Science, Technology and Innovation
ISSN 2544-9125 • 2026; 25 (2): 18-26
doi: 10.55225/sti.757

STI • 2026; 25 (2): 18-26

Evaluation of the effectiveness of three disinfectants used for chicken hatching egg disinfection based on microbiological analyses and hatchability results

Ewelina Węsierska[1] A-C, Magdalena Trela[2] D-G

[1]University of Agriculture in Krakow, Faculty of Veterinary Medicine, Department of Infectious and Invasive Diseases and Public Health Protection, al. Mickiewicza 24/28, 30-059 Krakow, Poland
[2]University of Agriculture in Krakow, Faculty of Animal Breeding and Biology, Department of Zoology and Animal Welfare, al. Mickiewicza 24/28, 30-059 Krakow, Poland

Original article

Abstract

Objective: The aim of the study was to compare three commercial disinfection procedures for chicken hatching eggs with respect to eggshell microbial contamination, embryonic mortality, macroscopic signs of egg-content contamination, and hatchability.

Materials and methods: A single commercial batch of 2,250 hatching eggs from 59-week-old Ross 308 broiler breeders was divided into three groups of 750 eggs. Eggs were disinfected with 1% Virocid®, 1% Virkon S®, or Fumagri HA® applied by fumigation at 0.8 g/m^3. Due to the study being conducted under commercial hatchery conditions, where all hatching eggs undergo routine disinfection, it was not possible to establish an untreated control group comprising eggs that had not been subjected to disinfection. Microbiological samples were collected before disinfection and 15 min after treatment. Incubation outcomes were evaluated by candling, hatch records, and breakout examination of rejected and unhatched eggs. Categorical data were analysed using chi-square tests, pairwise comparisons of proportions, and relative risk (RR).

Results: Hatchability of fertile eggs was 83.3%, 86.0%, and 87.1% for Virocid®, Virkon S®, and Fumagri HA®, respectively, with no significant overall difference among treatments ($p = 0.140$). The distribution of embryonic mortality across incubation periods was also not significantly affected by the disinfection procedure ($p = 0.130$). Macroscopic signs of egg-content contamination were less frequent after Fumagri HA® than after Virocid® treatment (1.1% vs 3.3%; RR = 0.35). Microbiological analyses showed no consistent reduction in total microbial counts after disinfection. No colonies with morphology typical of *Salmonella* or *Pseudomonas* were detected on the screening media used.

Conclusions: The three disinfection procedures did not significantly differ in overall hatchability or embryonic mortality. However, Fumagri HA® was associated with the lowest frequency of macroscopically identified egg-content contamination. The lack

Keywords

- biosecurity
- eggs contamination
- mortality
- one day chick
- hatchery

Authors' contributions

A – Preparation of the research project
B – Assembly of data for the research undertaken
C – Conducting of statistical analysis
D – Interpretation of results
E – Manuscript preparation
F – Literature review
G – Revising the manuscript

Corresponding author

Magdalena Trela
e-mail: magdalena.trela@urk.edu.pl
University of Agriculture in Krakow
Faculty of Animal Breeding and Biology
al. Mickiewicza 21
31-120 Kraków, Poland

Article info

Article history

- Received: 2026-08-11
- Accepted: 2026-08-17
- Published: 2026-08-19

Publisher

University of Applied Sciences in Tarnow
ul. Mickiewicza 8, 33-100 Tarnow, Poland

Financing

This study was funded by the institutional subsidy of the Faculty of Animal Breeding and Biology, University of Agriculture in Krakow, Poland.

Conflict of interest

None declared.

of concordance between eggshell microbiological results and embryological outcomes indicates that hatchery disinfection should be evaluated using both microbiological and incubation-related criteria. Because the study involved a single commercial batch and limited microbiological replication, the findings should be regarded as preliminary.

Introduction

Modern poultry production is one of the most important sectors of the food industry, constituting one of the primary sources of animal protein in human nutrition. Poultry meat and eggs are characterized by high nutritional value and low fat content, and their relatively low price makes them among the most frequently chosen food products. Such a large scale of production requires a constant supply of healthy chicks, therefore the biological quality of hatching eggs, hygiene in handling them, and the proper incubation process are key elements of modern poultry production. Development of technologies used in poultry hatcheries enables high hatchability, but hatching efficiency depends not only on the previously mentioned parameters but also on the quality of the shell itself, storage and transport conditions, and the level of microbiological contamination of the shell and egg content [1,2]. Egg contamination can occur both vertically, during egg formation in the oviduct, and horizontally, after egg laying due to contact with a contaminated environment. In the latter case, microorganisms from the litter, air, equipment, or personnel settle on the shell and can penetrate through its pores. The composition and abundance of shell microbiota depend on the health of the flock, sanitary conditions, and the method of egg transport and storage [3]. The risk of penetration is increased by dirt, moisture, shell damage, and disruption of the egg's natural protective barriers [4–6]. The cuticle limits the penetration of microorganisms into the shell pores, while egg albumen contains, among other things, lysozyme, ovotransferrin, and other components that inhibit the growth of some bacteria. However, these barriers do not provide full protection at high levels of contamination or improper egg handling [4,7,8].

