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Ammonia Disinfection of Hatchery Waste for Elimination of Single-Stranded RNA Viruses ▽

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ABSTRACT

Hatchery waste, an animal by-product of the poultry industry, needs sanitation treatment before further use as fertilizer or as a substrate in biogas or composting plants, owing to the potential presence of opportunistic pathogens, including zoonotic viruses. Effective sanitation is also important in viral epizootic outbreaks and as a routine, ensuring high hygiene standards on farms. This study examined the use of ammonia at different concentrations and temperatures to disinfect hatchery waste. Inactivation kinetics of high-pathogenic avian influenza virus H7N1 and low-pathogenic avian influenza virus H5N3, as representatives of notifiable avian viral diseases, were determined in spiked hatchery waste. Bovine parainfluenza virus type 3, feline coronavirus, and feline calicivirus were used as models for other important avian pathogens, such as Newcastle disease virus, infectious bronchitis virus, and avian hepatitis E virus. Bacteriophage MS2 was also monitored as a stable indicator. Coronavirus was the most sensitive virus, with decimal reduction (D) values of 1.2 and 0.63 h after addition of 0.5% (wt/wt) ammonia at 14 and 25°C, respectively. Under similar conditions, high-pathogenic avian influenza H7N1 was the most resistant, with D values of 3.0 and 1.4 h. MS2 was more resistant than the viruses to all treatments and proved to be a suitable indicator of viral inactivation. The results indicate that ammonia treatment of hatchery waste is efficient in inactivating enveloped and naked single-stranded RNA viruses. Based on the D values and confidence intervals obtained, guidelines for treatment were proposed, and one was successfully validated at full scale at a hatchery, with MS2 added to hatchery waste.

INTRODUCTION

Hatchery waste (HW) is a by-product from the poultry industry consisting of egg shells and nonhatched eggs. HW may contain pathogens such as bacteria, parasites, and viruses (12, 32, 39, 51), so sanitation treatment is needed before its further use in biogas and composting plants. Treatment before use as fertilizer in agriculture is also common practice (18), due to the exposure and risk of disease transmission to wildlife, domestic birds, and other species, including humans, via the feed or food chain.

Hatchery waste disposal is a problem because of the potential presence of opportunistic pathogens, sometimes even of a zoonotic character. Incineration or pasteurization is expensive, and landfill is likely to become less attractive due to stricter environmental European Union regulations, increasing the costs (19). Another option is treatment through liming, which is a common disinfection method for HW in Sweden. However, liming leads to unsuitable working conditions and is technically complicated, e.g., due to the high pH level (>12) and formation of carbonate and phosphate sediments. The global production of poultry meat is increasing, with large-scale production units and geographical regions with dense poultry and human populations, especially in Asia. Therefore, appropriate disinfection of litter, including HW, is important in order to limit the transmission of pathogens to the environment, the human population, and subsequent flocks (19, 25).

Disinfection using ammonia (NH_3) has been studied regarding human feces (46) and bovine manure (31). Ammonia in its uncharged form is toxic to many microorganisms, including viruses (49, 50). Studies indicate that the mechanism of NH_3 inactivation of viruses involves cleavage of viral RNA in intact particles and that single-stranded RNA (ssRNA) viruses are more sensitive than double-stranded ones (8, 47). A number of epizootic poultry diseases are caused by ssRNA viruses, such as avian influenza virus (AIV), which causes avian influenza (AI), and Newcastle disease virus (NDV), which causes Newcastle disease (ND). During outbreaks, infectious virions are present in litter such as feces and unhatched eggs (9, 12, 35, 39, 41, 42), while during subclinical infections, viruses causing milder forms of ND could be present (12). Other important ssRNA viral diseases of poultry include avian infectious bronchitis (IB) and hepatitis-splenomegaly syndrome, caused by infectious bronchitis virus (IBV) and avian hepatitis E virus (AHEV), respectively. The presence of viable AHEV has been demonstrated in chicken eggs (20), but the risk of IBV presence in HW is low (11). However, even if virus is not vertically transmitted, i.e., from the hen to the chicken, poor sanitation practices are generally a problem if the virus is excreted in the feces, which could contaminate the eggs and then infect the hatching chicks (37).

