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Animal Health Protection – Assessing Antimicrobial Activity of Veterinary Disinfectants and Antiseptics and Their Compliance with European Standards: A Narrative Review

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Abstract

Disinfectants and antiseptics lead in reducing the number of microorganisms, including pathogenic ones, thus limiting the number of infections. In the veterinary field, disinfection prevents the transfer of pathogenic microorganisms from animals to humans and *vice versa*, as well as among animals. Several assays of disinfectant antimicrobial activity testing, often not standardized, without appropriate controls, and not validated, have been used and published. To unify these methods, nine European Standards (ENs) for the veterinary area have been prepared. These tests make it possible to examine whether a given disinfectant has bactericidal, fungicidal, or virucidal activity by the standard. This publication discusses ENs regarding the assessment of the above-mentioned antimicrobial activity of disinfectants used in veterinary medicine. Recent research on this topic has also been cited. According to ENs, tests are carried out using the suspension method or carriers in clean and dirty conditions. The decontamination of high-risk animal and zoonotic pathogens is also discussed. Selected publications on cattle, pig, poultry, and aquaculture farm disinfection are presented. Only valid methods of the described studies with appropriate statistical analysis can prove adequate antimicrobial activity. So far, the role of international standards in investigating the antimicrobial activity of disinfectants and antiseptics to reduce infections has been underestimated. This publication highlights gaps and irregularities in conducted research and aims to inform about existing EN standards dedicated to testing the biocidal activity of disinfectants and antiseptics intended for use in the veterinary area.

K e y w o r d s: European Standards (EN), antibacterial activity testing, antifungal activity testing, antiviral activity testing, disinfection in veterinary

Introduction

Disinfectants (chemical preparations used to inactivate or destroy microorganisms on inert, inanimate surfaces) and antiseptics (chemical preparations used to inactivate or destroy microorganisms on living tissue) significantly prevent infections in humans and animals. In the veterinary field, the preparations used should fulfill several functions as providing general hygiene conditions, especially for animals, but also for people who are in contact with animals, maintaining hygiene in animal husbandry and equipment, limiting the transmission of pathogenic microorganisms causing zoonosis and infections among animals, reducing the possibility of spreading microorganisms carrying various resistance genes. Large-scale animal disease outbreaks can cause substantial losses, affect human and animal health, and lead to high economic costs. Disinfectants and antiseptic preparations, depending on the application, should have appropriate antimicrobial activities, such as bactericidal, mycobactericidal/tuberculocidal, sporicidal, fungicidal/pesticidal, or virucidal activity. The effectiveness of these preparations has to be guaranteed by normative documents that must be elaborated in development studies before being approved and admitted to the market.

There are several international organizations, such as the American Society for Testing and Materials (ASTM International), the Association of Official Agricultural Chemists (AOAC International), the International

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Committee for Standardization (ISO), and the European Committee for Standardization (CEN) that develop test methods for assessing the antimicrobial activity of disinfectants (Wales et al. 2021).

The CEN is a non-profit association that gathers 34 European National Standard Bodies and supports the European economy in global trade by creating and distributing consistent European standards (ENs). These standards are elaborated by the Technical Committees (TCs). Almost 400 CEN Technical Bodies have been created so far, and each TC has its field of interest within which appropriate standards are developed. For example, TC 216, termed "Chemical Disinfectants and Antiseptics" was established in 1989 with the following scope: "*Standardization of the terminology*, *requirements*, *test methods including potential efficacy under in-use conditions*, *recommendations for use and labelling in the whole field of chemical disinfection and antiseptics. Areas of activity include agriculture (but not crop protection chemicals)*, *domestic service*, *food hygiene and other industrial fields*, *institutional*, *medical and veterinary applications*" (Holah 2003). This TC covers the whole field of chemical disinfectants and antiseptics and operates in four Working Groups (WGs): a Horizontal WG (HWG) and three WGs responsible for the Medical (WG1), Veterinary (WG2) and Food Hygiene, Domestic and Institutional (WG3) areas. The main objective of TC 216 is to elaborate test methods in the following three phase modes. In Phase 1, the ability of a product to demonstrate essential bactericidal, fungicidal, or sporicidal activity is tested. Phase 2 tests contain standard microorganisms and loading organic substances simulating contamination, added to the product sample. These tests are divided into two steps. Phase 2 Step 1 tests contain suspension tests to determine bactericidal, sporicidal, fungicidal, or virucidal activity under laboratory conditions that simulate practical conditions. Phase 2 Step 2 tests include other laboratory tests, e.g., carrier and surface tests, more representative of a practical application. Phase 3 tests are planned to guide product users regarding suitable field trials (Bolten et al. 2022).

In particular, EN 14885:2022/AC:2023 (2023) combines and presents all CEN laboratory methods for testing the chemical disinfectant and antiseptic products to support claims that they have specific antimicrobial activities appropriate to their intended application. The ENs for testing the bactericidal, fungicidal, and virucidal activities of preparations used in the veterinary area include tests with Phase 2 Steps 1 and 2.

The application of normative documents for determining the biocidal activity of disinfectants and antiseptics using in the medical area has recently been reviewed (Tyski et al. 2022). However, standards review has yet to be available in the veterinary field.

Although viral infections in humans caused by SARS-CoV-2 and influenza viruses, as well as respiratory syncytial virus, currently represent a significant threat among people, severe viral infections in animals caused by e.g., foot-and-mouth disease virus (FMDV), African swine fever virus (ASFV), and avian influenza, may also be epidemic (EFSA et al. 2023; Kang et al. 2024) which causes substantial financial losses for animal breeders. Besides, many species of bacteria and fungi can also cause infections in animals (livestock and pets). In this context, using appropriate disinfectants and antiseptics, along with adequate application procedures, can significantly reduce epidemics and common infections.

This publication aims to draw attention to the need to properly assess, by ENs, the biocidal activity of preparations that can be applied in the veterinary field. We present available articles in which the antimicrobial activity of disinfectants was tested in accordance with ENs as well as using own methods. Unfortunately, publications from the recent period in which appropriate EN was used to test the antimicrobial activity of disinfectants and antiseptics in veterinary medicine are scarce. In many publications, the authors either modify ENs by changing the test microorganisms, contact time, and organic load or develop their own methods (in-house, in-field, *in vivo*) for assessing the antimicrobial activity of disinfectants. Perhaps the use of methodology inconsistent with the recommended standards results from low and insufficient knowledge about the quality and antimicrobial specificity of disinfectants and antiseptics.

On the other hand, there is most likely no widespread knowledge about the standards, e.g., ENs, that disinfectants and antiseptics used in the veterinary area must meet. ENs are unavailable online, cannot be copied, and must be purchased from CEN. In particular, one should be aware of how the antimicrobial activity declared by manufacturers needs to be tested to determine the real biocidal potency of these preparations. So far, nine ENs specifically dedicated to veterinary areas have been developed. Besides, there is several other ENs of general application, which may also be relevant in veterinary. In order to promote correct research methodology, we present and briefly characterize the available ENs for the veterinary area in this review.

Determining biocidal activity according to European Standards

Determining bactericidal activity according to ENs. Among the EN Phase 2 standards, two Step 1 standards and three Step 2 standards have been developed to evaluate the bactericidal activity (including

Table I
The European standards regarding the bactericidal activity of chemical disinfectants and antiseptics used in veterinary practices.

Range	EN number	Title	Test bacteria	Reference
Phase 2, Step 1	1656	Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area	*Pseudomonas aeruginosa* ATCC® 15442™, *Proteus hauseri* ATCC® 13315™, *Escherichia coli* ATCC® 10536™, *Staphylococcus aureus* ATCC® 6538™, *Enterococcus hirae* ATCC® 10541™, *Streptococcus uberis* ATCC® 19436™	EN 1656:2019 (2019)
Phase 2, Step 2	14349	Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action	*Pseudomonas aeruginosa* ATCC® 15442™, *Proteus hauseri* ATCC® 13315™, *Staphylococcus aureus* ATCC® 6538™, *Enterococcus hirae* ATCC® 10541™	EN 14349:2012 (2012)
Phase 2, Step 2	16437	Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on porous surfaces without mechanical action	*Pseudomonas aeruginosa* ATCC® 15442™, *Proteus hauseri* ATCC® 13315™, *Staphylococcus aureus* ATCC® 6538™, *Enterococcus hirae* ATCC® 10541™	EN 16437:2014+ +A1:2019 (2019)
Phase 2 Step 2	17422	Quantitative surface test for the evaluation of teat disinfectants used in the veterinary area	*Escherichia coli* ATCC® 10536™, *Staphylococcus aureus* ATCC® 6538™	EN 17422:2022 (2022)
Phase 2, Step 1	14204	Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants and antiseptics used in the veterinary area	*Mycobacterium avium* ATCC® 15769™	EN 14204:2012 (2012)

mycobactericidal activity) of chemical disinfectants and antiseptics applied in the veterinary area (Table I).