The microbiota of eggshells includes environmental microorganisms and potentially pathogenic bacteria. The genera Staphylococcus, Enterococcus and Pseudomonas are most often associated with horizontal infections, whereas Escherichia can infect eggs both vertically and horizontally. Salmonella, on the other hand, is most commonly transmitted vertically [4,5,9]. Pathogenic strains of Avian Pathogenic Escherichia coli (APEC) are of particular importance, as they are responsible for embryonic deaths and chick infections. Bacteria from the genus Salmonella also pose a significant threat to poultry production, food safety, and public health [10–13]. One of the fundamental elements of biosecurity is effectively disinfecting hatching eggs before incubation. The methods used should reduce the number of microorganisms on the shell effectively without compromising its properties, and more importantly, without having any toxic effects on the embryo, humans or the environment. In practice, both physical and chemical methods are employed, but preparations applied as fogs or aerosols currently predominate. For many years, formaldehyde fumigation was the standard method, but due to risks to personnel and potential adverse effects on embryos, alternative methods are being sought. Practical applications include oxidising preparations, quaternary ammonium salt and aldehyde compounds, organic acid-based fumigants, ultraviolet radiation and plant-based substances [7,14–16].

Previous research indicates that the effectiveness of the procedure depends on the active ingredient, concentration, contact time, application method, shell contamination level, and environmental conditions. Reducing the number of microorganisms on the egg surface does not always translate directly into improved hatchability. A potent antimicrobial agent may be ineffective if applied incorrectly, and a procedure that limits microbiota may simultaneously affect the cuticle, shell conductivity, or embryo development [14,17]. Therefore, disinfection assessment should be based on both microbiological and embryological indicators.

The aim of this study was to compare three commercial preparations used in a poultry hatchery – Virocid®, Virkon S®, and Fumagri HA® – in terms of changes in the microbiological load on the shell surface, embryo mortality, egg content contamination, and the percentage of hatched chicks. It was hypothesized that the procedures used differ in their microbiological effectiveness and may have different effects on incubation.

Materials and methods

Study site and experimental material

The study was conducted at the commercial poultry hatchery (RenMar Hatchery, Poland) as part of a routine production process. Two thousand two hundred fifty (2,250) hatching eggs of Ross 308 broilers from a single 59-week-old parent flock were used. The eggs were divided into three groups of 750 eggs each, weighing (mean ± SD) 67.8 ± 0.44 g. Each group contained five hatching trays of 150 eggs each. The flock was under constant veterinary and zootechnical supervision, allowing for ongoing assessment of its condition and housing conditions. The experiment was conducted in a single commercial run.

Egg disinfection

As the study was conducted under commercial hatchery conditions, all hatching eggs were subjected to the disinfection procedure; therefore, an untreated control group could not be included. Immediately before incubation, the eggs were subjected to one of three disinfection procedures. Virocid® and Virkon S® were applied as 1% aqueous solutions by spraying in a disinfection chamber. Fumagri HA® was fumigated at a dose of 0.8 g/m^3. The chamber volume was 120 m^3. The eggs were exposed to each disinfection procedure for 20 minutes. These procedures were carried out in accordance with the manufacturers' instructions and the facility's current safety regulations (Table 1).

Table 1. Procedures of hatching egg disinfection

Preparat	Main active ingredients	Administration and dose	Exposure time
Preparat	Main active ingredients	Administration and dose	Exposure time
Virocid®	quaternary ammonium compounds, glutaraldehyde	spraying, 1% aqueous solution	20 min
Virkon S®	peroxygen compound with oxidizing action, organic acids and surfactants	spraying, 1% aqueous solution	20 min
Fumagri HA®	hydroxyacetic acid; ingredients supporting the fumigation process	fumigation, 0.8 g/m^3	20 min

Incubation and embryological evaluation

After disinfection, the eggs were incubated in a setter (Jartom, Poland) using a proprietary technological incubation programme, that automatically adjusted the temperature and relative humidity (RH) according to the stage of embryo development. From days 1 to 18, the eggs were placed on incubator trolleys on trays set at a 45° angle and automatically rotated 90° every hour. On day 10, control lighting was performed. On day 18, the eggs containing live embryos were transferred to baskets in the hatcher (Jartom, Poland), where they remained until hatching. All rejected eggs as well as all unhatched eggs, underwent breakout embryopathological analysis. Fertilisation was confirmed and the estimated time of embryonic death was determined (D1–D10, D11–D18 or D19–D21). The position of the embryo, the presence of visible developmental defects and macroscopic signs of infection of the egg contents were also assessed. Hatch rate and mortality were expressed in terms of the number of fertile eggs.

