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# Bestilling vedr.

‣ Biocidaktivstoffer til brug i det veterinære beredskab ved udbrud af alvorlige, virale, smitsomme husdyrsygdomme

## Abstract

When planning disinfection of infected premises, a preliminary cleaning is always required before using chemical disinfectants. Mechanical brushing of surfaces with soaps or detergents is essential for achieving effective chemical decontamination, as most decontamination agents are inactivated by organic materials and grease. The first part of this report (Sections 1 to 4) summarizes different physical and chemical methods of inactivating African swine fever virus (ASFV) and foot-and-mouth disease virus (FMDV) while the second part (Sections 5 to 9) addresses avian influenza virus (AIV) and Newcastle disease virus (NDV) and aims to indicate when a given method or product is recommended for use.

FMDV is a non-enveloped virus and has a protein shell (capsid) surrounding its genome while ASFV is a large and complex virus with both an envelope and a capsid. Thus, these viruses have different physical properties. Both AIV and NDV are enveloped viruses. Soaps and detergents have a virucidal effect on enveloped viruses due to their surfactant properties, emphasizing the advantages of a proper cleaning prior to disinfection. While many disinfection agents are effective against enveloped viruses, the use of Virkon® S is recommended for its high efficacy and ease of use. Exposure for 10 min to a 2% Virkon S solution is recommended for inactivation of AIV and NDV. The same solution is also useful for inactivation of ASFV and FMDV, but with longer exposure times, bearing in mind the significant negative effect of presence of organic material and high pH on the efficacy of Virkon S. Thus, optimal concentrations and exposure times may vary for these four types of virus depending on the matrix of the material and the surfaces to be disinfected.

With regard to disinfection of carcasses and slurry, due to the massive organic load and the need to break down the carcasses, heat, high pH (alkaline conditions), or better, combinations of these, for extended periods of time are recommended.

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# 1 Use of biocidal agents during African swine fever (ASF) outbreaks

## 1.1 Background

African swine fever virus (ASFV) is the only member of the family of Asfarviridae. It is a large (ca. 200 nm diameter) enveloped virus with icosahedral symmetry. Its complex molecular structure consists of four concentric domains: a core (dense structure containing the genome housed within a nucleoid), covered by a thin protein layer (the matrix), enclosed by a lipid envelope (the inner envelope), which is itself surrounded by the capsid that is the outer layer of the intracellular virions. The extracellular virions have an additional outer envelope obtained by budding from the surface of the plasma membrane. Both types of virions are infectious. The genome is a linear, double-stranded DNA molecule, covalently closed at its ends with a size which varies between isolates, i.e. between 170 and 193 kilobase pairs (kbp). It contains about 170 genes (again depending on the strain). The virus replicates within the cytoplasm of cells but there is some evidence for a nuclear involvement as well.

In addition to infecting pigs, ASFV is the only known DNA arbovirus and in Africa the virus is maintained in a cycle involving replication in both soft ticks (*Ornithodorus* spp.) and warthogs (apparently without symptoms). Domestic pigs and wild boar (e.g. in Europe) are highly susceptible to infection by ASFV and transmission between them occurs readily without the involvement of soft ticks. There are at least 24 different genotypes of the virus (distinguished by the VP72 gene). Excursions of the virus outside of Africa have occurred, a genotype I virus spread to the Iberian peninsula in the 1950's and then in 2007 a genotype II virus spread to Georgia. From there the virus spread to Russia, Ukraine and other Eastern European countries before moving eastwards to China and other Asian countries including Thailand, Cambodia, Vietnam and the Philippines. In the west, the virus has spread to some Eastern European countries as well as Germany and recently (2021) to Italy. Limited outbreaks also occurred in Belgium and Czech Republic but these countries have now been declared free again from ASFV. The virus has also recently spread to the Dominican Republic and Haiti in the Caribbean.

Different strains of the virus vary in their pathogenicity, most of the genotype II viruses that have spread widely across the world are highly pathogenic and cause very high (near 100%) levels of case fatality and thus ASFV has major economic importance.

The virus replicates in a range of different tissues including tonsils, spleen, liver and peripheral blood mononuclear cells (PBMCs) and high levels of infectious virus are present within blood. The virus is also present in all secretions/excretions from infected animals. ASFV is normally spread by direct or indirect (e.g. on contaminated materials) contact between animals but it can be transmitted via aerosols over short distances within buildings. However, the practical importance of airborne transmission is uncertain and likely very little.

### 1.1.1 Summary of ANSES opinion on survival of ASFV in suidae and the environment

According to studies reported in the literature, the ASFV is a resistant virus when combined with organic matter. Thus, it can persist in a viable form for several months in the blood and in the corpses (even putrefied) of infected animals, this is at so-called "ambient" temperature ranges (variable according to the seasons and / or the regions and / or the level of burial in the soil where appropriate). The virus remains infectious for much less time, of the order of a few days, in feces (feces, urine). Finally, its survival would be very limited in saliva as well as in the air, especially in humid and / or sunny regions (sensitivity to moisture and light). Information about the survival of the virus in water is very limited and difficult to interpret.

### 1.1.2 Transmission studies conducted by Danish institutions and the Pirbright Institute

It is noteworthy that there have been attempts to explore the transmissibility of ASFV to pigs from a contaminated environment (generated by infection of pigs with the virus and then removal of the infected animals before introduction of new pigs to the infected environment). Studies in Denmark (Olesen et al., 2018) showed that transmission only occurred if the pigs were introduced at 1 day after removal of the infected pigs. On later days (up to 7 days later) no transmission of ASFV was observed. Follow up studies have failed to show transmission even at 1 day after removal of the infected animals. Similarly, at the Pirbright Institute in the UK, transmission was not observed from a similarly contaminated environment even when animals were introduced on the same day as the infected animals were removed (LK Dixon, personal communication). Thus, there seems to be some variance between the known long-term survival of the virus in various matrices (e.g. meat) and the ability of virus excreted into the environment to be transmitted. This clearly has implications for determining the efficacy of disinfection procedures.

## 1.2 Inactivation of ASFV

### 1.2.1 Inactivation at elevated temperatures

Different health agencies all mention inactivation of ASFV by heat, e.g. 60°C for 30 min (EFSA Panel on Animal Health Welfare 2010), 56°C for 70 min or 60°C for 20 min (USDA 2013, OIE 2013), but without specifying the nature of the strain and the matrix considered.

The Danish legislation BEK nr 1001 af 27/06/2018 (Existing) ”Bekendtgørelse om anvendelse af affald til jordbrugsformål” provides different options for obtaining a controlled hygienisation of organic waste, including exposure to elevated temperature conditions for different periods of times. Some of these options include use of thermophilic biogas digesters (EPA, 2018). BEK nr 1001 assumes that all viruses are inactivated following controlled hygienisation, however, manure or slurry from a farm with an ASF outbreak cannot be transported to a biogas plant for treatment due to high contamination risks during transport and handling at the biogas plant.