The leading standard in Phase 2, Step 1 is EN 1656:2019 (2019), from which the antimycobactericidal effectiveness test was separated to create the EN 14204:2012 (2012) standard. The suspension test described in EN 1656:2019 (2019) is carried out on two products dedicated to surface disinfection and teat antisepsis. The strains of different species used in the assays are selected to represent the species causing the most frequent contamination of inanimate or teat surfaces. Notably, *Proteus hauseri* (former *Proteus vulgaris*) ATCC® 13315™ and *Streptococcus uberis* ATCC® 19436™ strains are not intended to be used in the EN for preparations in the medical area. Also, different loading substances, depending on the intended practical use of the product, are selected in veterinary, as compared to the medical area (He et al. 2024). In the veterinary area, for surface disinfection and low-level soiling, 3.0 g/l bovine albumin (BSA) is recommended, whereas, for high-level soiling, 10 g/l yeast extract with 10 g/l BSA is recommended.

In veterinary medicine, attention is paid to the antisepsis of teats, especially those of cows, sheep, and goats that produce milk. Teats are generally decontaminated before and after milking, and appropriate procedures have been developed to test preparations for this purpose. For pre-milking teat antisepsis, 3.0 g/l BSA is recommended, whereas for post-milking antisepsis, 10.0 g/l milk powder may be used. These loading substances differ from the material used in the medical area, where 0.3 g/l BSA solution under clean conditions and 3.0 g/l BSA solution with 3 ml/l sheep erythrocytes under dirty conditions are used, simulating not only contamination with common organic substances but also blood contamination. The proposed contact time of the preparation with bacteria is vast, from 1 minute to 2 hours for surface disinfection.

In contrast, the contact time for pre-milking teat antiseptics ranges from 30 seconds to 3 minutes, and for post-milking antiseptics, it ranges from 1 to 30 minutes. A neutralizer is added to terminate the antimicrobial activity. The range of acceptable test temperatures is also wide, with 5–40°C for preparations for surface disinfection and 20–30°C for teat antiseptics preparations. The applied product meets the requirements of EN 1656:2019 (2019) if it reduces the number of bacteria by at least 5 $\log_{10}$.

It should be underlined that much attention is paid to teat disinfection, which significantly reduces intermammary infections and mastitis cases. Different suspension methods, disc diffusion assays, and swabbing techniques have been used to assess the antibacterial efficacy. Recntly, Fitzpatrick et al. (2022) analyzed different methods, including EN 1656:2019 (2019) to evaluate teat disinfectant products. The authors noticed that ten teat disinfectants available on the market, containing different active components (lactic acid, chlorhexidine, diamine, chlorine oxide, iodine), were highly effective and caused over 5 $\log_{10}$ reductions of three bacterial strains indicated in EN 1656:2019 (2019) for teat antisepsis after a contact time of 5 minutes

at 30°C. In the same study, laboratory methods, such as the disc diffusion assay and the in-field method as an experimental challenge teat swabbing method, have also been shown to help determine the effectiveness of teat disinfectants, especially against *S. uberis* ATCC® 19436™. However, such high agreement between the results of these three methods was not demonstrated for the *Staphylococcus aureus* ATCC® 6538™ test strain. On the other hand, the experimental challenge teat swab method allowed for the direct evaluation of teat disinfectant products when applied to teat skin. Not compliant with EN 1656:2019 (2019), the in-field method of experimental challenge teat swabbing is the most frequently described method to determine the effectiveness of testing disinfectants in reducing the number of contaminated bacteria (Gleeson et al. 2009; Mišeikienė et al. 2015; Fitzpatrick et al. 2021) or udder infections (Gleeson et al. 2018).

In addition to the classic use of disinfectants, they can also be used in an aqueous environment. Disinfection is one of the methods of preventing the emergence and development of diseases in aquaculture, which is defined as farming fish, mollusks, crustaceans, aquatic plants, algae, and other marine organisms. It covers cultivating fresh- and salt-water populations under controlled conditions. In addition to disinfecting farming and fishing equipment, antimicrobials are used in baths of roe, hatchling and adult aquatic organisms. However, there is no ENs exception for the microorganisms testing in an aqueous environment; only standard-classical strains are recommended, and typical aquaculture pathogens are not included. Taking into account the specificity of the disinfection process in aquaculture and the presence of microorganisms other than those in poultry and mammal populations, Verner-Jeffreys et al. (2009) evaluated bactericidal and virucidal testing standards for aquaculture disinfectants. The authors estimated the bactericidal activity according to the modified EN 1656:2019 (2019), using standard strains of aquaculture pathogens: *Carnobacterium piscicola* ATCC® 35586™, *Yersinia ruckeri* ATCC® 29473™, *Aeromonas salmonicida* subsp. *salmonicida* ATCC® 14174™, and *Lactococcus garvieae* NCIMB 702927, and a contact time of 30 minutes at 4°C. Loading substances dedicated to dirty conditions were applied. The products were tested: a) peroxygen compounds, b) a mixture of peracetic acid and hydrogen peroxide, c) acidic iodophore and d) chloramine T. The neutralization-dilution method was applied. The species *C. piscicola* and *L. garvieae* were the most resistant bacterial strains tested, requiring concentrations between 0.1% and 0.5% of each of the four products investigated to achieve 5 $\log_{10}$ bacteria reduction.

In addition to the standard Phase 2 Step 1 mentioned in EN 1656:2019 (2019), CEN has developed two Phase 2 Step 2 standards for the veterinary area. Preparations dedicated to surface disinfection could be tested on two different surfaces, according to EN 14349:2012 (2012) on smooth, non-porous surfaces and EN 16437:2014+A1:2019 (2019) on porous surfaces. In the first case, bacteria are coated on stainless-steel discs; in the second case, contaminated pieces of poplar wood are used. As in the suspension method EN 1656:2019 (2019), loading substances and testing bacterial strains are recommended. The obligatory contact time is 30 minutes for the first standard EN 14349:2012 (2012) and 60 minutes for the second EN 16437:2014+A1:2019 (2019). An additional contact time may be selected, up to 60 minutes for EN 14349:2012 (2012) and up to 2 hours for EN 16437:2014+A1:2019 (2019). Such an extended contact period is unique in European Standards. In both standards, the test temperatures are the same, with an obligatory temperature of 10°C; additionally, temperatures of 4, 20, or 40°C may be used. The requirements of both standards for reducing the number of bacteria are lower than those in the suspension test EN 1656:2019 (2019). Therefore, the tested product meets the requirements of EN 14349:2012 (2012) and EN 16437:2014+A1:2019 (2019) if it reduces the number of bacteria by at least 4 $\log_{10}$.

To unify the teat disinfection methods and apply an effective disinfection procedure, a particular carrier standard, Phase 2 Step 2, was developed in 2022 to evaluate teat disinfectants used in the veterinary area EN 17422:2022 (2022). As is defined in this standard: "*method applies to teat disinfectants that are used on teat skin without mechanical action as pre-milking and/or post-milking teat disinfectants in veterinary area – i.e., in breeding, husbandry, production, veterinary care facilities, transport and disposal of all animals except when the food chain following death and entry into processing industry*". The product should demonstrate at least 3 $\log_{10}$ (pre-milking disinfectant) or 4 $\log_{10}$ (post-milking disinfectant) reductions of *S. aureus* ATCC® 6538™, and *Escherichia coli* ATCC® 10536™ cells, compared to the water control, when tested at 30°C at a contact time for 30 seconds to 3 minutes (pre-milking disinfectants) or 1 to 5 minutes (post-milking disinfectants). The following substances are recommended: 3.0 g/l bovine albumin for pre-milking disinfectants or 10.0 g/l milk powder for post-milking disinfectants. In this Phase 2 Step 2 standard, synthetic skin is used as the carrier inoculated with a suspension of test bacteria mixed with the loading substance. After the conditioning period, the test surface is immersed in the product for a specified contact time, and subsequently, a neutralizer is added to terminate the antimicrobial activity. The number of surviving bacteria removed from the surface by ultrasound treatment is estimated, along with the number of bacteria on a surface treated with

Table II
The European Standards concerning fungicidal activity of chemical disinfectants and antiseptics used in the veterinary medicine.