Ammonia disinfection may serve as an adequate sanitation method in cases of viral epizootic disease outbreaks (AI and ND), instead of, e.g., the calcium hydroxide or formaldehyde treatments currently recommended (2). Ammonia disinfection may also be suitable for minimizing the risk of transmission of other highly infectious poultry diseases. However, in order to formulate recommendations on how to process contaminated litter, such as HW, it is important to obtain scientific data on the effect of the disinfectant on the actual material. The kinetics of virus inactivation by NH_3 have been reported for poliovirus and bacteriophage f2 in suspensions (47). The relatively low reaction rate, compared with that of chlorine, makes NH_3 unsuitable for disinfection of wastewater. However, it may be practical for the disinfection of organic material, such as wastewater sludge (13), and therefore might be adequate for HW treatment. The addition of NH_3 also increases the fertilizer value of the HW through the amount of available nitrogen.

In ammonia treatment, unionized NH_3 is the active substance. The equilibrium of NH_3 and its ionized form, NH_4^+, depends on the pH and the temperature and is shifted toward NH_3 when either of these increases. The present study determined the inactivation rates for various types of ssRNA

viruses in HW after addition of increasing levels of NH_3 at different temperatures. The overall aim was to devise full-scale treatment regimes for hatchery waste, especially during disease outbreak situations. The F-specific bacteriophage MS2 was used as a model indicator organism for stable ssRNA viruses, and its reduction in HW was examined to assess NH_3 treatment according to European legislation (3-log reduction; EC 208/2006) for animal by-products (ABP) to be used as source material in biogas and composting plants (18). MS2 has previously been used as an indicator organism for assessing the effect of NH_3 in urine (45).

MATERIALS AND METHODS

Microorganisms. The following relevant and model viruses were used: low-pathogenic AIV (LPAIV), strain A/mallard/Sweden/1174/05(H5N3) isolated at the National Veterinary Institute (SVA, Uppsala, Sweden) (52); high-pathogenic AIV (HPAIV), strain A/turkey/Italy/1387/00(H7N1), (Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy); bovine parainfluenza virus type 3 (BPIV-3), strain 1878/88, isolated at SVA; feline calicivirus (FCV), strain 2280 (ATCC VR-2057); and feline coronavirus (FCoV), strain DF2 (ATCC VR-2004). The bacteriophage used was enterobacteria phage MS2 (ATCC 15597-B1). Table 1 presents an overview of viral properties. Experiments with the HPAIV strain A/turkey/Italy/1387/00(H7N1) were conducted using a biosafety level 3 facility.

Table 1.
Properties of the viruses/bacteriophage used in the study[a]

Propagation of microorganisms. Cultivation conditions are summarized in Table 2. In short, the cell lines were grown to confluence in 25-cm^2 cell culture flasks (Corning, NY) in their respective cell culture medium (CCM), and the virus was inoculated and cultivated to a 80 to 100% cytopathogenic effect (CPE), freeze-thawed once, centrifuged using centrifuge 5702 R (Eppendorf, Hamburg, Germany) for 20 min at 4°C at 2,500 × *g* to remove cell debris, aliquoted, and stored at −70°C until use. The fetal bovine serum (FBS) used tested free of bovine viral diarrhea virus by a sensitive PCR method (16). The MS2 bacteriophage was cultivated according to the standard ISO 10705-1 (3).