Montgomery (1921) found that heating of ASFV at 60°C for 10 min completely removed infectivity detected by injection into pigs, while treatment at 56°C for 15 min did not suffice. Plowright and Parker (1967) found half-lives of around 1 min at 60°C and 3-5 min at 56°C, but also found that a small fraction of the virus would survive for 30 min and 2-3½ hrs, respectively, under these conditions. Based on these studies, an opinion by ANSES concluded that several factors seem to influence the time required for virus inactivation, i.e. the starting virus titre, the strain of the virus, the number of passages in cell cultures and the presence of pig serum. This may explain recent findings by Bourry et al. (2018) that showed the inactivation of a virus stock of the Georgia 2007/01 strain was incomplete after heating in a water bath at 60°C for 30 min as demonstrated by culture on alveolar macrophages, although 60°C for 120 min prevented virus multiplication in vitro., However, inactivation was not complete since one of two experimentally inoculated pigs with this heated material became infected.

Recently, preliminary Danish studies of ASFV in cell culture medium (Eagles Minimum Essential Medium (MEM)) dried onto plastic and glass surfaces and kept at 70°C indicated that infectivity of dried cell culture virus is lost within 24 hrs, however, a small fraction of virus may have survived for more days and these results need to be confirmed (K. Tjørnehøj, personal communication).

### 1.2.2 Inactivation under acidic or alkaline conditions

Various health agencies mention a wide range of pH values tolerated by ASFV but without referring to specific experimental work supporting such values. For EFSA, the tolerated pH range varies from 4 to 13 (EFSA Panel on Animal Health Welfare 2010). Both the USDA and OIE mention increased resistance of ASFV in the presence of serum. For example, inactivation occurred at pH 13.4 after 21 hrs in the absence of serum, but required 7 days in the presence of serum (OIE, 2013; USDA, 2013). These figures seem to originate from a study by Plowright and Parker (1967) that showed that ASFV is stable for at least 20 hrs between pH 3.9 and pH 10.1, and that a small fraction of the virus is resistant down to pH 3.1 for at least 20 hrs and up to pH 12.6 for at least eight days.

The Danish legislation BEK nr 1001 af 27/06/2018 (Existing) "Bekendtgørelse om anvendelse af affald til jordbrugsformål" provides different options for obtaining a controlled hygienisation of organic wastes, e.g. treatment with slaked lime (Ca(OH)2) where all material has obtained a pH of >12 for a minimum of 90 days. The time and pH should be measured and documented for all treated material.

### 1.2.3 ANSES report conclusion on inactivation by heat and acidic-alkaline conditions

"In summary, the use of physicochemical methods for the inactivation of ASF virus is not well documented by experimental publications. It appears that heat seems to be the only effective way when the core temperature reaches the necessary threshold, which is variable depending on the matrix and exposure time. Heat has been mentioned as useful for decontaminating slurry but this has only been experimentally verified in one study. The inactivation of the virus in liquid manure must therefore be followed by a verification of its effectiveness taking into account the variations related to the viral titre, the viral strain, the nature of the organic matter present, etc. The inactivation of the virus by alkaline pH does not appear to be very effective and there are no experimental studies to validate the decontamination of different matrices by acidic pH although the virus seems to be sensitive".

### 1.2.4 Inactivation using biocidal products

According to the OIE Technical card for ASFV, the virus is susceptible to ether and chloroform. ASFV is inactivated by 30 min exposure to 8 ppt (parts per thousand) sodium hydroxide; 30 min exposure to between 0.03-0.5% hypochlorite; 30 min exposure to 3 ppt formalin; 30 min exposure to 3% ortho-phenylphenol and generally to iodine compounds (OIE, 2021). The activity and effectiveness will vary depending on the pH, time of storage and organic content.

According to the ANSES opinion from 2019, "there is relatively little scientific data available on the efficacy of biocidal active substances with respect to ASFV and they all come from laboratory studies. In 2013, the USDA proposed a list of US Environmental Protection Agency (EPA) approved products for ASFV and recommended in particular the use of products based on potassium peroxymonosulphate (as in Virkon S). Nevertheless, the test conditions of these products are not indicated in the EPA document ".

Krug et al. (2011) evaluated the effect of three chemicals on pathogens of several important transboundary diseases of animals and found that for ASFV on non-porous surfaces, 10 min treatment with ≥1% citric acid and 500 or 1000 ppm (parts per million) sodium hypochlorite could be used for disinfection, while 4% sodium carbonate was less effective. Extending this work to ASFV dried onto a wooden surface, a material more typically found in the field, Krug et al. (2012) showed that exposure to a higher concentration of citric acid

(2%) for a longer time (30 min) was necessary, while sodium hypochlorite was less effective and even at 2000 ppm would not completely inactivate ASFV.

Krug et al. (2018) investigated the effect of three commercial disinfectants and citric acid for inactivation of ASFV dried onto non-porous (plastic, steel, CO2-treated sealed concrete) surfaces, without and with addition of blood or faeces. This study showed that within 10 min, 1% citric acid inactivated ASFV dried without organic material on stainless steel, while although 600 ppm sodium hypochlorite (pH 10.8) removed 4 log of the virus titre, a small fraction of the virus survived. A third disinfectant, 800 ppm quaternary ammonia (pH 1.8), was effective to inactivate the virus after 10 min exposure, possibly due to the low pH.

However, drying ASFV in a mixture with organic material (swine blood) abolished the inactivating effect of 1% and 5% citric acid, 800 ppm quaternary ammonia, and 600 and 1500 ppm sodium hypochlorite solutions. For virus dried in swine faeces, the effect of 1500 ppm sodium hypochlorite vanished, while 2% citric acid was still effective.

Two % potassium peroxymonosulfate (the main component in Virkon S) was found to inactivate ASFV in liquid blood, but not in dried blood.

Another study of effect of Virkon S on ASFV in MEM dried on carbonated concrete (Gabbert et al., 2020) showed virus inactivation when using 2% and 5% Virkon S for 10 min.

Virkon S 2% is a broad-spectrum disinfectant with a low pH (Virkon S Product Information Sheet (mccaskie.co.uk)). When used correctly, Virkon S 2% is non-toxic for human and animals (Virkon S Pharmaxmin IFU (hattingagro.dk)). It can be used to disinfect stables, tools, footwear, and drinking water. It can also be used to wash animals. It may be administered by different spraying systems, high-pressure cleaning equipment and other means. The supplier should be consulted about whether Virkon S 2% also can be used to disinfect soil, manure or other wastes with a high organic content concentration.

For the Lindholm project, Virkon S 2% solution was chosen for inactivation of ASFV (K. Tjørnehøj, personal communication).