Range	EN number	Title	Test fungi	Reference
Phase 2, Step 1	1657	Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area	*Candida albicans* ATCC® 10231™, *Aspergillus brasiliensis* ATCC® 16404™	EN 1657:2016 (2016)
Phase 2, Step 2	16438	Quantitative surface test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action	*Candida albicans* ATCC® 10231™, *Aspergillus brasiliensis* ATCC® 16404™	EN 16438:2014 (2014)

water in place of the disinfectants, and the reduction is calculated. Lu et al. (2023) investigated the antimicrobial activity of a film-forming polyhexamethylene biguanide (PHMB) teat disinfectant using, in addition to the *S. aureus* ATCC® 6538™ recommended by EN 1656:2019 (2019) and EN 17422:2022 (2022), also other strains: *Streptococcus agalactiae* ATCC® 12386™ and *Streptococcus dysgalactiae* subsp. *equisimilis* ATCC® 35666™. The authors used their own methodology to determine the effectiveness of the disinfectant, both using the suspension method and a rabbit skin disinfection test. Finally, it was found that the tested PHMB-based disinfectant had an excellent killing effect on four indicator bacterial strains that colonized the rabbit skin (Lu et al. 2023). However, for this new PHMB-based disinfectant to be introduced to the market as a certified product, it should undergo tests per CEN standards. Furthermore, using synthetic skin in EN 17422:2022 (2022) does not raise ethical concerns, as using rabbits for testing.

Another approach has been developed to address the disinfection of mycobacterial contamination. Mycobacteriosis is a disease of small and large animals, and especially tuberculosis is a severe infection. A special standard, EN 14204:2012 (2012), has been developed to test the mycobactericidal activity of disinfectants and antiseptics in the veterinary area, using a *Mycobacterium avium* subsp. *avium* ATCC® 15769™ strain as the test organism. Notably, for the determination of the mycobactericidal and tuberculocidal activity of preparations used for the disinfection of instruments in the medical area, according to EN 14563:2008 (2008), the CEN recommends the use of two strains, *M. avium* subsp. *avium* ATCC® 15769™ and *Mycobacterium terrae* ATCC® 15755™. It should be underlined that the tuberculocidal activity of a product in the medical area is indirectly determined by its ability to reduce the number of *M. terrae* ATCC® 15755™ cells, and only this single strain is recommended to determine the antimicrobial activity against tuberculosis. Therefore, it seems reasonable to use preparations for disinfecting instruments and equipment used to treat tuberculosis animals, according to EN 14563:2008 (2008). Considering that mycobacteria belong to the kingdom of bacteria, the test conditions, such as contact time, temperature, and organic contamination, are the same as in those in Phase 2 Step 2 standards described above EN 14349:2012 (2012) and EN 16437:2014+A1:2019 (2019). Also, the requirement of EN 14204:2012 (2012) is the same, and the tested product complies with this standard if it reduces the number of mycobacteria by at least 4 $\log_{10}$.

Determining fungicidal activity according to ENs. Fungicidal activity is defined in ENs as the ability of a compound to reduce the number of vegetative yeast-like cells and mold spores. In contrast, pesticidal activity is defined as the ability of the product to reduce only the number of viable cells of yeast-like fungi. Table II shows two standards concerning the fungicidal activity of chemical disinfectants and antiseptics in the veterinary area.

The same fungal strains are used in both suspension (EN 1657:2016 (2016)) and carrier stainless-steel disc (EN 16438:2014 (2014)) standards. For fungicidal activity testing, vegetative cells of *Candida albicans* ATCC® 10231™ and spores of *Aspergillus brasiliensis* ATCC® 16404™ are recommended. For yeasticidal activity testing, only vegetative cells of *C. albicans* ATCC® 10231™ are used. It is worth noting that preparations for teat antisepsis may be tested against only vegetative cells of *C. albicans* ATCC® 10231™. These mentioned fungal strains are also recommended in tests on the fungicidal activity of preparations dedicated to disinfection and antisepsis in the medical area. However, different are selected loading substances added to tests of preparations for medical or veterinary areas when tests are conducted in clean and/or dirty conditions. In the veterinary area, low-level soiling is simulated by adding 3.0 g/l BSA. In contrast, high-level soiling is achieved by adding 10 g/l yeast extract and 10 g/l BSA, irrespective of the standard. In the case of testing the yeasticidal activity of teat antiseptics, supplementation with 10.0 g/l reconstituted skimmed milk is recommended.

The contact times indicated in the standards are different. In EN 1657:2016 (2016), for testing fungicidal and yeasticidal activities, 30 minutes is obligatory, with additional 5 minutes, 1 hour, or 2 hours. Regarding yeasticidal activity, the obligatory contact times are 5 minutes for post-milking preparations and 30 seconds for pre-milking antiseptics, with an additional 1-minute. The obligatory contact time in EN 16438:2014 (2014) is 60 minutes, with an additional 5 minutes, 30 minutes, or 2 hours.

The recommended temperatures for fungicidal activity testing are the same as those in the bactericidal assays: an obligatory 10°C and an additional 4, 20, and 40°C. In the case of teat antiseptics testing, the obligatory temperature is 30°C, with an additional 20°C.

The product meets the requirements of EN 1657:2016 (2016) if it reduces the number of yeast-like cells and mold spores by at least 4 $\log_{10}$. However, a more limited reduction in the number of fungi is required in Phase 2 Step 2 EN 16438:2014 (2014), with only 3 $\log_{10}$.

Antiseptics can play an important role in eradicating topical fungal infections caused by dermatophytes. CEN standards should be used to determine the fungicidal activity of disinfectants. A short contact time between the microorganisms and the fungicide is recommended in this case. Commonly, investigators need to correct a mistake when applying the CLSI guideline (2017), determining the MIC of disinfectants and recommending the obtained value as the recommended concentration of the disinfectant. In the case of the CLSI guideline (2017), the contact time is extended up to 72 hours. For instance, Gomes et al. (2015) investigated the susceptibility of 14 dermatophytes from species *Microsporum gypseum* and *Microsporum canis* isolated from clinical cases of dermatophytosis of cats and dogs to commonly used disinfectants applying the CLSI guideline (2017) rather than the appropriate CEN standard EN 16438:2014 (2014). Chloro-phenol derivate and chlorhexidine digluconate showed fungicidal activity against dermatophytes at concentrations below those recommended by the manufacturer. However, when tested at recommended concentrations, hypochlorite sodium was ineffective against most dermatophytes. Therefore, this was a study of the sensitivity of dermatophytes to commonly used disinfectants and not a determination of the effectiveness of disinfectants against the tested isolates at the concentrations recommended by the manufacturer.

Determining virucidal activity according to ENs. Recently, the interest in and demand for disinfecting virucidal preparations in the medical area have increased significantly due to the COVID-19 pandemic. However, the spread of many viral animal diseases, sometimes of an epidemic character, such as foot-and-mouth disease (FMD), African swine fever (ASF), avian – A/H5N1 or – A/H9N2, swine – A/H1N1 influenza, as well as rabies, can be limited by the use of disinfectant and antiseptic preparations, in line with their appropriate application. Due to the importance of viral animal pathogens and their destruction of agents, methods used to evaluate virucidal activity are highlighted. Regarding the structure of the virus particle, the susceptibility of enveloped viruses (such as ASFV and influenza viruses) to chemical disinfectants and antiseptics is much greater than that of non-enveloped viruses such as FMDV. Table III shows the standards concerning examining the virucidal activity of chemical disinfectants and antiseptics applied in the veterinary area regarding animal breeding, production, transport, and disposal of animals.

The obligatory test organism used in suspension test EN 14675:2015 (2015) is a picornavirus, a non-enveloped bovine enterovirus Type 1 (ECBO – Enteric Cytopathogenic Bovine Orphan Virus) ATCC® VR-248™. It is the model virus for applications such as the disinfection of instruments and surfaces of rooms and boxes for animal accommodation. According to EN 17122:2019 (2019), using stainless-steel discs as carriers for complete virucidal activity testing, the porcine parvovirus NADL-2 strain is recommended. In contrast, only the feline coronavirus Munich strain is used to test the virucidal activity against enveloped viruses. The tests can be conducted under clean and/or dirty conditions, according to the practical application of the product. Low-level soiling is simulated by

Table III
The European Standards regarding virucidal activity of chemical disinfectants and antiseptics used in the veterinary area.