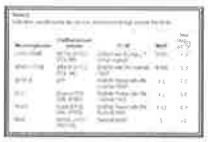

Table 2.
Cultivation conditions for the viruses and bacteriophage used in the study

Analysis of microorganisms. Viruses were analyzed by endpoint titration through the CPE using 96-well plates (Nunc, Roskilde, Denmark) containing the respective cell line essentially according to that used in reference 36 in 10-fold dilutions and by assaying eight 50-μl replicates per dilution. The respective cell line according to those listed in Table 2 was seeded 24 to 48 h before titration, with the longer time used for MDCK in which the method using trypsin according to that described in reference 40 was used. All CCMs for titrations were supplemented with 0.75 mg $liter^{-1}$ of amphotericin B (Fungizone; Bristol-Myers Squibb, Bromma, Sweden). The virus titers after 6 to 8 days were calculated according to Kärber (23) and expressed as the log_{10} 50% tissue culture infectious dose ($TCID_{50}$) in g^{-1} HW. The standard ISO 10705-1 (3) was used to enumerate the MS2 bacteriophage, and the titers were expressed as log_{10} PFU g^{-1} HW.

Hatchery waste. The material used for the disinfection studies was untreated HW, consisting of egg shells and tissue from developing embryos obtained from a commercial hatchery in southern Sweden, and was transported overnight at 2 to 8°C to the laboratory. The HW was minced and homogenized in a Stomacher 80 (Seward, Worthing, United Kingdom) to a particle size of about 5 mm. The same batch was used for the whole study and kept at −70°C until the start of the experiment. This batch had a dry matter content of 60% and a pH of 8.0. HW for the large-scale study at a commercial hatchery at Flyinge in southern Sweden also consisted of egg shells and tissue from developing embryos and had an initial pH of 7.4. This HW was not preminced and was used during normal production at the hatchery.

Experimental design. The homogenized HW was divided into 0.9-g portions in airtight 15-ml Falcon tubes (Becton Dickinson, NJ) and separately spiked with 0.1 ml of the respective microorganism (Table 1) to an initial concentration of between 5 and 7 log_{10} g^{-1}. Ammonia (28% in aqueous solution) (Rectapur; Prolabo, Stockholm, Sweden) was added to a concentration of 0.25, 0.5, or 0.75% (wt/wt), and the tubes were incubated at 5, 14, or 25°C. As controls, spiked HW and CCM (without NH_3) were used. Sampling for microorganism analysis was performed at regular intervals for 0 to 72 h in two separate experiments for each virus. Incubation took place in controlled refrigerators except at 25°C, when a water bath (Grant, Cambridge, United Kingdom) was used. The pH and total NH_3 concentrations were analyzed in duplicate samples initially after 18 h and 72 h of incubation. The pH was analyzed by diluting the sample by 10-fold in distilled water and adjusting it to room temperature, using a SevenEasy pH meter (Mettler Toledo, Schwerzenbach, Switzerland). Total NH_3 (17) was analyzed spectrophotometrically on a Thermo AquaMate (Thermo Electron

Ltd., Cambridge, United Kingdom) using the indophenol blue method (Merck, Whitehouse Station, NJ). The fraction of unionized NH_3 depends on the dissociation constant K_a, which is temperature dependent, according to the following, $K_a = 10^{-(2{,}729.92/T + 0.09018)}$, where T is the temperature in degrees Kelvin. Virus titers were determined by diluting the contents of each tube by 10-fold in CCM supplemented with 10% FBS, except when trypsin was added (H5N3 analyses), followed by extraction for 1 min using a shaking vortex (Vortex Genie 2; Scientific Industries). After centrifugation at 3,000 × g for 10 min at 4°C, the supernatant was gel filtered through Sephadex G-25 columns (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) in CCM to remove NH_3 and other cytotoxic low-molecular-weight substances.