Sample collection and microbiological analysis

Microbiological samples were collected before disinfection and 15 minutes after its completion. Two samples were collected from each group before the procedure and four samples after the procedure. Each sample consisted of four eggs, with each egg used for a different assay: total microbial count, *Salmonella* screening, *Staphylococci*, and *Pseudomonas* screening (Table 2).

Table 2. Microbiological sampling scheme

Preparation	Control sampling [4 eggs]	Experimental sampling [4 eggs]
Preparation	Control sampling [4 eggs]	Experimental sampling [4 eggs]

Preparation	Control sampling [4 eggs]	Experimental sampling [4 eggs]
Virocid®	2 samples	4 samles
Virkon S®	2 samples	4 samles
Fumagri HA®	2 samples	4 samples

The total number of microorganisms was determined by the plate count method using Plate Count LAB-AGAR™ (BioMaxima, Poland). Swabs were placed in sterile sodium chloride solution, vortexed, and then decimal dilutions of 10^{-1}, 10^{-3}, and 10^{-5} were prepared. From each selected dilution, 1 ml of suspension was added to the plate and covered with medium. The plates were incubated at 37°C for 24–72 h, after which colonies were counted. Eggshell impressions were made on selective-differential media: Chromogenic Salmonella Selective LAB-AGAR™ for the isolation and differentiation of *Salmonella* bacteria; Baird-Parker LAB-AGAR™ with lecithin and Tween 80 for the isolation of *Staphylococcus* spp.; and Cetrimide LAB-AGAR™ for the isolation of *Pseudomonas* bacteria (BioMaxima, Poland). The plate was applied to the eggshell for approximately 10 seconds and then incubated at 37°C for 24 hours. Results were expressed as log CFU/25 cm^2. No growth was recorded as <1 log CFU/25 cm^2. Due to the screening nature of the culture without a multiplication stage and biochemical confirmation, the results for *Salmonella* and *Pseudomonas* were interpreted as the absence of colonies with typical morphology on the media used, and not as a complete diagnostic test.

Statistical analysis

The overall comparison of the effectiveness of the preparations, which determined whether the preparations differed in the final outcome (the percentage of hatched chicks), was performed using the chi-square test of independence (χ^2) for a 2 × 3 table, i.e., category (hatched chicks, dead embryos) × group (Virocid®, Virkon S®, Fumagri HA®). Detailed analysis was performed using the χ^2 test for a 4 × 3 table, i.e., category (deaths in subsequent incubation phases D1–D10, D11–D18, D19–D21, and hatched chicks) × group (Virocid®, Virkon S®, Fumagri HA®). Pairwise comparisons were performed using the Z-test for the following categories: percentage of embryos that died in subsequent incubation phases D1–D10, D11–D18, and D19–D21, the proportion of hatched chicks, and the proportion of infected eggs relative to fertilized eggs. Furthermore, a Relative Risk (RR) analysis was performed for the key outcomes: the proportion of hatched chicks and the proportion of infected eggs, using Virocid-disinfected eggs as the reference group.

Analyses were performed using SigmaStat for Windows 3.5 (Systat Software Inc., USA).

Results and discussion

Under natural conditions, a multi-layered barrier comprising the cuticle covering the shell and the microbiota inhabiting its surface provides egg protection against infection. This barrier is composed primarily of commensal microorganisms that limit the development of pathogens [18–19]. The composition of the shell microbiota is influenced by various factors, including the physiological state of the individual chick, the condition of its parent flock, environmental conditions and the location of egg laying. This is particularly relevant for eggs laid on litter [20]. Furthermore, eggs incubated by birds have been shown to have a lower proportion of pathogenic bacteria than those incubated in commercial hatcheries [19, 21]. Therefore, in production practice, hatching eggs are routinely disinfected to reduce the number of microorganisms present on their surface. The procedures used may vary between hatcheries and are adjusted according to the degree of shell contamination.

The effectiveness of disinfection can depend on various factors, such as the concentration of the disinfectant, the duration of contact with the disinfected material, and the temperature and humidity conditions during the process [14]. In the experiment conducted, these parameters were precisely controlled and the analysed eggs showed no contamination or visible damage to the shell surface. Microbiological analyses revealed no clear advantage of any of the tested preparations, nor a consistent reduction in any of the assessed microbial groups. The total number of microorganisms on the surface of the hatching eggs in any of the groups decreased as a result of the disinfection procedures. The absence of *Salmonella* and *Pseudomonas* bacteria indicates that the initial sample was of good microbiological quality. The results obtained suggest that the preparations used in the experiment were not effective in reducing the microbiota present on the shell surface. This may be due to microorganisms having increased tolerance to the active substances used, or insufficient exposure time of the preparations before sampling for analysis. The influence of secondary contamination of samples during further handling of the eggs cannot be ruled out. Determining the presence of pathogenic *Staphylococci*