Range	EN number	Title	Test viruses	Reference
Phase 2, Step 1	14675	Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area	Bovine enterovirus Type 1 ECBO ATCC® VR-248™	EN 14675:2015 (2015)
Phase 2, Step 2	17122	Quantitative non-porous surface test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area	Porcine parvovirus strain NADL-2, Feline coronavirus strain Munich	EN 17122:2019 (2019)

adding 3.0 g/l BSA, and high-level soiling by adding 10.0 g/l yeast extract and 10.0 g/l BSA. The composition of soiling substances in both conditions is the same as in the standard EN 14675:2015 (2015). According to the first standard (EN 14675:2015 (2015)), the obligatory contact time is 30 minutes, with additional 1, 5, and 60 minutes. According to the second standard (EN 17122:2019 (2019)), the minimum contact time is 1 minute, with an additional 5, 15, 30, and 60 minutes; the maximum contact time is 120 minutes, which is an unusually long in disinfection according to ENs. The recommended temperature for virucidal activity testing in both standards is 10°C, and temperatures of 4, 20, or 20°C are acceptable. The product meets the requirements of the suspension method if it demonstrates a reduction of the viral titer of 4 $\log_{10}$ or more. The product meets the requirements of Phase 2 Step 2 EN 17122:2019 (2019) if it demonstrates a reduction in the titer of the tested viruses of 3 $\log_{10}$ or more. Notably, to confirm virucidal activity against enveloped viruses, the product shall pass the EN 17122:2019 (2019) with the coronavirus Munich strain only, whereas to confirm full virucidal activity, the product shall pass both EN 17122:2019 (2019) with the porcine parvovirus test strain and EN 14675:2015 (2015) with the bovine enterovirus test strain.

Sometimes, diseases of an epidemic nature make it necessary to kill a large number of sick animals and other animals with close contact, followed by safe disposal of their bodies and potentially contaminated products. Apart from the ethical considerations, such procedures cause severe economic damage to livestock industries and particular farmers, both from direct losses and the suspension of the international trade in animal products. Both the correct testing of the activity of disinfectants according to the indicated CEN standards and the proper use of adequate virucidal disinfection preparations are crucial to reducing the spread of viral diseases in animals. However, only a few studies have evaluated the virucidal activity of disinfectants used in the veterinary field, according to appropriate ENs (Harada et al. 2015; Juszkiewicz et al. 2020; Juszkiewicz et al. 2021; Beato et al. 2022).

Using the modified EN 14675:2015 (2015), Harada et al. (2015) tested several commercially available disinfectants and cleansers, including alcohol-based disinfectants, chlorine disinfectants, quaternary ammonium compounds, alkaline cleansers, and hand soaps against FMDV, considering not only antiviral efficacy but also human and environmental toxicity. After a contact time of 0.5 minutes, the acidic ethanol disinfectants were assumed to be non-toxic and effective for human use. In contrast, the alkaline cleansers were adequate for the control of FMD outbreaks, although they were not recommended for human use.

Many countries approved a list of biocides effective against ASFV, and only authorized virucidal agents should be applied according to the producer's instructions (DEFRA 2021; USDA APHIS 2023; WOAH 2023). However, the question arises of how and according to which guidelines the antiviral activity of these preparations was tested. Also, it is important to know whether international standards, such as the relevant ENs, were used. Preparations with low antiviral activity may not completely inactivate ASFV, and transmission of the viruses will not be totally inhibited. Besides, the proper chemical substance and its concentration but also the pH, temperature, contact time, and the presence of organic substances affect the effectiveness of the disinfection process. In this regard, attention should be paid to the publication of Juszkiewicz et al. (2020), who used modified EN 14675:2015 (2015) and statistical evaluation to investigate the following substances: sodium hypochlorite, sodium hydroxide, formaldehyde, glutaraldehyde, benzalkonium chloride, acetic acid, potassium peroxymonosulfate and phenol, at three concentrations, to evaluate their effectiveness against ASFV. The tests used low and high soiling levels, a contact time of 30 minutes and a temperature of 10°C were used in the tests. The highest activities against ASFV were shown by glutaraldehyde, sodium hydroxide, sodium hypochlorite, and potassium peroxymonosulfate. This virus was also inactivated in strong alkalic or acidic environments. The presence of organic substances inhibits virus inactivation, and therefore, the performance of cleaning procedures before disinfection is crucial to ensure an effective ASFV disinfection process (Juszkiewicz et al. 2020). The same group of authors (Juszkiewicz et al. (2021)) searching for anti-viral compounds of natural origin investigated the activity of 14 plant extracts against BA71V ASFV strain, according to EN 14675: 2015 (2015), taking into account solvent toxicity control and using statistical analysis. This research showed that peppermint extract (1.05%) was effective against ASFV. The remaining 13 plant extracts showed low or moderate activity against ASFV, and high soiling negatively impacted the disinfection effectiveness. Recently, Beato et al. (2022) reviewed reports on the efficacy of compounds and commercial disinfectants against ASFV, comparing methods adopted to assess the virucidal activity described in cited publications and international tests: EN 14675:2015 (2015), ASTM E1053-20 (2020), and OECD (2013) carrier test guidance. The authors did not refer to the EN non-porous surface test for the evaluation of the virucidal activity of disinfectants used in the veterinary area (EN 17122:2019 (2019)). The activity of tested compounds and preparations belonging to nine groups of antimicrobial agents against ASFV was described in detail. The authors also drew attention to biosecurity, which includes a set of measures and

procedures that have the common task of reducing the risk of the infection and spread of the disease.

Verner-Jeffreys et al. (2009) successfully modified EN 14675:2015 (2015) to test aquaculture disinfectants. The authors used an infectious pancreatic necrosis virus (IPNV) Spjarup serotype and disinfectants containing the following active substances: a) peroxygen, b) a mixture of peracetic acid and hydrogen peroxide, c) an acidic iodophore (2.8% w/w available iodine with a surfactant) and d) a chloramine T. The most significant issue was the cytotoxicity of some products, although using a chemical neutralizer or diluting the product could overcome that problem. However, an acidic iodophore disinfectant dilution or sodium thiosulphate did not reduce product toxicity. Modified dialysis was an alternative method for IPNV testing. The modified assay reliably tested the other three products for virucidal activity. The publication of Volpe et al. (2023) deserves great recognition because it assessed, using both applicable virucidal ENs for the veterinary area (EN 14675:2015 (2015); EN 17122:2019 (2019)) the activity of a commercial peroxy-acid biocide against the redspotted grouper nervous necrosis virus (RGNNV) and its reassortant RGNNV/striped jack nervous necrosis virus (SJNNV) strain, which is one of the most dangerous viral pathogens of aquaculture in Mediterranean. The obtained results presented the suitability of the tested biocide for nervous necrosis virus inactivation, being effective under some of the tested conditions.

Methods of airborne room disinfection according to ENs. In this narrative review, attention should also be paid to the recently developed CEN standard EN 17272:2020 (2020), which specifies the methods of testing the biocidal activity of airborne disinfectants spread upon the surface of mounted targets.

Notably, the CEN does not specify in which area appropriate disinfectants should be used; therefore, this standard may also applied in the veterinary area. Veterinary clinics and livestock premises are contaminated with microorganisms that can settle on the surfaces of materials, equipment, objects, and soil and constitute a reservoir of pathogens. The range of the antimicrobial activity of the tested preparations according to EN 17272:2020 (2020) is wide, unprecedented in other ENs for disinfectants and antiseptics testing. The reduction in the counts of the following microorganisms: bacteria (*E. coli*, *Acinetobacter baumannii*, *S. aureus*, and *Enterococcus hirae*), mycobacteria (*M. terrae* and *M. avium*), bacterial spores (*Bacillus subtilis*), yeast cells (*C. albicans*), fungal spores (*A. brasiliensis*), viruses (adenovirus type 5 and murine norovirus), and bacteriophages, is calculated. Three stainless-steel discs as carriers are used for each tested microorganism. The examination is carried out in clean and dirty conditions characteristic of the veterinary area described in Annex C of EN 17272:2020 (2020). Disinfection at two capacities, small (0.25–4 m^3) and large (30–150 m^3) is taken into account. The distance between the device spraying the disinfectant in forms such as gas, vapor, fog, or aerosol and the carriers has also been specified. After the assay, the carriers are moved to recovery liquid, and the analyzed organisms are removed by scraping the disc and then incubated in conditions suitable for the particular microorganism. Subsequently, the recovered organisms are counted, and the reduction is calculated. According to the Standard, tested preparation should reduce bacteria by at least a 5 $\log_{10}$, reduce mycobacteria, spores, and fungi, and reduce virus titer by at least a 4 $\log_{10}$ compared to microorganisms obtained from carriers not exposed to disinfection.

Misawa et al. (2023) investigated the inactivation of nontuberculous mycobacteria (*M. avium*, *Mycobacterium kansasii*, *Mycobacterium intracellulare*, *Mycobacterium abscessus* subsp. *massiliense* and *Mycobacterium abscessus* subsp. *abscessus*) by gaseous ozone treatment. These bacteria present in hospital or veterinary clinic aerosols can cause pulmonary mycobacteriosis in some people, especially those with underlying lung diseases and decreased immunity. It was proved, that gaseous ozone treatment at 1 ppm for 3 hours reduced the number of mycobacteria of all strains by more than 97%. Jiang et al. (2018) analyzed the effects of five disinfectant agents, namely ozone, chlorine, glutaraldehyde, quaternary ammonium salt, and "mixed disinfectant" (containing quaternary ammonium salt, aldehydes, and alcohol in spray form) on different types and quantities of airborne aerobic bacteria in broiler chicken houses. It turned out that the concentrations of bacteria in the empty broiler houses after using different disinfectants were reduced compared to the concentrations measured in a house not disinfected. However, the authors did not provide any numerical data quantifying the degree of microbial reduction after disinfection. Of the five tested agents, the "mixed disinfectant" had the best biocidal effect on the total microbial communities.

