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Original article

Survival rate of Suid Herpesvirus (SuHV-1, Aujeszky's disease virus, ADV) in composted sewage sludge

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Abstract

Sewage sludge constitutes a source of valuable biogenic raw materials, but it is a carrier of many pathogenic microorganisms and viruses. Subjected to an effective sanitization by means of the process of composting, it is suitable to use in agriculture as fertilizers. The aim of this study was to observe the survival rate of Suid Herpesvirus under the influence of the temperature alone (water bath) as well as in sewage sludge subjected to the process of composting (pile). The samples were taken at different time intervals, and the virus titres were determined. The viruses survived considerably longer under laboratory conditions: at 30°C as long as 21 days, at 40°C – 93 hours, and at 50°C – less than an hour. In the compost pile, in spite of the lack of the thermophylic phase, the total survival time of the viruses ranged from 34 to 44.5 hours, which indicates the vast importance of other physicochemical factors, apart from the temperature, contributing to virus inactivation.

Key words: Suid Herpesvirus, SuHV-1, Aujeszky's disease virus, ADV, sewage sludge, compost

Introduction

Beside pathogenic bacteria and parasite eggs, municipal sewage can contain many viruses (Paluszak et al. 2003a,b). Those most frequently found in it include adenoviruses, Coxackie viruses, enteroviruses and many others (Haas 1986). The spectrum of viruses shows large regional, seasonal and even daily fluctuations (Irving 1982). In the case when animal sewage flows down to the treatment plant, sewage sludge deriving from the slaughterhouse can also contain viruses occurring in farm animals. Rotaviruses, enteroviruses, Suid Herpesvirus and others are of great importance for epizootic reasons (Pejsak and Markowska-Daniel 1996). Enteroviruses are particularly often isolated from the slurry, and their titre can range from 10^3 to 10^6 $TCID_{50}$/ml (Strauch 1988).

As the process of sewage treatment does not cause the total elimination of viruses, some of then can get to soil and ground water, posing a threat to the environment (Strauch 1991, Straub et al. 1994, Olszewska et al. 1999). To minimize the hazards, sludge should

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be subjected to sanitization processes which will allow their safe use in the agriculture. An effective method of sludge utilization is the process of composting, during which oxidation of organic compounds occurs accompanied by a strong increase in the temperature of the composted material. Since in the Polish literature there are no data concerning the effect of processes of sludge composting on Suid Herpesvirus, we decided to observe the effect of meso- and thermophylic conditions on the inactivation rate of the virus both in the laboratory and technical scale. This allowed monitoring of the processes and, consequently, the elimination of virus transmission into the compost produced.

Materials and Methods

The virulent reference NIA-3 strain of Suid Herpesvirus (titre 9.5 log $TCID_{50}$/ml), obtained from the State Veterinary Institute in Pulawy was used in the study. The effect of the process of composting on the virus survival was examined in a compost pile, with the biomass composed of fermented sewage sludge and a mixture of straw and sawdust. Viruses before introducing into the waste biomass were adsorbed on special filters (the Filter-Sandwich method). 1 ml of the virus suspended in phosphate buffer of pH 6.5 was placed on the nylon membrane Zetapor (Cuno Inc.) with the thickness of 500 μm and pore diameter of 0.45 μm. Membranes were placed in polycarbonate sacks with pore diameter of 0.015 μm (Infiltec GmbH), which prevented penetrating virus particles beyond the carrier. Additionally, each carrier was placed in a plastic and metal net for protection against mechanical damage, and located at three levels of the pile (top, middle, bottom). The experiment was carried out in the winter period.

The effect of the temperature alone on the survival rate of viruses was observed under laboratory conditions. The virus suspension was diluted with a medium (Eagle Minimal Essential Medium – EMEM) with pH 7,2 in a ratio of 1:10, and then it was introduced into Eppendorf tubes and placed in the water bath with a temperature of 30, 40 and 50°C. Samples from the water bath and the piles were removed at set time intervals. Viruses were collected from membrane carriers by means of the action of ultrasounds, and their titres were determined using permanent porcine kidney cell line SK-6 (Kasza et al. 1972).