showed a reduction in bacterial counts only when the samples were disinfected with Virkon®. The results obtained indicate the selective effectiveness of Virkon S® in reducing the population of pathogenic staphylococci, as the tested preparations had no effect on the vegetative forms of *Staphylococcus* spp. that inhabit the surfaces of hatching eggs (Table 3). The difference in the biocidal effectiveness of the preparations primarily results from their chemical composition and the mechanism of action of the active substances. The main active ingredient in Virkon S® is potassium peroxymonosulfate (PMS), a strong oxidant. Its antimicrobial activity involves generating reactive oxygen species and sulfate radicals. This leads to oxidative damage to cell membrane lipids, proteins and other key bacterial cell components. This results in a loss of cell integrity and cell death [22–23]. Additionally, the organic acids present in the preparation stabilise the acidic environment, favouring the activity of potassium peroxymonosulfate. Meanwhile, the surfactants reduce surface tension and improve surface wetting, facilitating even contact of the disinfectant with bacterial cells [24]. The oxidative properties of potassium peroxymonosulfate may also affect the organic components of the eggshell cuticle. In support of this possibility, Sylte et al. (2017) [25] reported partial removal of the cuticle following treatment of turkey eggs with Virkon S®, suggesting that the oxidative activity of this disinfectant may affect the integrity of the cuticle. Such an effect may potentially alter the protective properties of the cuticle and, consequently, the conditions at the eggshell surface during disinfection.

The limited effectiveness of the tested agents against the vegetative forms of *Staphylococcus* spp. present on the shells of hatching eggs may be due to the eggshell's physical properties. The shell's porous structure and the presence of the cuticle create a complex microenvironment in which bacteria can localise in pores and microcavities, which limits their direct contact with disinfectants. This phenomenon may reduce the effectiveness of disinfecting egg surfaces chemically, despite the high activity of biocidal agents under laboratory conditions [4,26–27]. Furthermore, *Staphylococcus* bacteria demonstrate the ability to form biofilms on various surfaces. The extracellular polymeric substance (EPS), the main component of biofilms, constitutes a diffusion barrier that limits the penetration of antimicrobial and disinfectant agents, thereby increasing the tolerance of bacterial cells to their effects. While this phenomenon has not yet been clearly demonstrated for the surface of hatching egg shells, it cannot be ruled out as one of the mechanisms responsible for the limited effectiveness of disinfection [28–30].

Different results were observed in terms of the total microbial count, with an increase in the number of isolated microorganisms noted after the use of Virkon S®. However, the observed increase in microbial counts is most likely not indicative of actual microbial multiplication, but may instead be the result of the heterogeneous distribution of microorganisms on the eggshell surface and the biological variability of the tested samples. The literature emphasises that determining egg surface microbiota is characterised by significant variability resulting from uneven shell colonisation and difficulties in standardising sample collection [31]. Comparing the results of the *Staphylococcus* spp. count and total microorganism count with the risk analysis (Table 4) revealed no correlation between the degree of eggshell surface contamination and embryological results under the experimental conditions. The results obtained suggest that the level of microbial contamination observed was insufficient to significantly impact the incubation process and hatching parameters [14]. Virkon S® was found to have the safest effect on embryos during the early incubation period (D1–D10). In the group of eggs disinfected with this preparation, the percentage of embryo deaths was 4.4%, which was lower than in the Virocid® and Fumagri HA® groups by 1.6 ($p = 0.251$) and 0.9 ($p = 0.537$) percentage points, respectively. However, this beneficial effect did not persist in the later stages of incubation. This is particularly evident between D11–18 (Table 4). The safety of this preparation for chicken embryos was also studied by Mousa-Balabel et al. [32], who found that, of the agents used, it produced the highest hatchability results and the lowest percentage of weak chicks.

Data on the total number of microorganisms on the eggshell surface show that Virocid® was the most effective disinfectant. While it did not reduce the number of *Staphylococcus* bacteria, it effectively reduced the total number of microorganisms on the egg surface under the experimental conditions (Table 3). Similar results from disinfection with Virocid® were observed by Mohammadi-Aragh et al. [17], who also found this agent to be the most effective. This may suggest that other components of the eggshell microbiota are more sensitive to the active substances in Virocid®, namely quaternary ammonium compounds (QACs) and glutaraldehyde, than to *Staphylococcus* bacteria. QACs primarily act by damaging the cytoplasmic membrane and disrupting its permeability, while glutaraldehyde causes irreversible cross-linking of proteins and other cellular macromolecules. However, the literature describes cases where bacteria of the Staphylococcus genus have shown reduced sensitivity to quaternary ammonium compounds. This is related to the presence of active disinfectant efflux pumps (e.g. proteins encoded by the qacA/B or smr genes), as well as changes in cell sur-