Large-scale study. In order to examine NH_3 inactivation of hatchery waste (HW) under normal conditions, a large-scale trial was performed at Flyinge, Sweden. The mixing tanks were thoroughly rinsed with tap water before the trial to remove every trace of lime (normally used as standard treatment of the HW), and the pH was checked to ensure neutrality. In a vacuum container of about 3 m^3, approximately 0.5 m^3 of HW were spiked with 5 liters of MS2 bacteriophage diluted to 50 liters in tap water using a securely attached hose. Duplicate initial samples were taken from the top and bottom of the tank. A 60-liter volume of 28% NH_3 solution was diluted to 200 liters using tap water and gradually introduced to the HW. During the experiment, duplicate samples were taken at 0, 2, 4, 6, 7, and 24 h from the top and bottom of the tank. Hold control samples were kept in HW with deionized water instead of NH_3 at the same temperature. For each sampling occasion, the ammonia nitrogen, pH, temperature, and MS2 titer were analyzed. Samples for ammonia nitrogen and pH were withdrawn and stored at 4°C until analysis, whereas samples for MS2 analyses were stored and transported frozen.

Cytotoxicity and viral interference assays. To assess the cytotoxicity and the interference properties of the HW regarding virus infection of cells and to set the assay detection limits, cytotoxicity and viral interference assays were performed essentially according to those described in reference 22, as follows. A tube with 0.9 g of HW and 0.75% (wt/wt) NH_3 was extracted and treated as described above. The cytotoxicity of the effluent and the effluent diluted 1:5 were evaluated by inoculating cell microtiter plates using eight 50-μl replicates per dilution and by inspecting the cells daily for the time used in the titration assays. The viral interference assays were performed by titration of the viruses, as described in "Analysis of microorganisms," in the resulting effluent and in the effluent diluted 1:5, and these virus titers were compared to the virus titers obtained using only CCM as the titration medium. In samples in which no virus was detected, the detection limit of the titration assay was calculated according to the Poisson distribution by use of the following, $c = -\ln p/v$, where $1 - p$ is the 95% probability that the aliquot is free of infectious virus ($P = 0.05$), v is the volume tested, and c is the virus concentration (7).

Statistics. A general linear model was used to determine the effects of temperature and NH_3 additions on the reduction of viruses in hatchery waste, with time as a covariate, using the Tukey all-pairwise comparison. Linear regression was used to determine the inactivation rate constant k in different treatments. D values (the time required to reduce the population by 1 $\log_{10}$ [90%]) were derived by taking the reciprocal of k, and a 95% confidence interval of D was calculated from the t distribution of the standard error from the regression analysis. The correlation between MS2 and virus inactivation was determined using the Pearson product-moment correlation. Statistical analyses were performed in Minitab 15 (Minitab Inc., State College, PA). Diagrams were produced in SigmaPlot (SPSS, Chicago, IL).

RESULTS

The gel filtration procedure used in the extraction process was shown to not reduce the virus titers, and the results from the cytotoxicity and viral interference assays showed interference of the undiluted gel-filtered sample for bovine turbinate (BT) and Fcwf cells, where the effluent had to be diluted 1:5. For MDCK cells, the undiluted gel-filtered sample could be used. The detection limit was calculated to be 0.9 $\log_{10}$ g^{-1} HW by large-volume assays analyzing at least 3 ml of each sample. The pH was raised from 8.0 in the untreated HW to 9.2, 9.5, and 9.7 after the addition of 0.25, 0.5, and 0.75% (wt/wt) NH_3, respectively, and the pH remained stable for up to 72 h.