Other studies have shown that exposure for 30 min to 8/1000 (parts per thousand) sodium hydroxide and 30 min 0.03-0.5% hypochlorites were also found effective to inactivate ASFV.

The ANSES opinion summarizes the use of biocidal products as:

“In summary, the scientific literature and other relevant technical documents (ministerial instructions based on OIE recommendations, or EPA recommendations or relevant Belgian authorities) highlight the efficacy of several active substances of interest in association, notified to the biocidal examination program, such as chlorine, aldehydes, ortho-phenylphenol, potassium peroxymonosulphate, iodine, hydrogen peroxide and / or peracetic acid and quaternary ammonium but in conditions of use very varied and not always representative of the conditions of ground”.

The final comment from opinion of ANSES reference no 2018-SA-0237 (published at Maisons-Alfort 4th April, 2019) is:

“The use of physicochemical methods for the inactivation of the ASF virus is not well documented from published experiments. Heat seems to be the only effective way when the core temperature reaches the necessary threshold, variable depending on the matrix and the heating time. Heat has been mentioned as usable for decontaminating slurry but this has only been experimentally verified in a single study. The inactivation of the virus in liquid manure must therefore be followed by a verification of its effectiveness

taking into account the variations related to the viral titre, the viral strain, the nature of the organic matter present, etc.

The inactivation of the virus by alkaline pH does not appear to be very effective and there are no experimental studies to validate the decontamination of different matrices by acidic pH although the virus seems to be sensitive. Experts recommend further investigations on the effect of acid pH for inactivation of ASF virus".

### 1.2.5 Inactivation of ASFV in carcasses and slurry

With regard to carcasses, these should be burnt directly in a controlled process, heated for sufficient time to allow sufficient heat effect in the entire carcass, or broken down to or below the cell-level for the disinfectant to gain access to the virus. The latter two options are combined in both rendering and alkaline hydrolysis where carcasses are dissolved by treatment with a strong alkaline solution and heat, and in most cases also some form of cutting. These combined processes can also easily inactivate ASFV and other viruses, but the individual process need to be validated and monitored to ensure full inactivation.

As for carcasses, viruses in slurry can be inactivated by heat or by long-term treatment at high pH (see section 1.2.2), or by a combination process. Again, the individual process needs to be validated for the virus in question and monitored during the operation.

## 1.3 Summary for inactivation of ASFV

Heat and several biocidal products can be used for inactivation of ASFV.

Virkon S seems to be a good choice based on the reported effects and relatively user-friendly formulation, however, data for use in environments with organic matter is still very limited. Inactivation with other acidic disinfectants could also be explored further.

Thus, when planning disinfection of infected premises, it is important to start with thorough cleaning without the creation of aerosols before disinfection, and to ascertain the safe inactivation of ASFV in the virus-contaminated and dirty liquid waste produced by this cleaning, e.g. by heat treatment.

## 1.4 References for ASFV

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# 2 Use of biocidal agents during foot-and-mouth disease (FMD) outbreaks

## 2.1 Background

Foot and mouth disease (FMD) is caused by a virus infection (see OIE Technical card). The FMD virus (FMDV) is the prototypic member of the Aphthovirus genus within the picornavirus family. It has a single-stranded positive-sense RNA genome of about 8400nt which is enclosed by a protein shell, or capsid, comprised of 60 copies of 4 different virus encoded proteins (VP1, VP2, VP3 and VP4). The VP4 is not exposed on the outer surface of the virus. The virus exists in 7 different serotypes (O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1) and there is little or no cross protection between them. It is the sequence of the exposed capsid proteins, VP1, VP2 and VP3 which determines the antigenic and physical properties of the virus. Serotype O is most common globally and serotype C has not been found outside of laboratories for many years and is probably eradicated. The SAT (Southern African Territories) serotypes are normally confined to southern Africa and the Asia 1 serotype, as its name suggests, is mainly found in Asia but has reached as far west as Greece.

The virus can replicate very rapidly (a life cycle of about 5-6 hrs can be observed in cell culture). During virus replication, the virus generates variants due to an error-prone RNA-dependent RNA polymerase and thus the virus evolves very rapidly (0.5 -1% of the genome changes per year in the field) and this enables analysis of the pathways of virus transmission from farm to farm.

The virus is highly contagious and infects a variety of cloven-hoofed animals including pigs, cattle and sheep. In total about 70 different animal species have been found to be susceptible to the virus but most of these, e.g. deer, probably cannot maintain the infection in the field with the exception of the African buffalo.

In addition to the acute infection, many cattle (about 50%) become persistently infected with the virus. In this state the animals maintain low levels of infectious virus in the oropharynx for months or years without any clinical signs of disease. Pigs do not become persistently infected.

Europe has been free of FMD for over 10 years (since the last outbreak in Bulgaria) but the virus continues to circulate in the Middle East and North Africa and thus there continues to be a high risk of spread into Europe.

The virus is very easily transmitted between animals by direct and indirect routes. Cattle are highly susceptible to the virus whereas pigs appear to require a higher dose to become infected. Pigs exhale large amounts of virus in their breath and are important sources of infection for cattle, thus airborne transmission from pigs to cattle can occur readily. Under certain ideal climatic conditions this airborne spread of virus can occur over extended distances (>100km), e.g. over water.

## 2.2 Inactivation of the FMDV

At cool (4°C) or moderate (17-20°C) temperatures, FMDV can survive for an extended period of time (14-56 days) in swine and cattle slurry (Bøtner & Belsham, 2012; Haas et al., 1995). Thus, following a disease outbreak, it is necessary to apply chemicals, e.g. disinfectants, to ensure elimination of virus infectivity from premises that had infected animals.

### 2.2.1 Inactivation at elevated temperatures

The virus is inactivated by heating at 50°C or above for 1 hr (Bøtner & Belsham, 2012) when in suspension or in slurry. However, when dried on surfaces, the virus can survive for longer times and at higher temperatures. Kristensen et al. (2021) demonstrated the survival of certain FMDV for at least 24 hrs at 70°C when dried on plastic or glass surfaces; but complete inactivation was achieved by two days exposure at this temperature. At room temperature, the virus could survive for a week when dried on surfaces.

### 2.2.2 Inactivation under acidic or alkaline conditions

FMDV is readily inactivated by both alkaline and acidic conditions, thus it rapidly loses infectivity at pH >9.0 or below pH 6.0.

### 2.2.3 Inactivation using biocidal products

A review (Robinson et al., 2016) found that suitable disinfectants for inactivation of FMDV are already approved and pointed to research into their efficacy on various surfaces and conditions relevant to use in field conditions. Krug et al. (2011) evaluated the effect of three chemicals on pathogens of several important transboundary diseases of animals and found that for FMDV on non-porous surfaces, 10 min treatment with ≥1% citric acid, 500 or 1000 ppm (parts per million) sodium hypochlorite, or 4% sodium carbonate could be used for disinfection (Krug et al., 2011). Extending this work to FMDV dried onto a wooden surface, a material more typically found in the field, Krug et al. (2012) showed that exposure to a higher concentration of citric acid (2%) for a longer time (30 min) was necessary, while for sodium hypochlorite not even 2000 ppm would suffice to inactivate FMDV.