face properties [24,33]. However, the analysis (Table 4) shows that such a strong reduction in microbiota does not improve conditions for embryo development. Despite an effective reduction in the total number of microorganisms on the shell surface, the group of eggs disinfected with Virocid® had the lowest percentage of hatched chicks and the highest incidence of infection in the egg content and embryos (3.3%). This was higher than in the Virkon S® and Fumagri HA® groups by 1.0 and 2.1 percentage points, respectively ($p = 0.387$ and $p = 0.019$). The highest mortality was observed at the beginning (D1–D10) and end (D19–D21) of incubation, which may have been caused by infections during these periods (Table 4). The potential interaction of quaternary ammonium compounds with the eggshell cuticle may be of particular relevance to the observed lower hatchability and higher incidence of internal egg contamination in the Virocid® group. Previous studies have suggested that quaternary ammonium-based sanitizers may alter eggshell permeability through interactions with the cuticle, potentially affecting the respiratory properties of the eggshell and water loss during incubation [34]. Such alterations may influence embryonic development and, if the protective function of the cuticle is compromised, could potentially facilitate the penetration of microorganisms into the egg. This demonstrates that assessing the effectiveness of disinfectants based solely on the number of microorganisms living on the shell surface is not sufficient to determine the safety of the disinfection procedure.

In the case of Fumagri HA®, we observed a different effect compared to Virocid®. Despite the lowest disinfection effectiveness, as confirmed by the highest total microorganism counts after disinfection (Table 3) and the lack of reduction in Staphylococcus bacteria, the analysis showed that the group of eggs disinfected with Fumagri HA® had the highest hatching rate (87.1%) and the lowest incidence of egg content infection (1.1%). The highest mortality rate in this group was also observed in the periods (D1-D10) and (D19-D21), which may also indicate progressive infections during this time (Table 4). In this context, the method of application of Fumagri HA® may be of importance. Unlike Virocid® or Virkon S®, which was applied by direct spraying of the eggshell surface, Fumagri HA® was applied by fumigation, which does not involve direct wetting of the eggshell. Therefore, it cannot be excluded that the method of application, together with the chemical properties of the preparation, may have resulted in less intensive interaction with the eggshell surface and its natural protective barriers, including the cuticle. Preservation of the integrity of these barriers may have contributed to maintaining favourable conditions for embryonic development, which may partly explain the highest hatchability rate observed in the Fumagri HA® group. At the same time, preservation of the barrier function of the eggshell may have limited the penetration of microorganisms into the egg, as reflected by the lowest incidence of internal egg contamination. The obtained results may indicate that the presence of more abundant microbiota on the surface of hatching eggs does not necessarily lead to an increased risk of impaired development of the chicken embryo. The species composition of the microbiota, the ability of microorganisms to penetrate the shell, and interactions between microorganisms and the natural protective barriers of the egg may also be important [35].

Table 3. Results of microbiological tests of the shell surface before (BD) and after disinfection (AD)

Item	Period of disinfection	Virocid®	Virkon S® (log cfu/25 cm^2)	Fumagri HA®
Total microorganism count	BD	2.57	2.89	3.65
	AD	2.41	3.80	4.19
Staphylococcus	BD	1.58	2.68	<1
	AD	2.14	2.60	2.70
Salmonella – screening medium	BD	<1	<1	<1
	AD	<1	<1	<1
Pseudomonas – screening medium	BD	<1	<1	<1
	AD	<1	<1	<1

Table 4. Hatching results of eggs disinfected with Virocid®, Virkon S® or Fumagri HA®

Item	Virocid®	Virkon S®	Fumagri HA®
Set eggs, n	750	750	750
Fertilized eggs, n (%)	615 (82.0)	614 (81.9)	621 (82.8)
Embryo mortality D1–D10, n (% of fert.)	37 (6.0)	27 (4.4)	33 (5.3)
Embryo mortality D11–D18, n (% of fert.)	12 (2.0)^{ab}	16 (2.6)^{b}	5 (0.8)^{a}

Item	Virocid®	Virkon S®	Fumagri HA®
Embryo mortality D19–D21, n (% of fert.)	54 (8.8)	43 (7.0)	42 (6.8)
Contaminated eggs (% of fert.)	20 (3.3)[b]	14 (2.3)[ab]	7 (1.1)[a]
Mortality – total, n (% of fert.)	103 (16.7)	86 (14.0)	80 (12.9)
Hatched chicks, n (% of fert.)	512 (83.3)	528 (86.0)	541 (87.1)

[a, b] – values within the same row with different superscripts differ significantly ($p < 0.05$).