The virus titers were reduced following a linear decay in the ammonia-treated HW, and D values of the different treatments are presented in Table 3. In untreated HW, the ammonia concentration varied by about 10-fold in the two batches studied (Tables 3 and 4), having mean total ammonia concentrations of 0.4 and 2.8 g $liter^{-1}$. This was reflected in the D values of untreated HW, which ranged from 6.7 to 37 h at 14°C (Table 3). Thus, some inactivation of all viruses studied took place under these conditions. The D value was further significantly decreased by NH_3 addition ($P < 0.001$) and increasing temperature ($P < 0.001$). FCoV was the most sensitive virus, with D values of 1.2 and 0.63 h after addition of 0.5% NH_3 at 14 and 25°C, respectively. The corresponding figures for the other viruses were 1.3 and 0.67 h for BPIV-3, 2.1 and 0.78 h for LPAIV H5N3, 3.0 and 1.4 h for HPAIV H7N1, and 2.0 and 1.3 h for FCV (Table 3). The final virus reductions were >4.2 (LPAIV), >5.7 (HPAIV), >4.6 (BPIV-3), >4.7 (FCV), >5.0 (FCoV), and >5.0 (MS2) $\log_{10}$ g^{-1}, depending on the initial virus titer used. The detection limit under these conditions was reached after 3 to 24 h (viruses) and 18 to 72 h (MS2).

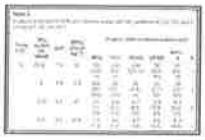

Table 3.

D values of viruses in CCM and hatchery waste with NH_3 additions of 0.25, 0.5, and 0.75% (wt/wt) at 5, 14, and 25°C

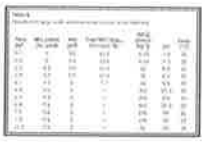

Table 4.

Results from large-scale ammonia treatment trial at the hatchery

Like the viruses, the MS2 bacteriophage was significantly affected by higher temperature ($P < 0.001$) and NH_3 concentration ($P < 0.001$). The *D* values were higher than those for the viruses, as follows: 7.7 and 2.3 h after the addition of 0.5% (wt/wt) NH_3 at 14 and 25°C, respectively. Addition of 0.75% ammonia decreased the *D* value to 5.3 and 1.3 h at 14 and 25°C, respectively (Table 3). The Pearson product-moment correlation on the *D* values showed a significant correlation ($P < 0.001$) in inactivation rates between the viruses and the indicator organism, with correlation coefficients between 0.801 (MS2/BPIV-3) and 0.977 (MS2/HPAIV) (data not shown). The *D* value for MS2 was always higher than those for every virus in all treatments (Fig. 1).

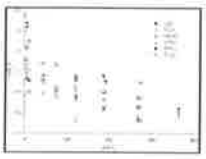

Fig. 1.

Log_{10} *D* values (h) of ssRNA viruses as a function of ammonia concentration (mmol kg^{-1}) in hatchery waste.

The volume and the mean titers of MS2 on each sampling occasion in the large-scale ammonia treatment trial at the hatchery are shown in Table 4, while the inactivation rate of MS2 in HW, together with the ammonia concentrations of the top samples during the trial, are shown in Fig. 2. As the volume of HW varied through new additions (Table 4), the MS2 concentration is expressed in total log_{10} $tank^{-1}$. The detection limit was 5.9 log_{10} PFU $tank^{-1}$. At a time of 6 h, NH_3 had been added to about 0.6% (wt/wt), resulting in 214 and 353 mmol kg^{-1} for the top and bottom samples, respectively. The inactivation rate of MS2, calculated by regression analysis, was 1.3 log_{10} per hour at a temperature of 19 to 22°C. The control with HW and water did not show any significant reduction during the 24-h experiment.

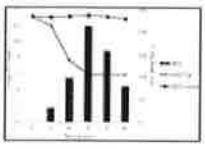

Fig. 2.

MS2 in hatchery waste, expressed as the number of PFU in the storage tank, as a function of time after addition of ammonia. After 6 h, MS2 levels fell below the detection limit ($10^{5.9}$ PFU $tank^{-1}$). The calculated ammonia concentration (mmol kg^{-1}...

DISCUSSION

This study investigated the virus inactivation capacity of NH_3 on a selected panel of viruses causing important animal diseases. All viruses tested were more rapidly inactivated by increasing the concentration of NH_3 added to the hatchery waste at each individual temperature studied.