Hand hygiene and handrub disinfection according to ENs. One more aspect related to disinfection in the veterinary area should be noted. Hand hygiene related to hand washing and hand disinfection procedures is essential as it reduces the transmission of pathogenic microorganisms and the number of infections. Especially hand disinfection methods have recently been very popular and recommended due to the COVID-19 pandemic. This issue, which draws widespread attention in the medical area, is also crucial in the veterinary area to ensure the safety of people in contact with animals and *vice versa*, as well as the safety of animals in contact with other animals (Verwilghen et al. 2011; Schmitt et al. 2021). Makovska et al. (2024) drew attention to the importance of hand disinfec-

Table IV
The European Standards concerning bactericidal activity testing of antiseptics used in hand hygienic and surgical procedures, which may be applied in the veterinary area.

Range	EN number	Title	Test bacteria	Reference
Bactericidal Phase 2, Step 2	1499	Hygienic handwash	*E. coli* K 12 NCTC 10538	EN 1499:2013 (2013)
Bactericidal Phase 2, Step 2	1500	Hygienic handrub	*E. coli* K 12 NCTC 10538	EN 1500:2013 (2013)
Bactericidal Phase 2, Step 2	12791	Surgical hand disinfection	Normal skin bacteria	EN 12791:2016+A1:2018 (2018)

tion on pig farms in European countries to reduce the incidence of diseases in these animals. Developing relevant standards for testing preparations used for hand hygiene, the CEN does not limit their application to any area. Notably, three ENs should also be applied for preparations used in the veterinary field (Table IV).

EN 1499:2013 (2013) presents hygienic hand washing using water-based preparations. The standard nontoxic *E. coli* K 12 strain is applied on the hands of volunteers and hand washing is carried out according to the classical six-step hand washing scheme. Contact time of the test product is 30–60 seconds. The mean value of the reduction in the release of bacteria obtained with the test product for hygienic hand washing should be statistically significantly greater than that obtained with the comparable ordinary liquid soap. No antibacterial substance is added.

The following two standards concern disinfection by hand alcoholic preparations. In the standard relating to hygienic hand rub EN 1500:2013 (2013), the assay is similar to the above standard EN 1499:2013 (2013), with the difference that more volunteers take part in the test (18–22 versus 12–15) healthy adults. The mean cell reduction value of the bacteria obtained with the hygienic hand sanitiser by rubbing is not significantly lower than that obtained with the reference 2-propanol 60% (v/v), 3 ml 2-propanol rubbed for 30 seconds, repeated once.

Surgical hand disinfection is described in the third standard, EN 12791:2016+A1:2018 (2018). The activity of the product is tested against the resident skin bacteria, not on specified test organisms. The following samples from the hands are taken for bacterial counts: the number of bacterial cells is assessed in the following three steps: a) just after pre-washing the hands with ordinary soap (before hand rub with a test product or reference hand rub), b) immediately after the surgical hand rub with the test product, just before putting on the surgical gloves, and c) 3 hours after surgical hand rub and holding the hands in gloves. The ratio of the resulting values represents the product's antimicrobial activity. Reduction in the microbial count between steps a) and b) corresponds to immediate effect. Reduction in the microbial count between steps a) and b), correspond to immediate effect. Reduction between a) and c) indicates prolonged actions. The reference product is 1-propanol 60% (v/v), and several 3 ml portions of 1-propanol are rubbed into the hands within 3 minutes. Surgical handrub disinfection procedure lasts no longer than 5 minutes and is carried out in a way like in other standards. The antibacterial activity of the tested disinfectant should be non-inferior to that of 1-propanol 60% v/v.

Other methods not compliant with the ENs for the assessment of the antimicrobial activity of veterinary disinfectants

Disinfectants play a leading role in reducing the spread of pathogens among animals and their transfer from animals to humans as well as limiting the infections in animals and humans. Sufficient decontamination of infected environments, facilities, objects, and products protects against animal disease outbreaks. Attention should also be paid to the economic aspect related to animal diseases, especially those of an epidemic. Animal breeders, especially pigs, cattle, poultry, and aquaculture in such cases, may suffer substantial financial losses. In the above situations, effective disinfectants may be crucial to reducing the number of sick animals. As mentioned, CEN has developed 9 ENs for testing disinfectant preparations specifically intended for use in the veterinary area. However, the knowledge of ENs in the veterinary and animal breeding community is limited, as evidenced by the few publications that cite disinfectant tests performed according to ENs. There are, however, a significant number of non-standard procedures, often of an on-field nature, developed for livestock breeders. An additional problem is the use of disinfectants in aquaculture. There is a great difficulty in determining the biocidal effectiveness of preparations added to water and thus diluted.

Severe and highly contagious animal diseases are primarily FMD, ASF, and classical swine fever (CSF)

caused by viruses. These transboundary diseases cause severe production losses and have profound impacts on livestock trade because a single outbreak of FMD or ASF may stop international trade for a certain period (USDA APHIS 2023; 2024). Disinfection of the environment, including roads, contaminated ground parts, and surfaces of animal premises, pens, vehicles, and equipment that come into contact with infected animals, is crucial and of great importance in reducing the spread of diseases. It should be underlined that the viruses mentioned above can persist in the environment, especially on clothes, utensils, utility items, and feed supplies (Krug et al. 2012).

Disinfection in cattle farming. In cattle breeding, the most significant economic losses result from diseases caused by viruses and prions. The FMDV infects cows, goats, sheep, pigs, and other artiodactyl animals. This virus is destroyed by a low or high pH and by disinfectants, which, in their concentrated form, might be toxic but also caustic or corrosive. The possibility of the eradication of FMD by the use of agents inactivating the FMDV has been intensively studied, mainly based on own methods (Krug et al. 2012; Kim et al. 2013; Harada et al. 2015; Bui et al. 2017; Onodera et al. 2023). Krug et al. (2012), prior to the development of ENs, to evaluate the efficiency of porous surface disinfection, applied and dried FMDV and ASFV suspensions on birch wood carriers and exposed them to citric acid and sodium hypochlorite. Based on the results, 2% citric acid effectively inactivated both viruses after 30 minutes of contact time at 22°C, whereas 2,000 ppm sodium hypochlorite inactivated ASFV but did not cause a 4 $\log_{10}$ decrease in the FMDV titer.

Several scientists from institutions in the Far East assessed the virucidal effectiveness of the disinfectants. In a review, Kim et al. (2013) compiled information on 60 active components of disinfectants used in South Korea to eradicate FMD, considering their antiviral activity and human and ecological toxicities. The ecological properties (acute fish toxicity, daphnia, and algae toxicity) and health effects (acute oral, inhalation, and dermal toxicity, as well as the results of the bacterial mutation, chromosome aberration, and micronucleus tests) of the chemicals were summarised. Some disinfectants contained several toxic substances, such as phenol, cresol, xylene, sulfuric acid, formaldehyde, hydrogen peroxide glutaraldehyde, benzalkonium compounds, and methyl benzyl ammonium chloride. According to the authors, almost 90% of the disinfectants were sprayed on the roads, and approximately 10% were used to disinfect cattle sheds and other sites where FMD occurred.

Gabbert et al. (2020) also conducted a study and developed a laboratory method to recover FMDV and ASFV loads from porous concrete coupons. Validating disinfection procedures performed on porous materials like wood, paper, cloth, etc., is challenging. Unsealed concrete coupons were fabricated from industrial sources and carbonated by exposure to 5% CO_2, lowering the matrix pH. This study demonstrated a simple, low-cost and reproducible assay to recover sufficient viral loads from porous concrete coupons to enable quantitative evaluation of disinfectant efficacy.

Water electrolysis has emerged as an interesting approach to finding new effective but non-toxic substances in disinfectants. Bui et al. (2017) using a non-standard method developed by the authors, studied the potential of electrolyzed water for the eradication of FMDV. Although acidic electrolyzed water at pH 2.6 showed virucidal effects, the obtained results were not statistically processed, and the disinfection method was not validated. Recently, Rhee's group developed a test to evaluate the effectiveness of disinfectants against FMDV, with higher safety and improved performance, and investigated the usage as a surrogate the viruses like ECBO and bacteriophage MS2 (MS2), also according to the Korean Animal and Plant Quarantine Agency (APQA) guidelines for efficacy testing of veterinary disinfectants (Rhee et al. 2022). What is interesting in South Korea is that the disinfectants are officially approved when tested according to the APQA guidelines for efficacy testing of veterinary disinfectants. However, the tests are written in Korean, which limits their global application.