A more targeted study into the use of three commercial disinfectants and citric acid for inactivation of FMDV dried onto non-porous (plastic, steel, CO2-treated sealed concrete) surfaces, without and with addition of blood or faeces, was performed by Krug et al. (2018). This study confirmed that within 10 min, 1% citric acid inactivated FMDV dried without organic material (in phosphate buffered saline) on stainless steel, while 600 ppm sodium hypochlorite (pH 10.8) did not completely inactivate the dried virus. A third disinfectant, 800 ppm quaternary ammonia (pH 1.8), was effective to inactivate the virus after 10 min exposure.

Drying FMDV in a mixture with organic material (swine blood) abolished the inactivating effect of 1% and 5% citric acid, 800 ppm quaternary ammonia, and 600 and 1500 ppm sodium hypochlorite solutions. For virus dried in swine faeces, the effect of 1500 ppm sodium hypochlorite vanished, while 2% citric acid was still effective.

Two % potassium peroxymonosulfate (the main component in Virkon S) was found to inactivate FMDV in liquid blood, but not in dried blood.

To overcome the difficulties of inactivating the virus when dried in the presence of organic matter, thorough cleaning prior to disinfection has been suggested as has heat treatment to inactivate the virus in any virus-contaminated and dirty washing solutions. Another study of the effect of Virkon S on FMDV dried on carbonated concrete (Gabbert et al., 2020) showed virus inactivation when using 1%, 2% and 5% Virkon S for 10 min.

### 2.2.4 Inactivation of FMDV in carcasses and slurry

The principles for inactivating carcasses and slurry are as for ASFV (see section 1.2.5).

## 2.3 Summary for inactivation of FMDV

In conclusion, when planning disinfection of infected premises, it is important to start with thorough cleaning without the creation of aerosols before disinfection, and to ascertain the safe inactivation of FMDV in the liquid waste produced by this cleaning. When disinfecting cleaned surfaces, 2-3% Virkon S and 1-2% citric acid for 10-30 min exposures can be used to inactivate the virus, depending on nature of surfaces and efficiency of cleaning. Higher concentrations of the two disinfectants and longer exposure times are necessary for porous surfaces and surfaces that cannot be completely cleaned. Future research is needed to confirm if quaternary ammonia and 4% sodium carbonate solutions could be possible alternatives; in particular the fat-dissolving/emulsifying effect of 4% sodium carbonate could be of value for treatment of porous surfaces that are difficult to clean. Sodium hypochlorite cannot be recommended for inactivation of FMDV in the field setting.

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# 3 Use of biocidal agents during high pathogenicity avian influenza virus (HPAIV) outbreaks

## 3.1 Background

Avian influenza (AI), or more commonly known as avian flu or bird flu, is a disease affecting both domestic and wild birds. The disease is caused by avian influenza viruses (AIVs) which are negative- and single-stranded RNA-viruses. The virus particles contain an envelope which is obtained from the cell plasma membrane at budding. The viruses belong to the *Orthomyxoviridae* family within the *Influenza A virus* genus and can be further categorized into subtypes by their hemagglutinin (HA) and neuraminidase (NA) genes. To this day, 18 HA and 11 NA types have been identified (Sato et al., 2019) in which 16 HA and 9 NA types have been detected in birds (Munster et al., 2007). AIVs of the H5Nx and H7Nx subtypes can be further divided into two groups depending on their pathogenicity, low pathogenicity AIVs (LPAIVs) or high pathogenicity AIVs (HPAIVs). References used in this section are listed in Section 4 combined with those used for Newcastle Disease virus.

Wild water birds belonging to the order Anseriformes (mainly ducks, geese, swans) and Charadriiformes (gulls, terns, shorebirds) are the natural reservoir of LPAIVs. Spillover to domesticated birds with AIVs may occur through direct or indirect contact with infected waterfowl. LPAIV infections in laying chickens can be entirely asymptomatic or cause mild symptoms such as acute respiratory disease and egg production losses. The circulation of LPAIV over time increases the possibility of mutations, which can result in the emergence of a highly pathogenic avian influenza virus (HPAIV) (Abdelwhab et al., 2013). HPAIV causes an acute, severe, fulminating disease in most chickens accompanied by high mortality rates approaching 100% in many gallinaceous birds. Economic losses occur directly from disease and indirectly from loss of trade and restrictions. Additional economic hardship comes with the cost of disease control (flock depopulation and clean up). From wild water birds, AIVs can also be transmitted to other wild and domestic animals. Avian influenza viruses also pose a significant threat to human health as these viruses have been shown to be able to infect humans. As of today, more than 2600 human cases have been recorded (World Health Organization, 2022).

## 3.2 Inactivation of AIV

Prior to the disinfection of poultry premises after a HPAIV outbreak, cleaning the farm is the necessary first step of any decontamination process. Cleaning removes organic matter, salts, and visible soils, all of which interfere with virus inactivation. The physical action of scrubbing with detergents and surfactants and rinsing with water removes substantial numbers of virus particles. If a surface is not cleaned, the efficacy of the disinfection process can be compromised.

### 3.2.1 Physical methods

#### *3.2.1.1 Ultraviolet light and ionizing radiation*

Previous studies have shown that exposure to UV light decreases virus infectivity in comparison to unexposed virus samples (Sutton et al., 2013). The use of sunlight, or an artificial UVB source, as an addition component of a post-outbreak decontamination routine could be implemented at minimal cost. Any residual virus

present on an exposed surface of equipment or fomites may have its infectivity greatly reduced by this treatment, however, the inactivating properties of UVB require direct, unimpeded exposure to the radiation source, which is difficult to ensure in the field. For the disinfection of premises, exposure to UV radiation may therefore not be appropriate as the sole method as it is efficacious only when the surfaces are well cleaned and the source of light is positioned very close to the surfaces to be disinfected (Samberg and Meroz, 1995). It has also previously been shown that ultraviolet radiation light is not able to inactivate AIVs in a timely manner. UV-exposure of 45 min was not sufficient for complete inactivation of a HPAIV H7N3 (Muhmmad et al., 2001).

Ionizing radiation, neutrons and especially gamma photons penetrate into and through most biological and non-biological materials deeper than ultraviolet light or chemical agents (Lowy et al., 2001). It has been demonstrated that gamma photon radiation is the most efficacious in inactivating influenza viruses. Ionizing radiation is widely used in industry for sterilization of laboratory and clinical equipment, of biological reagents and also for preparation of non-infectious antigens (Lowy et al., 2001).