It should also be emphasised that comparing the effectiveness of Virocid®, Virkon S® and Fumagri HA® did not reveal any effect on overall hatchability ($p = 0.140$). A detailed analysis, which included cases of embryo death in subsequent incubation phases, also failed to confirm the hypothesis that the preparations affect embryo development ($p = 0.130$) (Table 4). The results obtained did not confirm the expected relationship between reducing the total number of microorganisms on the eggshell surface and hatchability. The analysis demonstrated that the crucial factor is not the number of microorganisms present on the shell itself, but rather the effect of the disinfectant on the eggs' natural protective barriers and the ability of microorganisms to penetrate the interior of the egg [4]. It can be suggested that agents with stronger chemical action, such as formaldehyde, despite being more effective in reducing the number of microorganisms present on the shell surface, may increase the egg's susceptibility to damage and weaken its natural protective structures, such as the cuticle, which may lead to internal infections during incubation [36–38].

Therefore, it is crucial to search for disinfectants that are gentle on the structures that make up the eggshell. Al-Shammari et al. [15] and Kubaczyński et al. [3] demonstrated the safety of individual plant extracts as disinfectants and that their use helps maintain hatchability. Ibrahim et al. [38] conducted an experiment demonstrating that selected concentrations of silver nanoparticles can reduce the microbiological contamination of quail eggshells and improve hatchability. Batkowska et al. [39] confirmed that ultraviolet radiation has no negative effect on the viability of chicken embryos or egg hatchability. Nevertheless, it is necessary to continue searching for solutions that are as safe as possible for chicken embryos. Periodically repeating the studies described in this paper in hatcheries would also be worthwhile for monitoring the impact of the tested disinfectants.

The results obtained indicate that the effectiveness of disinfectants should not be assessed solely in terms of reducing shell microbiota. It is equally important to analyse the incubation process and the frequency of infections in the egg contents, as this may provide a more accurate reflection of the safety of the procedure for the developing embryo. However, it should be emphasised that these results are from an experiment conducted on a single set of commercial eggs from a single parent flock. Furthermore, the number of microbiological samples was limited and the Salmonella and Pseudomonas assays were screening tests. Therefore, the results require confirmation in studies involving a larger number of flocks and repeat experiments.

Conclusions

1. In a single commercial run, no overall difference was observed in the percentage of hatched chicks or in the distribution of embryonic mortality between eggs disinfected with Virocid®, Virkon S®, and Fumagri HA®.
2. Fumagri HA® was associated with a lower frequency of macroscopically detected egg contents infections than Virocid® and with lower mortality during the D11–D18 period than Virkon S®.
3. The results of microbiological assays of the shell surface did not demonstrate a consistent reduction in microbial counts and did not directly correspond to embryological results.
4. Validation of hatching egg disinfection should simultaneously include microbiological indicators: embryonic mortality, egg contents infections, and hatching performance.
5. Due to the single run, the lack of a non-disinfected group, and limited microbiological replication, the presented results are preliminary and require confirmation in an experiment with independent replicates.

Acknowledgments

The authors gratefully acknowledge the staff of the RenMar Hatchery for their cooperation and assistance in conducting this study. The authors would like to express their sincere thanks to Milena Rosół for her contribution to the experimental work and assistance with the collection and processing of research material conducted as part of her undergraduate engineering thesis, the results of which contributed to the present publication.

Approval of ethics committee

The study was conducted on commercial hatching eggs. The disinfection procedure corresponded to standard commercial hatchery practice and did not involve any experimental procedures on live animals. The study included only monitoring of the incubation process and analysis of unhatched eggs; therefore ethical approval was not required.

Data available

The data presented in this study are available from the corresponding author upon reasonable request.

References

[1] Stańko S, Mikuła A. Tendencje na rynku mięsa drobiowego na świecie i w Polsce w latach 2000–2016. Zeszyty Naukowe SGGW w Warszawie Problemy Rolnictwa Światowego. 2017;17(3):268–278. https://doi.org/10.22630/PRS.2017.17.3.72.

[2] Adame MM, Ameha N. Review on egg handling and management of incubation and hatchery environment. Asian Journal of Biological Sciences. 2023;16(4):474–484. https://doi.org/10.3923/ajbs.2023.474.484.

[3] Kubaczyński A, Widz P, Batkowska J. Dezynfekcja jaj wylęgowych – tradycje i innowacje. In: Nowakowicz-Dębek B, Pawlak H, editors. Ergonomia. bezpieczeństwo i higiena pracy w praktyce. Lublin: TWN Libropolis; 2016. p. 243––253. Available from: https://www.researchgate.net/publication/335189519_Dezynfekcja_jaj_wylegowych_-_tradycje_i_innowacje.

[4] Berrang ME, Cox NA, Frank JF, Buhr RJ. Bacterial penetration of the eggshell and shell membranes of the chicken hatching egg: A review. Journal of Applied Poultry Research. 1999;8(4):499–504. https://doi.org/10.1093/japr/8.4.499.

[5] De Reu K, Messens W, Heyndrickx M, Rodenburg TB. Uyttendaele M. Herman L. Bacterial contamination of table eggs and the influence of housing systems. World's Poultry Science Journal. 2008;64(1):5–19. https://doi.org/10.1017/S0043933907001687.

