella spp.* While **Miguel Ruano *et al*., (2001)** found that $H_2 O_2$ 2% concentration had excellent bactericidal effect in the presence of organic matter.

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