#### *3.2.1.2 Heat treatment*

Heat treatment for the inactivation of AIV-infected premises has been investigated in several studies. Because AIV is not environmentally stable, heating poultry houses may provide an alternative disinfection method.

Low pathogenicity AIV was inactivated in one day at 26.7°C or above. At 10.0°C, 15.6°C and 21.1°C, the inactivation times increased to 2 to 5 days. Highly pathogenic AIV followed a similar trend; the virus was inactivated after one day at 43.3°C and 32.2°C and required two and five days for inactivation at 21.1°C and 10.0°C, respectively (Stephens and Spackman, 2017). These studies document that AIV can be inactivated with heat prior to cleaning a poultry house during outbreak recovery, which will improve the safety for workers from a potential zoonotic infection with AIV and it will reduce the likelihood of lateral spread of virus during clean-up.

### 3.2.2 Chemical methods

A list of disinfectants suitable for the decontamination of farms and equipment is presented in Table 1. The table is based on recommendations from the Animal Health Australia, Australian Veterinary Emergency Plan (AUSVETPLAN), 2022.

#### *3.2.2.1 Soaps and detergents*

This category of chemical agents is recommended for the removal of organic materials and dirt from surfaces to be decontaminated. In addition, they also have an effect on the lipid components of virus particles through their surfactant property. This characteristic makes these agents efficacious disinfectants against all enveloped viruses, including AIVs (Animal Health Australia, 2022a), Newcastle disease virus and coronavirus (Animal Health Australia, 2022b). Further disinfection should follow the use of soaps and detergents.

#### *3.2.2.2 Alkaline conditions*

Within this group of chemicals, sodium hydroxide and sodium carbonate have been extensively used as disinfectants in the food and dairy industry. Alkalis can also be used during cleaning procedures because they have a natural saponifying action on lipids and organic material (Animal Health Australia, 2022a).

An in vitro study showed that the HPAIV H5N1 and the LPAIVs H3N2, H6N1 and H9N2 was inactivated by treatments with alkaline solution (from pH 12.2 up to concentration of 0.1 % at room temperature for two

hours (Seo et al., 2007). Another study revealed that sodium hydroxide was capable of inactivating HPAIV H5N1 at 0.1, 0.2 and 0.3% concentration after only five minutes (Shahid et al., 2009).

#### *3.2.2.3 Acid conditions*

Acids have a high virucidal activity and through the correct choice of acid, or acid mixture, this class of disinfectants can be used for several purposes including liquid effluent treatment, decontamination of structures and for personal use. Inorganic acids inactivate viruses through decreasing pH values, whereas organic acids inactivate viruses also by the interaction of lipophilic structures with membranes of enveloped viruses (Haas et al., 1995). In decontamination practices, two compounds are generally used: hydrochloric acid which is an easily available strong acid, which have a low toxicity index, and citric acid which is a weaker acid available in solid form that can be used safely for personal use and clothing decontamination (Animal Health Australia, 2022a).

The efficacy of a commercial disinfectant belonging to the organic acids category against an LPAIV, A/Carduelis/Germany/72, H7N1 was investigated in the presence and absence of proteins at different concentrations (0.1%, 0.5%, 1%, 2%), at different temperatures (4, 10 and 20°C) and for different contact times (5, 10, 15, 30, 60 and 120 min) (Yilmaz et al., 2004). It was shown that the disinfectant was efficacious at 20°C in suspensions without proteins, but its efficacy was negatively influenced by protein contamination and low temperature. In order to achieve complete virus inactivation at 4°C, it was necessary to extend the contact time up to 120 min (1% concentration). Another study investigated the virucidal efficacy of Clean-Zone®, which contains citric acid (20% w/v), malic acid (10%, w/v) and phosphoric acid (10%, w/v), against AIV (H9N2) (Chae et al., 2018). The disinfectant and AIV were mixed in hard water (HW) and with added organic matter suspension (OM) to mirror situations with inappropriate cleaning prior to disinfection. AIV was inactivated with 200- and 50-fold dilutions of the disinfectant under HW and OM conditions after a virus-disinfectant contact time of 30 min, respectively.

#### *3.2.2.4 Oxidizing agents*

The disinfectant activity of oxidizing agents results from the development of a free hydroxyl radical that oxidizes lipids and nucleic acids (De Benedictis et al., 2007). The efficacy of any oxidizing agent decreases in the presence of organic material. Therefore, surfaces must be properly cleaned prior to disinfection with these agents. Oxidizing agents are corrosive for metals and irritating for mucus membranes, eyes and skin.

One study investigated disinfectants from chlorine and non-chlorine oxidizing agents for their virucidal ability against two Egyptian H5N1 HPAIVs, namely sodium hypochlorite, calcium hypochlorite (bleaching powder), Virkon® S and Peraclean® (Rohaim et al., 2015). The disinfectants were tested individually for effectiveness against HPAI H5N1 for 5, 10, 15 and 30 min. It was revealed that all of these disinfectants were effective when the contact time was increased to more than 15 min and Virkon® S was effective even at a contact time of only five min.

#### *3.2.2.5 Aldehydes*

Aldehydes are organic compounds. Although several compounds in this group have a virucidal effect, only three agents are widely used for disinfection: glutaraldehyde, formaldehyde and formalin (De Benedictis et al., 2007). The mechanism by which viral inactivation is achieved by this group of chemicals is through the alkylation of amino and sulphydrilic groups of proteins and purinic bases. Their efficacy decreases in the presence of organic matter.

The disinfectant effect of glutaraldehyde is achieved at 2%, at an exposure time of 10–30 min. It is chemically stable and mildly corrosive for metals. It has been widely used for chemical sterilization of medical

instruments, however, for large-scale decontamination, it is not recommended due to its cost (Animal Health Australia, 2022a).

Formalin diluted in water is an active disinfectant against many viruses belonging to different families. Formalin is, however, toxic for humans and is a more unstable compound than formaldehyde. One study tested the AIV inactivation effectiveness of formalin at three different dilutions (0.06%, 0.12% and 0.24%) at different incubation times (6, 12, 18 and 24 h). After 6 h, formalin was not able to inactivate AIV at a concentration of 0.06% and 0.12% and at a concentration of 0.24% no virus was detected by virus isolation. At least 12 h exposure time was necessary to inactivate AIV at all the tested concentrations (Muhmmad et al., 2001).

Gaseous formaldehyde can be used to decontaminate air spaces and equipment that must be kept dry. As it is difficult to control optimal conditions of gas distribution (concentration and distribution, time of contact with surfaces, temperature, humidity), gaseous formaldehyde is not considered sufficient to decontaminate a closed air space. Formaldehyde gas must be used with care as it is toxic even at low concentrations. In addition, it can generate dangerous compounds in association with other agents (i.e. potassium permanganate) (Animal Health Australia, 2022a).