HPAIV H7N1 is a notifiable disease (14), and since H7 vaccines are known to fail to provide cross-protection (42), there is a risk from infected materials such as HW. HPAIV H7N1 was the most resistant of the enveloped viruses studied here, with *D* values about 75% higher than those for FCoV and BPIV-3 after 0.5% NH_3 addition at 14 and 25°C. LPAIV H5N3, which is classified as "low-pathogenicity notifiable avian influenza" due to its mutation rate in poultry (14), was the next most resistant of the enveloped viruses studied, except at 5°C. The difference in NH_3 resistance between these two influenza viruses might be a consequence of different fatty acid compositions of the envelope (6), affecting virion permeability to molecules such as NH_3. Similarly, it has been shown that virulent NDV has fatty acid content dissimilar to that of avirulent NDV (5). NDV is excreted in large amounts in feces (1) and may also be present in eggs (12, 35). BPIV-3, a virus in the same virus subfamily (*Paramyxovirinae*) as NDV, was almost as sensitive as the coronavirus in the present study (FCoV). However, at 5°C, BPIV-3 had higher *D* values than AIV and FCV (Table 3). Since treatment of HW in Sweden is normally conducted at ambient temperature, this is an important finding. Infectious bronchitis is one of the most important infectious diseases of poultry, leading to great economic losses all over the world. As IBV titers are high in feces during the acute phase of infection (21), the virus could contaminate eggs or be spread by ingestion of feces or contaminated water. Due to the risk of fecal-oral transmission, litter must

be taken care of and disinfected properly. Ammonia treatment may serve as an adequate disinfection method considering the fast inactivation of FCoV, which was the most sensitive of the viruses tested. It was used as a model for IBV since they are from the same genus (*Coronavirus*) and thus share common physicochemical properties.

The enveloped viruses tested in this study were readily inactivated by addition of 0.25% (wt/wt) NH_3, so this treatment may be an adequate sanitation method in the event of outbreaks of avian influenza, Newcastle disease, or infectious bronchitis. For NDV, however, treatment at 5°C may not be applicable due to the lower inactivation rate, with higher uncertainty in the inactivation kinetics for BPIV-3 (Table 3). In this case, a longer time period would be needed.

In a recent survey performed in Europe, 89.7% of chicken flocks tested positive for AHEV (33), which can cause hepatitis-splenomegaly syndrome. AHEV can give rise to subclinical infections (4) and may therefore be present in HW despite the absence of symptoms. However, AHEV cannot be cultured, and therefore, FCV was used as a model for inactivation, as also suggested by Martens and Bohm (28), as the two viruses were formerly classified in the same virus family. However, AHEV is now classified as a single member of the *Hepeviridae* virus family (29). *D* values were higher than those for enveloped viruses (Table 3), and in cases of hepatitis-splenomegaly syndrome, extended exposure of HW to NH_3 treatment might be required.

The fact that MS2 was more tolerant to all treatments (Fig. 1) makes it an excellent indicator of ssRNA virus reduction during NH_3 treatment. MS2 and the viruses were affected by NH_3 in similar ways, as confirmed by the correlation analysis. Furthermore, MS2 is harmless to humans and animals and is easy to propagate to large volumes of high-titered virus. Thus, MS2 can be used to validate full-scale treatment of hatchery waste, which was the objective in the full-scale trial. MS2, added to 2 m^3 hatchery waste, which was treated with NH_3 to up to 214 mmol kg^{-1}, was inactivated at a rate of 1.3 $\log_{10}$ h^{-1} ($D = 0.77$ h), i.e., three times higher in the full-scale process compared with the that in the present bench-scale process. Some differences in temperatures, pH values, and ammonia concentrations prevent direct comparisons with the bench-scale study. However, the inactivation was faster in the full-scale experiment, probably as an effect of NH_3 being introduced to the HW during vacuum mixing, accelerating NH_3 diffusion and resulting in faster inactivation.