[6] Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Gast R, Humphrey TJ, Van Immerseel F. Mechanisms of egg contamination by Salmonella Enteritidis. FEMS Microbiology Reviews. 2009;33(4):718–738. https://doi.org/10.1111/j.1574-6976.2008.00161.x.

[7] Motola G, Hafez HM, Brüggemann-Schwarze S. Assessment of three alternative methods for bacterial disinfection of hatching eggs in comparison with conventional approach in commercial broiler hatcheries. PLoS One. 2023;18(3):e0283699. https://doi.org/10.1371/journal.pone.0283699.

[8] Lesnierowski G, Borowiak R. Wpływ warunków środowiskowych na zmianę właściwości lizozymu w białku jaj kurzych. Żywność. Nauka. Technologia. Jakość. 2012;19:77–87.

[9] Olsen R, Kudirkiene E, Thøfner I, Pors S, Karlskov--Mortensen P, Li L, Christensen J. Impact of egg disinfection of hatching eggs on the eggshell microbiome and bacterial load. Poultry Science. 2017;96(11):3901–3911. https://doi.org/10.3382/ps/pex182.

[10] World Health Organization. Salmonella (non-typhoidal). World Health Organization; 2018. Available from: https://www.who.int/en/news-room/fact-sheets/detail/salmonella-%28non-typhoidal%29.

[11] Food Standards Agency. Salmonella risk profile of UK-produced hen shell eggs: Exposure assessment. Food Standards Agency; 2023. Available from: https://www.food.gov.uk/research/salmonella-risk-profile-of-uk-produced-hen-shell-eggs-exposure-assessment.

[12] Watts A, Wigley P. Avian Pathogenic Escherichia coli: An overview of infection biology. Antimicrobial Resistance and Vaccination. Antibiotics. 2024;13(9):809. https://doi.org/10.3390/antibiotics13090809.

[13] Sharma S, Kaur S, Naguib M, Bragg A, Schneider A, Kulkarni RR, Nazmi A, Abdelaziz K. Major foodborne bacterial pathogens in poultry: Implications for human health and the poultry industry and risk mitigation strategies using probiotics. Microorganisms. 2025;13(10):2363. https://doi.org/10.3390/microorganisms13102363.

[14] Oliveira GDS, McManus C, Salgado CB, Dos Santos VM. Effects of sanitizers on microbiological control of hatching eggshells and poultry health during embryogenesis and early stages after hatching. Animals. 2022;12(20):2826. https://doi.org/10.3390/ani12202826.

[15] Al-Shammari KIA, Batkowska J, Gryzińska M, Wlazło Ł, Ossowski M. Nowakowicz-Dębek B. The use of selected herbal preparations for the disinfection of Japanese quail hatching eggs. Poultry Science. 2022;101(10):102066. https://doi.org/10.1016/j.psj.2022.102066.

[16] Korowiecka K, Trela M, Tombarkiewicz B, Pawlak K, Niedziółka JW, Swadźba M, Lis MW. Ocena wpływu wybranych substancji stosowanych do dezynfekcji jaj wylęgowych na wyniki lęgu piskląt kurzych. Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego. 2017;13(2):25–35. https://doi.org/10.5604/01.3001.0013.5221.

[17] Mohammadi-Aragh MK, Linhoss JE, Evans JD. Effects of various disinfectants on the bacterial load and microbiome of broiler hatching eggs using electrostatic spray. Journal of Applied Poultry Research. 2022;31(3):100278. https://doi.org/10.1016/j.japr.2022.100278.

[18] Board RG. Course of microbial infection of hens' egg. Journal of Applied Bacteriology. 1966;29(2):319–323. https://doi.org/10.1111/j.1365-2672.1966.tb03482.x.

[19] Cook MI, Beissinger SR, Toranzos GA, Arendt WJ. Incubation reduces microbial growth on eggshells and the opportunity for trans-shell infection. Ecology Letters. 2005;8(5):532–537. https://doi.org/10.1111/j.1461-0248.2005.00748.x.

[20] Potter B, Hyde E, Pier HN, Rutter MA, Voss MA. A comparison of the bacterial microflora found on the surface of American kestrel and house wren eggs. The Open Ornithology Journal. 2014;7:40–48.

[21] Ruiz-De-Castañeda R, Vela AI, González-Braojos S, Briones V, Moreno J. Drying eggs to inhibit bacteria: Incubation during laying in a cavity-nesting passerine. Behavioural Processes. 2011;88(2):142–148. https://doi.org/10.1016/j.beproc.2011.08.012.

[22] Hernández A, Martro E, Matas L, Martín M, Ausina V. Assessment of in-vitro efficacy of 1% Virkon® against bacteria. fungi. viruses and spores by means of AFNOR guidelines. Journal of Hospital Infection. 2000;46(3):203–209. https://doi.org/10.1053/jhin.2000.0818.