#### *3.2.2.6 Alcohol*

Alcohols are efficacious against AIVs and other enveloped viruses. Their efficacy decreases rapidly at concentrations below 50% (v/v). Alcohols are inflammable and damage plastic objects and therefore they are not recommended for wide disinfection of poultry premises, but only for restricted use on persons (hands) and for laboratory decontamination (Animal Health Australia, 2022a).

To determine the inactivation effectiveness of the avian influenza A (H7N9) virus, one study treated two H7N9 viruses with ethanol (Zou et al., 2013). The viruses were completely inactivated after only 5 minutes when treated with ethanol at the recommended concentrations.

## 3.3 Inactivation of virus in carcasses and slurry

With regard to carcasses, these should be burnt directly in a controlled process, heated for sufficient time to allow sufficient heat effect in the entire carcass, or broken down to or below the cell-level for the disinfectant to gain access to the virus. The latter two options are combined in both rendering and alkaline hydrolysis where carcasses are dissolved by treatment with a strong alkaline solution and heat, and in most cases also some form of cutting. These combined processes can also easily inactivate the viruses in question, but the individual process need to be validated and monitored to ensure full inactivation.

Similar to viruses in carcasses, viruses in slurry can be inactivated by heat (Bøtner et al., 2010) or by long-term treatment at high pH, or by a combination process. Again, the individual process needs to be validated for the virus in question and monitored during the operation.

## 3.4 Summary for inactivation of AIV

Avian influenza virus are enveloped viruses. Soaps and detergents have a virucidal effect on enveloped viruses due to their surfactant properties, emphasizing the advantages of a proper cleaning prior to disinfection. While many disinfection agents are effective against enveloped viruses, the use of Virkon® S is recommended for its high efficacy and ease of use. A 2% Virkon S solution for 10 min is recommended for inactivation of avian influenza virus.

# 4 Use of biocidal agents during Newcastle disease virus (NDV) outbreaks

## 4.1 Background

Newcastle disease is a notifiable, WOAH (World Organization of Animal Health) listed diseased. The disease is caused by the virus avian orthoavulavirus serotype 1 (AOaV-1), but is often referred to as its former name avian paramyxovirus serotype 1 (APMV-1). AOaV-1 is an enveloped virus with a single-stranded, negative-sense, non-segmented RNA genome. The virus can infect all avian species, but especially chickens are sensitive to Newcastle disease viruses. The viruses can be divided into two classes; Class I and Class II with genomes sizes of 15,198 nucleotides and 15,186 or 15192 nucleotides, respectively (Czeglédi et al., 2006). Class I consists of only genotype 1, while Class II can be subdivided into 21 different genotypes (Dimitrov et al., 2019).

Only the virulent variants of the virus are referred to as Newcastle disease virus (NDV) and the term Newcastle disease refers to the disease caused by a virulent variant. However, in everyday language, the term Newcastle disease (ND) is often used interchangeably, whether virulent or not. All AOaV-1 strains whether virulent or non-virulent are referred to as NDV is this document. NDV can be further allocated into pathotypes depending on clinical signs observed in chickens. The three pathotypes listed with decreasing virulence are velogenic, mesogenic and lentogenic (Hu et al., 2022). Some authors further distinguishes between viscerotropic velogenic and neurotropic velogenic variants and a fifth pathotype called subclinical (WOAH (OIE), 2021). NDV is spread by aerosols, faeces and other secretions. Clinical signs of the disease include depression, diarrhoea, reduced egg production, thin-skinned eggs and neurological signs such as paralysis and head tilt. Mortality can reach 100% (WOAH (OIE), 2021). The virus is not dangerous to humans, but has in rare cases caused mild eye infections (Keeney and Hunter, 1950). NDV has caused at least four panzootics globally, and Class II genotype VII viruses, which are all predicted to be virulent, are currently endemic in many countries in Asia and Africa, posing a great threat to the poultry industry (Dimitrov et al., 2016). As chickens in particular are sensitive to NDV, it is mandatory by law to vaccinate commercial chickens in a range of countries including Denmark (BEK nr 1391 af 12/12/2019). No NDV outbreaks has been encountered in chickens in Denmark since the implementation of mandatory vaccination, but in 2022 an outbreak occurred in pigeons with a Class II genotype VI strain (Fødevarestyrelsen, 2022).

## 4.2 Inactivation of the NDV

As for AIV, thorough cleaning prior to decontamination is essential in order to ensure a proper inactivation of NDV as the presence of fat, grease and organic material reduces the effectiveness of most disinfectants (Samberg and Meroz, 1995). Therefore, cleaning of surfaces with a detergent is recommended prior to disinfection. The use of warm water is important as disinfectants work best at 20°C (Meroz and Samberg, 1995). All waste material should be decontaminated. Equipment and fixtures should be dismantled for thorough disinfection and fomites such as footwear and clothing should be disinfected or destroyed. As NDV is mostly secreted through faecal matters, surfaces and equipment in contact with faecal matter should be prioritized for destruction or disinfection to reduce the risk of viral spread. NDV has been found to survive in faecal matter and poultry houses for up to 30 days after depopulation of the farm (Fortner, 1952; Kinde et al., 2004). Additionally, viral spread should be minimized by control rodents and wild birds at the farm. In Denmark, all birds situated in an epidemiological unit where NDV is detected must be euthanized (BEK nr

1390 af 12/12/2019). Recommended methods for destruction of animal carcasses include burning or burying (Animal Health Australia, 2022a).

## 4.2.1 Physical methods

### *4.2.1.1 Ultraviolet light and ionizing radiation*

Several studies have demonstrated that ultraviolet (UV) light reduces viral infectivity and UV light—natural or artificial—could easily be implemented as a decontamination tool. Under field conditions, however, it can be challenging to secure direct, unhindered exposure to the radiation source, which is necessary for the inactivating effect of UV light (Deshmukh and Pomeroy, 1969; Ghrici et al., 2013; Sutton et al., 2013). Nevertheless, sunlight is a natural UV light source, and should always be considered in the decontamination planning, as viruses survive longer in damp cool environments that in warm settings exposed to radiation from the sun (Fortner, 1952; Mo et al., 2022).

A continuous UV light exposure has been shown to decrease the aerogenic spread of NDV (Perek and Heller, 1970). In that study, two experiments were conducted where groups of chickens were housed in different houses together with a cage containing four NDV infected chickens. Half of the houses had a continuous UV light exposure and these houses showed a significantly lower mortality than the other half of the houses without UV light exposure after 20-30 days. The environmental load of NDV was not investigated, so it is not clear whether the UV light had a decontaminating effect on the environmental contamination. Another study tested the exposure time and power needed for inactivation of NDV in a laboratory setting and found that a UV light power of minimum 50 mW/cm$^2$ was needed for complete NDV inactivation. Exposure times of 5, 10, 15 and 30 min at a 5 cm distance from the UV light, all had the ability to inactivate 500 HA units of NDV when using 50 mW/cm$^2$ UV light power (Ghrici et al., 2013). Another study found that 69 min of 90 mW/cm$^2$ UVB light exposure, which models solar ultraviolet radiation, reduced the titer of NDV by 1 $\log_{10}$ (90%), when the radiation source was kept 14 cm from the infectious material. These findings emphasize that using UV radiation alone to disinfect a space may not be the best option because the light source needs to be placed very close to the surfaces.