The obtained results revealed that the active substances in disinfectants, such as sodium dichloroisocyanurate, citric acid, malic acid, potassium peroxymonosulfate glutaraldehyde and benzalkonium chloride, were effective against MS2 and ECBO viruses, at higher concentrations than against FMDV, confirming their applicability as potential surrogates for FMDV in efficacy testing of veterinary disinfectants.

Looking for an ideal antiviral disinfectant, non-irritating, non-toxic, broad-spectrum, noncorrosive, and safe for humans, animals, and the environment, Kirisawa et al. (2022) evaluated the activity of electrically charged calcium bicarbonate mesoscopic crystals (named CAC-717) against six groups containing 22 animal viruses, most of them causing bovine diseases. Viruses differed in the presence or absence of an envelope, genomic structure (DNA or RNA), and the genomic strand (single or double). The American ASTM E1052-20 (2020) test method demonstrated the virucidal activity of test substances with viruses in suspension. The authors considered CAC-717 a candidate disinfectant for universal viral inactivation, safe for humans, animals, and the environment.

Similar studies were conducted by Sobhy et al. (2024) to find adequate preparations against viruses causing diseases of various animals. Commercial disin-

fectant consisting of 12% glutaraldehyde and 10% quaternary ammonium compounds (containing 7% benzalkonium chloride and 3% other quaternary ammonium compounds) diluted 1:100 (active components applied: 0.12% glutaraldehyde and 0.1% quaternary ammonium compounds) were tested against several single and double-stranded, enveloped and non-enveloped, DNA and RNA viruses, causing infections mostly in cattle, but also in dogs, pigs, and birds (poultry). The preparation dilution method and contact times from 5 minutes to 1 hour were used. Several suitable cell lines were applied for virus cultivation. Percent virus inactivation was calculated by comparing virus titers in disinfectant-treated samples versus negative control. The research method used was not based on EN tests. However, the investigated product could be helpful to in endemic disease control programs on farms, animal shelters, kennels, and veterinary clinics.

Besides viral infections in cattle, which create the most significant problems, bacterial infections in dairy animals, especially cows, also require attention. Gleeson et al. (2009) investigated the effect of six pre-milking teat preparation on lowering the staphylococci, streptococci and coliform count on teat skin prior to cluster application. The number of staphylococci and streptococci on cow teat surfaces was significantly reduced when disinfection products were applied. The use of wipes with disinfectant was particularly effective when physical wiping action and disinfectant application were simultaneously applied. Further research by Gleeson et al. (2018) confirmed their earlier *in vivo* observations. Furthermore, the application of disinfectant to un-cleaned teats may influence the antibacterial effectiveness of the disinfectants.

Mišeikienė et al. (2015) investigated the influence of three pre-milking teat antiseptic solutions on bacterial and fungal contamination of teat skin. Cow teats were swabbed before and after the application of disinfection preparations. Lactic acid and iodine reduced the number of coliforms, coagulase-negative staphylococci, and *S. uberis* to the greatest extent. However, no high effect was found in reducing *Candida* sp. count.

Fitzpatrick et al. (2021) tested the effectiveness of 96 commercially available teat disinfectants distributed in Ireland against bacterial isolates on teat skin. Teat disinfection products were applied directly to cow teats. Swab samples were plated onto three different selective agars to enumerate bacterial counts of streptococci, staphylococci, and coliform. It turned out that the bactericidal activity of the preparations is varied and not all of them effectively eradicate mentioned above groups of bacteria.

As described above, in-field studies of the antibacterial activity of teat disinfectants differ significantly from laboratory tests conducted by EN 1656:2019 (2019) and EN 17422:2022 (2022), where the method is validated, and standard strains are used. A similarity of the standard method EN 17422:2022 (2022) to teat wiping performed in in-field methods is using synthetic skin as the carrier inoculated with a suspension of test bacteria. After conditioning, the test surface is immersed in the disinfectant for a specified contact time, and subsequently, neutralizer is added to terminate the antimicrobial activity. The number of surviving bacteria removed from the surface is estimated, along with the number of bacteria on a surface treated with water in place of the disinfectants, and the reduction is calculated.

When discussing methods of testing disinfectants dedicated to cattle farming, issues with decontaminating proteinaceous infectious particles (prions) should also be mentioned. In humans, prion disease causes severe neurological diseases, including Kuru and Creutzfeldt-Jacob Disease (CJD). Prions can also cause several transmissible spongiform encephalopathies (TSEs) in animals, including bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in deer and elk, scrapie in sheep and goats, transmissible mink encephalopathy (TME), feline spongiform encephalopathy (FSE) and spongiform encephalopathy of exotic ungulates in zoos (Orge et al. 2021; EDQM 2023; EFSA 2023). Prions are long lasting and resistant to disinfection in the environment. No EN method of testing disinfectants for their efficacy against prions has been developed.

According to a review of Alarcon et al. (2021) concerning cleaning and disinfection guidelines and recommendations following an outbreak of classical scrapie, treatment with sodium hypochlorite containing 20,000 ppm free chlorine or 2 M sodium hydroxide for 1 hour is recommended for prion decontamination-based on laboratory experiments. The authors also gathered several actions for cleaning and disinfecting farms against prions recommended by five selected countries (USA, Great Britain, Norway, Iceland, and Australia), along with their estimations of difficulty and cost.

Baune et al. (2023) compared the efficacy of two phenolic disinfectants for prion inactivation. The older one, Environ LpH, which turned out to be insufficiently effective against prions, was composed of ortho-benzyl-para-chlorophenol (6.4%), *o*-phenylophenol (0.5%), hexylene glycol (4%), isopropanol (8%) glycolic acid (12.6%) and p-tertiary-amylophenol (3%). The composition of a new disinfectant, Wex-cide 128, was less complicated: ortho-benzyl-para-chlorophenol (3.03), *o*-phenylophenol (3.4%), hexylene glycol (10–30%), isopropanol (1–5%). The anti-prion activity of the preparations mentioned above was determined against prions derived from four different species – cervid CWD, mouse-adapted scrapi 22L, hamster-adapted scrapi 263K, and human sporadic CJD. Preparation Wex-code

128 highly inactivated the three animal prions but was less effective against human CJD.

Eraña et al. (2020) described the preparation of beads coated with infectious prions applied to different assays commonly used to decontaminate equipment, materials, and surfaces. Different ways decontaminated the coated beads: autoclaved in different conditions, exposed to ultraviolet irradiation, and submerged in different antimicrobial solutions such as 37% chlorine, 1 N sodium hydroxide, 1% Virkon™ (Zotal Laboratories, Spain), and a mixture of 1% sodium dodecyl sulfate, and 0.5% acetic acid. A set of beads was treated with PBS as a negative control. The level of the remaining prions after decontamination was determined according to the special Protein Misfolding Shaking Amplification method. The bead material affected covering beads with prions and the effectiveness of decontamination.

There is a big problem with the disinfection of medical devices – especially prion-contaminated endoscopes after their use on confirmed or suspected prion-infected humans or animals. Kampf et al. (2020) reviewed procedures for prion-contaminated endoscope reprocessing. Different methods are used depending on the decontamination center and country. Sodium hydroxide (1 M) and sodium hypochlorite (10,000–25,000 g/l) solutions are commonly applied, as well as single-use brushes and cleaning solutions. Williams et al. (2019) also investigated the inactivation of prions that causes CWD by sodium hypochlorite. The 40% of sodium hypochlorite after 5-minute treatment effectively inactivated CWD seeding activity from stainless-steel wires and CWD-infected brain homogenates; however, it did not inactivate CWD seeding activity from solid tissues.

Disinfection in pig farming and transport. Particular attention should be paid to disinfection in pig farming because diseases are also associated with substantial financial losses in these animals. Makovska et al. (2024) assessed cleaning and disinfection measures on pig farms in 10 European countries during 2019–2022. While hygiene standards were met on several farms, there is generally a need to improve practices, especially in the areas of hand and boot cleaning between rooms and compartments.

The ASFV is highly contagious and lethal and can cause hemorrhagic infections of animals from the *Suidae* family, affecting pigs of all ages. This virus can be transmitted not only by domestic pigs but also among wild pigs, such as boars, warthogs, bush pigs and feral pigs. Infections are spread orally or nasally via cutaneous wounds and tick vectors (Gallardo et al. 2015). Efficient cleaning and disinfection preparations, as well as their appropriate application, are fundamental for ASFV inactivation and prevention of the spread of the disease and to facilitate repopulation after an outbreak (De Lorenzi et al. 2020; Juszkiewicz et al. 2023). However, Frost et al. (2023) evaluated the efficacy of 24 commercial disinfectants against ASFV without applying the methodology described in the relevant ENs. Preparations containing oxidizing agents (peroxygen, iodophors), as well as formic acid and phenolic compounds, reduced titers of ASFV by over 4 $\log_{10}$ within 30 minutes at temperatures 4–20°C.