To achieve the intended target of 3 $\log_{10}$ reduction (18) with a high degree of certainty, addition of 0.25% (wt/wt) NH_3 (~10 liters of 28% NH_3 [aq.] m^{-3} material) should be followed by storage for 2 days at 14°C. After addition of 0.5% NH_3 (~20 liters of 28% NH_3 [aq.] m^{-3} material), the storage time can be shortened to 31 h. In comparison, the use of 5% (wt/wt) quicklime on hatchery waste tested using the picornavirus ECBO as a standard resulted in a recommended treatment time of 3 to 7 days at ambient temperature and pH 12 (34). If NH_3 treatment is used for material falling below European Union regulations in ABP, a reduction in *Ascaris* ova is required, as it is a chemical treatment (18). However, studies have shown high efficiency of NH_3 under similar conditions and concentrations for *Ascaris suum* in human feces (30). Furthermore, the effect of NH_3 on the inactivation of other viruses needs to be validated. For example, in another study (48), reovirus (double-stranded RNA) was not as sensitive to NH_3 as ssRNA viruses. At 24°C, pH 9.5, and an NH_3 concentration of 313 mmol $liter^{-1}$, three types of polioviruses, two coxsackieviruses, and an echovirus were inactivated at >6 $\log_{10}$ in 24 h ($D < 4$ h), whereas the reovirus was inactivated at <2 $\log_{10}$ ($D > 12$ h) in the same amount of time. Thus, if viruses other than ssRNA viruses pose a risk in exposure to treated hatchery waste, the effects reported here need to be validated.

Breakage of RNA as the mechanism by which NH_3 inactivates viruses was supported by this study, considering the principle that the larger the genome, the more sensitive the virus. Since NH_3 is the active component, it is important that it is not lost as gaseous emissions, and therefore, treatment must take place in a closed container. The present study was performed in tubes for 72 h, with pH and NH_3 measured after 18 h and 72 h. A stable pH and no or minor NH_3 losses were recorded. The easiest way of controlling NH_3 losses is by measuring the pH online, or after treatment, as a control to ensure the hygiene quality of the end product. Larger pieces of material such as embryos and culled chicks that can be present in the HW (15) should not constitute a problem, since NH_3 diffuses easily through biological material (50). However, longer treatment times might be needed if the material is not preminced. Ammonia-treated HW is best used as fertilizer, as, e.g., composting would lead to ammonia emissions (44). To avoid major ammonia losses, field application must be performed close to the soil, using hose spreaders, with direct or subsequent incorporation into the soil (31). As the addition of NH_3 to HW increases the fertilizer value, and the cost of ammonia disinfection is equivalent to that of lime, which means that large-scale usage would be favorable due to both the fertilizer value and the lower pH of the treated material (31).

In conclusion, the present study indicates that NH_3 treatment of hatchery waste is efficient in inactivating enveloped and naked ssRNA viruses. During an epizootic outbreak situation, e.g., of HPAIV, the advice would be to add NH_3 to a concentration of 0.25% (wt/wt) at a temperature of >14°C and store it for 3 days or add NH_3 to a concentration of 0.5% (wt/wt) and store it for 2 days. Under normal conditions, based on MS2 inactivation, our recommendation is to add 0.25% (wt/wt) NH_3 and store it for 2 days at >14°C or to add 0.5% (wt/wt) NH_3 and store it for 31 h minimum. However, to comply with European Union regulations on ABP (EC 208/2006), the effect of ammonia on parasites and double-stranded viruses needs to be validated. Considering the risk of epizootic and zoonotic outbreaks, a constant and serious threat to the poultry industry and human health all over the world, it is very important to develop strategies to handle and control outbreak situations. The disinfection strategies described here provide useful support to other measures, such as stamping out or mass vaccination, in the control of infectious diseases.

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FOOTNOTES

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