Electromagnetic radiation can be divided into non-ionizing (lower frequency) radiation and ionizing (higher frequency) radiation. UV is a form of non-ionizing radiation, whereas ionizing radiation includes gamma rays, x-rays and neutron radiation (Ravanat and Douki, 2016). Compared with chemical reagents and UV radiation, ionizing radiation has higher ability to penetrate through most biological and non-biological materials. Ionizing radiation is used for sterilization of laboratory and clinical supplies, biological reagents and for food (Animal Health Australia, 2022a). (Farkas, 1998; White et al., 1990). Only one study investigated the amount of gamma radiation necessary to reduce the number of viral PFU/m by 90%. This was found to be 0.52 Mrad (Sullivan et al., 1971). More studies on inactivation of NDV by ionizing radiation are needed, before this method can be recommended. Furthermore, radiation exposure can be harmful to humans and all radiation sources and radioactive materials should be handled with outmost care, making other decontamination methods preferable (International Atomic Energy Agency, 2011).

### *4.2.1.2 Heat treatment*

Inactivation of NDV by heat was measure in both high and low moisture litter (Mo et al., 2022). In low moisture litter, inactivation occurred at 43.3˚C, 37.8˚C, 32.2˚C, 26.7˚C, 21.1˚C and 15.5˚C after 1, 2, 2, 18, 30 and 70 days, respectively. At 10˚C, the virus was still active after 90 days, but was predicted to reach inactivity at day 112. In high moisture, litter inactivation occurred at 43.3˚C, 37.8˚C, 32.2˚C, 26.7˚C, 21.1˚C, 15.5˚C and 19˚C after 1, 2, 5, 18, 26, 60 and 80 days, respectively. Heat inactivation was faster in high moisture litter than

low moistures litter at 10°C to 26.7°C (Mo et al., 2022). Another study also found that the environmental conditions such as moisture had an impact on the persistence of NDV. They found that NDV persisted for 30 days in winter, 14 days in spring and 7 days in summer when housed in poultry houses previously occupied by infected stock (Fortner, 1952).

NDV strains may vary in their sensitivity to heat inactivation (Animal Health Australia, 2021). A study investigating thermostability of several different NDV strains found that heat sensitivity was unrelated to pathotype as the velogenic and lentogenic NDV groups contained strains with both high and low thermostability (Lomniozi, 1975). Adequate heat must be applied during heat inactivation to ensure complete viral inactivation to secure that strains of both high and low thermostability are inactivated. Another study investigating a lentogenic and a velogenic strain found the strains to have very similar thermostabilities. In this study, a 6 $\log_{10}$ viral reduction was found at 59°C and 70°C after 10 and 5.5 min and 16 and 5 min, respectively. At 82.2°C the velogenic and lentogenic strain showed a 8.8 $\log_{10}$ and 6 $\log_{10}$ reduction, respectively, in less than 30 sec (Chmielewski et al., 2011).

## 4.2.2 Chemical methods

Contact times and concentrations for disinfectants suitable for the decontamination of farms and equipment are presented in Table 1. The table is based on recommendations from Animal Health Australia, Australian Veterinary Emergency Plan (AUSVETPLAN), 2022.

### *4.2.2.1 Soaps and detergents*

Soaps and detergents are recommended as the first step of decontamination to remove organic materials, grease and fat, which reduce the effectiveness of most disinfectants (Samberg and Meroz, 1995). Using hot water and scrubbing enhances the cleaning (Meroz and Samberg, 1995).

Soaps are anionic detergents. If a cationic detergent is to be used for decontaminating, soaps and anionic detergents should not be used for cleaning as they may neutralize the detergent. Additionally, they neutralize quaternary ammonium compounds and chlorhexidine (Animal Health Australia, 2022a).

Soaps and detergents are very effective against enveloped viruses such as NDV because of their surfactant property. Commercial detergents containing alkylbenzene sulfonates have been shown to successfully inactivate NDV in concentrations of 1%, 1.5% and 2% with contact times of 5, 10 and 15 min, respectively (Nuradji et al., 2021). Though soaps and detergents are effective on their own, the sole use of these is not recommended (Animal Health Australia, 2021).

### *4.2.2.2 Alkaline conditions*

Alkalis are virucidal and have a natural saponifying effect that make them ideal for cleaning procedures, especially as they work well in the presence of organic matter and fats and can be mixed with soaps and detergents without decreasing their virucidal effect (Jeffrey, 1995; Sellers, 1968). Sodium hydroxide and sodium carbonate are examples of alkalis. NDV is more susceptible to alkalis than to acids (Animal Health Australia, 2021).

### *4.2.2.3 Acid conditions*

Acids are virucidal and by selecting the right acid or acid mixture, it can be used for a variety of purposes, ranging from liquid effluent treatment to structure decontamination and personal use. Acids can be divided into organic and inorganic acids. The virucidal effect of organic acids is achieved by destruction of the lipid membranes of enveloped viruses. Inorganic acids achieve the virucidal effect by lowering the pH value (Haas

et al., 1995; Poli et al., 1979). As inorganic acids have a strong corrosive effect, organic acids such as hypochloric acid and citric acid are typically employed in decontamination procedures. Hypochloric acid is a strong acid with a low toxicity index while citric acid is a weaker acid available in solid form that can be used safely for personal use and clothing decontamination (Animal Health Australia, 2022a).

No available experimental studies have investigated the effect of hypochloric and citric acids on NDV. Nevertheless, it is thought to be effective against NDV due to its well-known impact on enveloped viruses (B. Haas et al., 1995; Jang et al., 2014; Poli et al., 1979). The effect of peracitic acid, an organic acid, was investigated and found reduce the viral titer by 3 $\log_{10}$ (99%) when used at 50%, 100% and 200% of the manufacturers' recommended concentration. The same result was found for contact times of 1 to 10 min (Patnayak et al., 2008).

#### *4.2.2.4 Oxidizing agents*

Examples of oxidizing agents include chlorine, hypochlorite and Virkon S. Oxidizing agents have a low toxicity and have a high virucidal effect, but the effect decreases significantly in the presence of organic material. Therefore, proper cleaning most be implemented before disinfection with oxidizing agents (Animal Health Australia, 2022a). Furthermore, they corrode metals and irritate mucus membranes, eyes, and skin. Although oxidizing agents are sold as a powder that can be applied directly to contaminated surfaces, they are usually prepared as a solution according to the manufacturer's instructions (FAO, 2015).