Rhee et al. (2021b) studied the activity of acidic electrolyzed water against ASFV and avian influenza virus A/H9N2. The effective free chlorine concentration of acidic electrolyzed water at pH 5.0–6.5 against ASFV under low-level organic soiling and high-level organic soiling conditions was 40 ppm and 80 ppm, respectively, whereas those against avian influenza virus were 60 ppm and 100 ppm, respectively, after 30 min of contact time.

A group of Chinese scientists from Harbin Veterinary Research Institute studied the possibility of using oxidizing agents, such as highly complexed iodine (HPCI) (Pan et al. 2021; Qi et al. 2022) and ozonized water (Zhang et al. 2020), as disinfectants inactivating ASFV under laboratory conditions. However, they did not use a standardized and validated method. 0.25% HPCI completely inactivated the ASFV in 5 minutes, whereas 5% povidone-iodine (PVP-I) required at least 15 minutes to inactivate ASFV (Pan et al. 2021) entirely. Further research allowed a reduction in the concentration of HPCI when used with compounds of organic acids (COAs), which are acidifiers created from short- and medium-chain fatty acids, indicating a synergistic effect in the inactivation of ASFV (Qi et al. 2022). The best inactivation effect was obtained when the ratio of HPCI and COAs was 5:1 and the optimal assay temperature was 25°C. However, the results obtained are difficult to evaluate because an un-validated and non-standardized virucidal activity assay was used. In the third publication in this series, the viral reduction was determined by using an infectivity assay on porcine primary alveolar macrophages and a non-standardized assay (Zhang et al. 2020).

Another critical problem in veterinary severe infections of the gastrointestinal tract of many animal species can be caused by spirochetes of the genus *Brachyspira*. *B. hyodysenteriae*, *B. pilosicoli*, and *B. hampsonii* strains can cause severe mucohemorrhagic diarrhea in pigs. Almost 20 years ago, the effectiveness of disinfectant agents against *Brachyspira* sp. was tested (Corona-Barrera et al. 2004; Lobova and Cizek 2004). Recently, the efficacy of different disinfectant sanitizers against isolates of *B. pilosicoli* was tested by Gómez-Garcia et al. (2022), who investigated eight different commercial disinfectants and 70% ethanol as a control against 10 *B. hyodysenteriae* isolates derived from pig diarrhea outbreaks in Spain and the reference strain *B. hyodysenteriae* ATCC® 31212™. The tests were slightly based on EN 1656:2019 (2019) but with significant modifica-

tions. Sterile feces were used as the loading substance, the contact time between the bacteria and the disinfectant was 30 minutes, and the incubation temperature was 10°C. Dey-Engley neutralizer was used to stop activity of the disinfectant against bacteria. A reduction of at least 5 $\log_{10}$ in the number of bacterial cells of the tested *B. hyodysenteriae* strains was demonstrated, meeting the requirements. Six out of eight disinfectants tested reduced the number of bacterial counts of at least 7 $\log_{10}$. However, peroxygen (49.7% pentapotassium-bis(peroxymonosulphate) bis(sulphate) and organic acids preparation) in 1% of working concentration did not reduce the number of bacteria of 3 out of 11 tested *B. hyodysenteriae* strains, in accordance with EN 1656: 2019 (2019). A similar situation occurred in the case of a preparation containing 5% glutaraldehyde and 4.5% didecyldimethylammonium chloride applied in 1% of working concentration, which was not effective, at least 5 $\log_{10}$ reduction of bacteria was not achieved in the case 1/11 of the tested strains. Very enlightening are described the on-farm studies concerning the cleaning and disinfection effectiveness of two preparations (Gómez-Garcia et al. 2022). The effectiveness of these processes was assessed by the microbiological method and real-time PCR. Although a statistically significant reduction in the number of bacteria was observed after the disinfection process, using real-time PCR, a large number of residual bacteria was still detected on the disinfected surfaces.

Disinfection also applies to animal transport. The problem of the spread of the above-mentioned *B. hyodysenteriae* strains causing diarrhea in pigs was discussed by Giacomini et al. (2018). Using molecular methods, such as multilocus sequence typing and multiple locus variable number tandem repeat analyses, isolates obtained from trucks were characterized. Sampling was performed before and after cleaning and disinfection. After sanitization, bacteria were still found in individual swabs taken from over several hundred swabs. Importantly, trucks transporting pigs from numerous farms play a crucial role in spreading *B. hyodysenteriae* of different genetic profiles, and the efficient disinfection of trucks may reduce the problem.

The efficacy of different cleaning and disinfection procedures to reduce *Salmonella* and other *Enterobacteriaceae* in the lairage environment of a pig abattoir was investigated by Walia et al. (2017). Eight different methods were evaluated, including high-pressure cold water wash alone and followed by detergent and disinfectant preparations with and without rinsing. Swabs were taken from the floor and walls and examined for the presence of *Salmonella* and other *Enterobacteriaceae*. Although the bacterial recovery of these two groups of rods was different, the greatest bacterial reduction was achieved after washing with water, cleaning with detergent and disinfection with a chlorocresol-based preparation.

Disinfection in poultry farming. Hernández (2020) underlines the significance of the cleaning and disinfection of poultry facilities and equipment and describes popular antimicrobial agents and methods to evaluate the contamination level in poultry houses.

The presence of pathogenic bacteria, including multidrug-resistant *E. coli* (Benameur et al. 2023), New Delhi metallo-β-lactamase-producing Gram-negative bacteria (Zhai et al. 2020), and *Campylobacter* spp. (Urdaneta et al. 2023), was detected in facilities related to poultry farming. Ohashi et al. (2022) drew attention to *Salmonella* strains growing in the form of a biofilm. In their study, more than 80% of *Salmonella* strains from farms in Yamagata prefecture in Japan produced biofilms. To establish efficient disinfection protocols on farms, including biofilm destruction, the authors developed *in vitro Salmonella*-contaminated poultry house models by depositing bacteria on stainless-steel and ceramic carriers in created houses. *Salmonella* cells were not efficiently removed from the models even by cleaning with a surfactant at 25 and 65°C and disinfection with quaternary ammonium compound or hypochlorous acid at 25°C. However, the cited publications do not refer to the standards published by the CEN.

Because there are concerns that the use of disinfectants would select for resistance of microorganisms to antibiotics and disinfectants, Maertens et al. (2020) determined and monitored the effect of the repeated use of different disinfectants (formaldehyde, benzalkonium chloride and a mixture of peracetic acid 55 g/l and hydrogen peroxide 220 g/l) on the bacteria antibiotic and disinfectant susceptibility under practical conditions in a broiler farm and in pig pilot farms over a period of 1 year. All tested *E. coli* isolates (n = 67 from the broiler houses and n = 72 from the pig nursery units), obtained after disinfection, remained susceptible to these agents, indicating that the use of disinfectants did not select bacteria for disinfectant resistance under those conditions and time-scale. In their other publication, Maertens et al. (2018) demonstrated a significant association among several parameters of cleaning and disinfection and the hygienic status of the poultry house. The following disinfectant active substances were used: peracetic acid, formaldehyde, chlorine, glutaraldehyde, hydrogen peroxide and quaternary ammonium component. The use of peracetic acid and hydrogen peroxide-based disinfectants had the strongest positive impact on the hygienogram score, emphasizing the type of disinfectant product. Furthermore, other parameters, such as the use of a cleaning solution, a higher temperature, prolonged ventilation and disinfection by a specialist contractor, also have a significant beneficial effect on the hygienogram score,

which may be the basis to reduce the persistence of bacteria in poultry houses.

Another method used by researchers to determine the activity of disinfectants is the time-kill test. Looking for an agent limiting avian favus (dermatophytosis), Thongkham et al. (2022) evaluated the antifungal activity of several chemical disinfectants against arthroconidial and mycelial suspensions of the dermatophyte *Microsporum gallinae* ATCC® 90749™. However, the authors also did not use the tests recommended by the CEN but determined the MIC value of disinfectants and applied the time-kill method to assess fungicidal activity. Ethanol (400 µl/ml), chlorhexidine (97.5 µg/ml), benzalkonium chloride (156.3 µg/ml), glutaraldehyde (1,250 µg/ml), formaldehyde (3,125 µg/ml), phenol (16,000 µg/ml), sodium hypochlorite (1,600 µg/ml) and povidone-iodine (4,000 µg/ml) could eradicate *M. gallinae* arthroconidia by decreasing the number of viable cells in suspension test without organic soil at 30°C, by 5 $\log_{10}$ within 10 min. The issue concerning superficial mycosis caused by *M. gallinae* in poultry investigated Junnu et al. (2021) taking into consideration fact, that standard antifungal treatment can leave disinfectant residues in farm products. So, authors used clove essential oil ointment (3%, w/w) for the treatment of *M. gallinae* infection in chickens. A time-kill assay showed that tested ointment reduced the number of *M. gallinae* ATCC® 90749™ cells by 99.99% (4 $\log_{10}$) within 1 hour.