[23] Kunanusont N, Punyadarsaniya D, Jantafong T, Pojprasath T, Takehara K, Ruenphet S. Bactericidal efficacy of potassium peroxymonosulfate under various concentrations. organic material conditions. exposure timing and its application on various surface carriers. Journal of Veterinary Medical Science. 2020;82(3):320–324. https://doi.org/10.1292/jvms.19-0562.

[24] Gerba CP, Boone S, Nims RW, Maillard JY, Sattar SA, Rubino JR. et al. Mechanisms of action of microbicides commonly used in infection prevention and control. Microbiology and Molecular Biology Reviews. 2024;88(3):e00205-22. https://doi.org/10.1128/mmbr.00205-22.

[25] Sylte MJ, Chandra LC, Looft TO. Evaluation of disinfectants and antiseptics to eliminate bacteria from the surface of turkey eggs and hatch gnotobiotic poults. Poult Sci. 2017;96(7):2412–2420. https://doi.org/10.3382/ps/pex022.

[26] Samiullah S, Roberts JR. The eggshell cuticle of the laying hen. World's Poultry Science Journal. 2014;70(4):693–708. https://doi.org/10.1017/S0043933914000786.

[27] Kulshreshtha G, D'Alba L, Dunn IC, Réhault-Godbert S, Rodriguez-Navarro AB, Hincke MT. Properties, genetics and innate immune function of the cuticle in egg-laying species. Frontiers in Immunology. 2022;13:838525. https://doi.org/10.3389/fimmu.2022.838525.

[28] Otto M. Staphylococcal biofilms. Microbiology Spectrum. 2018;6(4). https://doi.org/10.1128/microbiolspec.gpp3-0023-20.

[29] Flemming HC, Wingender J. The biofilm matrix. Nature Reviews Microbiology. 2010;8(9):623–633. https://doi.org/10.1038/nrmicro241.5.

[30] Wu X, Wang H, Xiong J, Yang GX, Hu JF, Zhu Q, Chen Z. *Staphylococcus aureus* biofilm: Formulation, regulatory, and emerging natural products-derived therapeutics. Biofilm. 2024;7:100175. https://doi.org/10.1016/j.bioflm.2023.100175.

[31] Jones DR, Musgrove MT, Northcutt JK. Variations in external and internal microbial populations in shell eggs during extended storage. Journal of Food Protection. 2004;67(12):2657––2660. https://doi.org/10.4315/0362-028X-67.12.2657.

[32] Mousa-Balabel TM. Mohamed RA. Al-Midani SA. Abd El-Samad MS. Impact of boiler breeders hatching eggs disinfection time on some hatchability parameters. International Journal of Sciences: Basic and Applied Research. 2016;30(2):230–240.

[33] Buffet-Bataillon S. Tattevin P. Bonnaure-Mallet M. Jolivet-Gougeon A. Emergence of resistance to antibacterial agents: the role of quaternary ammonium compounds – a critical review. International Journal of Antimicrobial Agents. 2012;39(5):381–389. https://doi.org/10.1016/j.ijantimicag.2012.01.011.

[34] Brake J, Sheldon BW. Effect of a quaternary ammonium sanitizer for hatching eggs on their contamination, permeability, water loss, and hatchability. Poultry Science. 1990;69(4):517–525. https://doi.org/10.3382/ps.0690517.

[35] De Reu K. Grijspeerdt K. Messens W. Heyndrickx M. Uyttendaele M. Debevere J. Herman L. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria including Salmonella Enteritidis. International Journal of Food Microbiology. 2006;112(3):253–260. https://doi.org/10.1016/j.ijfoodmicro.2006.04.011.

[36] Cadirci S. Disinfection of hatching eggs by formaldehyde fumigation – a review. European Poultry Science. 2009;73(2):116–123. https://doi.org/10.1016/S0003-9098(25)00849-5.

[37] Cabanillas-Beltrán H, González-Estrada RR, Gutiérrez-Martínez P, Hernández-López SM. Quality and microbiological protection of table eggs by ultrasonic application of acidic electrolyzed water and chitosan. Acta Agronómica. 2020;69(2):97–105. https://doi.org/10.15446/acag.v69n2.77436.

[38] Ibrahim FA, El-Mousafa MK, El Sabry MI, Badr JM. Hassan ASI. Effect of egg disinfection by silver nanoparticles on eggshell microbial load. hatchability and post-hatch performance of quail chicks. International Journal of Poultry Science. 2018;17(5):234–242. https://doi.org/10.3923/ijps.2018.234.242.

[39] Batkowska J, Al-Shammari KIA, Gryzińska MM. Assessment of ultraviolet light effect in hatching eggs disinfection on hatchability traits of two breeds of quails and chicken. Acta Scientiarum Polonorum Zootechnica. 2015;14(2):33–44.