A study testing the virucidal effect of Virkon S and Sodium hypochlorite on NDV found a reduction of 3 $\log_{10}$ (99%) in viral titer by both oxidizing agents at concentrations of 50%, 100% and 200% of the manufacturer's recommended concentration at all tested contact times (1, 3, 5 and 10 min) (Patnayak et al., 2008).

#### *4.2.2.5 Aldehydes*

For decontamination, three aldehyde agents are widely used: glutaraldehyde, formaldehyde and formalin. Aldehydes are organic compounds. Cleaning of the infected premises prior to decontamination with aldehydes is encouraged as their virucidal effect is decreased by contact with organic compounds.

Glutaraldehyde is effective against all viruses at concentrations of 1-2%. It is used for sterilization of medical instruments, but is often not recommended for large-scale decontamination because of its cost (Animal Health Australia, 2022a; Jeffrey, 1995).

Formaldehyde can be used as a liquid and as a gas. Formalin is a 40% liquid solution of formaldehyde gas that is very effective against all viruses. Formalin is toxic for humans and more unstable that formaldehyde. One study tested the effect of different concentrations of formalin on NDV infectivity when incubated for 16 hours and found that concentrations of 0.04% and higher were needed for inactivation of NDV (King, 1990).

Formaldehyde gas is a very effective disinfectant. Formaldehyde fugimation can be applied to air spaces and equipment that needs to be kept dry, including electrical equipment. However, it is difficult to control the gas distribution and requires that the infected premises can be completely sealed as the gas is toxic and exhibits residual toxic activity. Dangerous compounds can be generated if formaldehyde is combined with other decontamination agents. Unfortunately, no satisfactory alternative to formaldehyde for gaseous decontamination is available (Animal Health Australia, 2022a; De Benedictis et al., 2007; Samberg and Meroz, 1995). The virucidal effect of formaldehyde fumigation on two strains of NDV has been investigated (Ide, 1979). A commercial pesticide fogger delivered formalin (37% formaldehyde) at a flow rate of 40 ml per min and a volume of 36 ml per cubic meter of space. Two cycles of fumigation were applied consisting of 18 hrs and 6 hrs, respectively. A 99% reduction in infectivity was achieved after the first cycle of formaldehyde

fumigation, however, residual infectivity of both NDV strains was detected and the second cycle of fumigation was needed for complete NDV inactivation (Ide, 1979).

#### *4.2.2.6 Alcohol*

Alcohol is effective against NDV due to its well-known impact on enveloped viruses (Kampf, 2018). Alcohol's effectiveness declines aberrantly at concentrations below 50% (v/v) (De Benedictis et al., 2007; Watts et al., 2021). Due to their flammability, alcohols are only advised for restricted personnel use and laboratory decontamination, not for widespread disinfection of poultry facilities (De Benedictis et al., 2007). A study examining the effect of commercially available hand sanitizers against NDV found that all three tested sanitizers were effective in inactivating NDV. In this study, 100µl of the hand sanitizers were applied to hands that had been smeared with 50µl NDV inoculum. The hand sanitizers were rubbed on the hands until completely dried to achieve the virucidal effect (Patnayak et al., 2008).

## 4.3 Inactivation of NDV in carcasses and slurry

With regard to carcasses, these should be burnt directly in a controlled process, heated for sufficient time to allow sufficient heat effect in the entire carcass, or broken down to or below the cell-level for the disinfectant to gain access to the virus. The latter two options are combined in both rendering and alkaline hydrolysis where carcasses are dissolved by treatment with a strong alkaline solution and heat, and in most cases also some form of cutting. These combined processes can also easily inactivate the viruses in question, but the individual process need to be validated and monitored to ensure full inactivation.

Similar to other RNA viruses, NDV in slurry is inactivated by heat (Bøtner & Belsham, 2012) or by long-term treatment at high pH, or by a combination process. Again, the individual process needs to be validated for the virus in question and monitored during the operation.

## 4.4 Summary for the inactivation of NDV

NDV are enveloped. Soaps and detergents have a virucidal effect on enveloped viruses due to their surfactant properties, emphasizing the advantages of a proper cleaning prior to disinfection. While many disinfection agents are effective against enveloped viruses, the use of Virkon® S is recommended for its high efficacy and ease of use. Exposure to a 2% Virkon S solution for 10 min is recommended for inactivation of NDV.

**Table 1: A list of disinfectants suitable for the decontamination of farms and equipment infected with AIV and ND (modified from Benedictis et al, 2006 and Ausvetplan, 2021)**

| Chemical category | Method of action | Limitations | Chemical compound/product | Recommended concentration | Recommended contact time | Recommended use |
|---|---|---|---|---|---|---|
| **Soaps and detergents** | Surfactant propriety against lipidic components | | | | 10 min | Animal housing/equipment, humans, human housing, machinery and vehicles, clothing, aircrafts |
| **Alkalis** | Protein denaturation | The activity increases at high temperatures. Not efficacious at room temperature | Sodium hydroxide | 1% (w/v) | 10 min | Animal housing/equipment, effluent and manure, machinery and vehicles, clothing |
| | | | Sodium carbonate | 4% (w/v) if anhydrous, 10% (w/v) if washing soda | 20 min | Animal housing/equipment, effluent and manure, machinery and vehicles, clothing |
| **Acids** | Inhibition of enzymatic reactions, protein and nucleic acid denaturizing | Can be corrosive | Hydrochloric acid | 2% (v/v) | 10 min | Effluent and manure |
| | | | Citric acid | 0.2% (w/v) | 15 min | Effluent and manure |
| **Oxidizing agents** | Denaturizing activity on lipids and DNA | Corrosive. Inhibited by organic materials and by basic values of pH | Calcium hypochlorite | 5000 ppm | 10-30 min | Animal housing/equipment, human housing, clothing |
| | | | Sodium hypochlorite | 5000 ppm | 10-30 min | Eggs, animal housing/equipment, human housing, clothing |
| | | | Virkon® | 2% (w/v) | 10 min | Animal housing/equipment, humans (not approved for use on skin), human housing, clothing, aircrafts |
| **Aldehydes** | Alchilation of amino and sulphydrilic groups of protein and nitrogen of the purinic bases | Decreasing efficacy in presence of organic compounds. Some aldehydes are corrosive and can also be toxic or unstable | Formalin | 8% (v/v) | 10-30 min | |
| | | | Glutaraldehyde | 2% (w/v) | 10-30 min | Animal housing/equipment |
| | | | Formaldehyde gas | | 15-24 hours | Electrical equipment |
| **Alcohols** | Protein denaturizing in presence of $H_20$ | | Ethanol | 70% (v/v) | 5-15 min | |

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