Not only mold fungi but also yeast-like pathogenic fungi can cause problems in the veterinary area. *Cryptococcus neoformans* is a encapsulated yeast that can cause systemic and cutaneous cryptococcosis in animals as well as humans. These yeasts can infect pigeons and pose a significant risk to public and animal health. Krangvichain et al. (2016) tested the susceptibility of *C. neoformans* strains isolated from pigeon droppings in Bangkok, Thailand, against disinfectants based on sodium hypochlorite, potassium monopersulphate and benzalkonium chloride compounds. The *C. neoformans* cells were resuspended in sterile pigeon feces, and the authors used the time-kill method and different agent concentrations, according to significantly modified EN 1657:2016 (2016). Based on the results, 0.12% benzalkonium chloride rapidly eradicated the tested yeast strains.

The research methodology used by Thongkham et al. (2022) and Krangvichain et al. (2016), based on time-killing tests, undoubtedly reflects to some extent the effectiveness of disinfectants, but it does not maintain all the test conditions included in the ENs.

Avian influenza is a highly pathogenic and contagious viral disease causing substantial mortality of birds and serious economic problems. The stability of virucidal activity of seven disinfectants on the market in Japan were tested under cold and organic contaminations, simulating the environmental surroundings conditions during winter (Khalil et al. 2023). Authors using two-fold serial dilutions of disinfectants tested conclude, that preparation containing ortho-dichlorobenzene (75%) and cresol (7%) is the most effective disinfectant among all tested against avian influenza. Also in this case, no tests were carried out according to the relevant EN. Rhee et al. (2021a) investigated seven representative active substances present in commercial disinfectants used against avian influenza virus in South Korea. They found that when the tests were conducted according to Korean APQA guidelines for efficacy testing of veterinary disinfectants, the minimal virucidal concentrations were as follows: glutaraldehyde 0.01%, sodium dichloroisocyanurate 0.1%, potassium peroxymonosulfate 0.2%, didecyldimethylammonium chloride 0.2%, citric acid 0.4%, benzalkonium chloride 0.4% and malic acid 0.5%.

Disinfection in aquaculture. Bacterial and viral infections are the biggest problem in aquaculture animals (Ina-Salwany et al. 2019; Dayana Senthamarai et al. 2023; Vega-Heredia et al. 2024), although parasites and fungi can also cause infections in these animals (Yanong 2003; Buchmann 2022). *Vibrio* spp. strains are opportunistic human and animal pathogens, found ubiquitously in aquatic environments, where they survive predominantly in biofilm form. de la Peña et al. (2024) tested the efficacy of commercial disinfectant containing: surfactant, potassium peroxymonosulphate, sodium polymetaphosphate, and hypochlorous acid, against *Vibrio parahaemolyticus* and *Vibrio harveyi*, which cause acute hepatopancreatic necrosis disease in the shrimps and significant decline in aquaculture industry, using a non-standard and non-validated method. Tested disinfectant showed positive bactericidal effects at 2,500 ppm (0,25%) and 5,000 ppm (0.5%) depending on tested strains. Mougin et al. (2024) investigated the adaptation and survival of *Vibrio* spp. strains in biofilm form in different concentrations of benzalkonium chloride. The authors point out that misuse and overuse of this agent in seafood industries may result to discharge of persistent benzalkonium chloride residues in the environment, which promotes *Vibrio* bacteria adaptation and the formation of a thick biofilm. It should be noted that commonly used disinfectant, bleaching powder (calcium hypochlorite, chlorine dioxide and sodium hypochlorite), is also used to disinfect water in marine aquaculture (e.g. shrimp farming in canvas ponds) to prevent diseases. The recovery of microorganism population and it composition were examined after disinfectant treatment, indicating that after 16 hours of microorganisms reduction, their number started to recovers to the initial value after 72 hours, what indicates the need for repeated disinfection of water, which undoubtedly affects the health of farmed animals (Tang et al. 2023).

Some viruses can also cause difficult to eradicate or treat infections in aquaculture animals. He et al. (2024) paid attention to *Micropterus salmoides* rhabdovirus (MSRV), which is significant pathogen causing high morbidity and mortality in largemouth bass, leading to high economic losses. Four disinfectants containing, potassium permanganate, trichloroisocyanuric acid, povidone iodine, and glutaraldehyde in the concentrations from 5 mg/l to 500 mg/l for were tested against MSRV up to 30 minutes. Viral nucleic acid was quantified by qPCR and infectivity was tested by challenge assay. The lowest concentration of disinfectant agents was estimated, which significantly reduced the level of viral nucleic acid within 5–20 minutes and had no significant effect on the survival rate of largemouth bass juveniles. It was found that potassium permanganate 5 mg/l applied for less than 24 hours could be used for control of MSRV.

Disinfection of surfaces contaminated by the rabies virus. Discussing in this review various aspects of the study of biocidal activity of disinfectants used in veterinary field, one cannot ignore the danger associated with rabies virus. Rabies is a disease infecting wild mammals, mainly foxes, wolves, raccoons, bats, but also pets, like dogs and cats, which can serve as reservoirs or vectors. The enveloped RNA rabies virus can be dangerous not only to animals but also for humans, especially for people working in a laboratory with this virus. Wu et al. (2017) presented a publication concerning the inactivation of the rabies virus. Virkon™ (1%), which contains sodium dodecylbenzenesulfonate, sulfamic acid, potassium peroxymonosulfate (an oxidizing agent) and inorganic buffers, caused more than a 4 $\log_{10}$ reduction of the rabies virus in culture medium supplemented with 10% fetal calf serum within 1 minute. Isopropyl alcohol at 70% caused a more than 3 log10 reduction of the rabies virus titer within 20 seconds, contrary to ethanol at 70%, which was ineffective. Paraformaldehyde (3% or 4%) with a treatment period of 30 minutes effectively inactivated the rabies virus. However, the method used for antiviral activity testing was neither standardized nor validated, similar to the method applied by Abd-Elghaffar et al. (2016) who investigated the inactivation of the rabies virus by hydrogen peroxide. The author demonstrated that H_2O_2 at 3% caused a complete and irreversible inactivation of the fixed rabies virus when incubated for 2 hours at 2–8°C. Such long contact time is completely not practical for field disinfection.

Conclusions

Disinfectants and antiseptics play a key role in preventing infections not only in humans but also in animals. There are many aspects and conditions of using disinfectants and antiseptics in veterinary medicine and practice. Effective antimicrobial preparations and properly carried out hygienic procedures can guarantee the reduction of the level of contaminating microorganisms. The quality of disinfectants and antiseptics should be guaranteed by normative documents, such as ENs. Appropriate bactericidal, mycobactericidal, fungicidal, yeasticidal or virucidal activity should be achieved in suitable environment. This biocidal effect should be precisely defined and evaluated. Only products meeting the validated methods outlined in normative documents should be used during effective disinfection and antiseptic procedures.

If appropriate ENs are applied for disinfectant tested and the test microorganisms reduction values required by the standards are obtained, we can expect that after using a given disinfectant, the level of pathogenic microorganisms will also be significantly reduced under normal conditions of preparation use, which should prevent the spread of infections.

As we have presented above, despite the availability of ENs, which are validated methods, and the results obtained with these tests can be compared between different centers around the world, performing tests in accordance with ENs is not widespread. Researchers either use modifications of ENs standards, trying to bring the test conditions closer to those encountered in a given environment, changing standard conditions sometimes in significant way, or develop their own methods based on isolates of microorganisms currently causing animal health problems. The characteristics and properties of these microorganisms are not known. The second issue is the availability of EN standards, according to the CEN statement, ENs cannot be copied and distributed. They are not available in the internet, and can only be purchased in CEN-CENELEC Management Centre, Brussels, Belgium, and this is an additional difficulty for potential users. Tests performed according to non-standard methods are not validated, rarely repeated, and the results obtained usually are not statistically analyzed. For this reason, the results obtained using non-standard methods should be considered only as indicative and not binding for a given disinfectant, proving its documented effective antimicrobial effect.

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Ethical statement

No ethics commission approval for this narrative review was required.

Author contributions

ST and AEL designed manuscript; ST, EB and AEL wrote the draft manuscript. ST, EB and AEL edited and reviewed the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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