The analysis was done with the microplate method and to multiply the cells the Eagle's medium supplemented with 7% foetal calf serum (FCS) and antibiotics was used. Virus suspensions in the solution was diluted in the medium (EMEM) at logarithmic progression from 10^{-1} to 10^{-8}. To determine the titre of strains they were placed in 4 microplate wells each in a volume of 50 μl and supplemented with 100 μl cells' suspension in the growth medium (with 7% FCS as above). As a control microplate wells containing 50 μl of maintenance medium (without FCS) each were supplemented with uninfected SK-6 cells suspended in Eagle's growth medium (100 μl/well). Incubation was conducted at 37°C in an atmosphere with 5% CO_2. Infected cultures were observed after 4 and 5 days of incubation under the inverted light microscope. SuHV-1 titres were determined with the Kärber method (1931) and presented as $\log_{10}TCID_{50}$/ml. The results were verified and subjected to statistical analysis.

Results

Effect of various temperatures on average titres of Suid Herpesvirus placed in the water bath was presented in Table 1. A considerable relationship between the virus survival rate and the temperature was observed. At the highest temperature (50°C) viruses were eliminated in less than 1 hour. Virus particles were definitely more slowly inactivated at lower temperatures. Namely, at 40°C the complete inactivation occurred after 4 days, whereas at 30°C on 14th day the virus titre still amounted to 3.05 $\log TCID_{50}$/ml. Thus, the longest theoretical time of survival of AD viruses was at the lowest temperature, since it was almost 21 days, whereas the shortest at 50°C – less than an hour

Table 1. Effect of various temperatures on average titres of Suid Herpesvirus under laboratory conditions.

Temperature [°C]	Time	Virus titre ($\log_{10}TCID_{50}$/ml)
30	0	9.5
	5	6.05
	8	5.8
	14 (days)	3.05
	21	0
40	0	9.5
	12	9.3
	24	8.8
	30	7.3
	36 (hours)	5.05
	60	4.55
	72	1.55
	96	0
50	0	9.5
	15	4.8
	30 (min)	3.05
	45	2.8
	60	0

Table 2. Regression lines describing the survival rate of Suid Herpesvirus in various temperatures under laboratory conditions.

Temperature (°C)	Regression equation	Total survival time of viruses	Decrease in virus titre
30	y= -0.43x + 9.04	x = 21.0 days	0.43 $log_{10}TCID_{50}$/day
40	y= -0.11x + 10.23	x = 93.0 hours	0.11 $log_{10}TCID_{50}$/hour
50	y= -0.14x + 8.23	x = 58.8 min	0.14 $log_{10}TCID_{50}$/min

Table 3. Average titres of Suid Herpesvirus [$log_{10}TCID_{50}$/ml] adsorbed on filters in different places of the compost pile.

Location of samples	Range of temperatures (°C)	Time (hours)				
		0	6	12	18	24
		Virus titre ($log_{10}TCID_{50}$/ml)				
Top	36-48	4.55	3.05	2.55	1.8	1.55
Middle	32-40		3.8	3.05	2.8	2.05
Bottom	26-33		2.8	2.05	1.8	1.8

Table 4. Regression lines describing the survival rate of Suid Herpesvirus in different places of the compost pile.

Location of samples	Regression equation	Total survival time of viruses (hours)	Decrease in virus titre ($log_{10}TCID_{50}$/hour)
Top	y = -0.1208x + 4.15	34.35	0.12
Middle	y = -0.1x + 4.45	44.5	0.10
Bottom	y = -0.1083x + 3.9	36.01	0.11

(Table 2). A decrease in the titre of virus suspension in the compost pile was presented in Table 3. It proceeded the fastest at the top layer – after 18 hours a decrease in titre by 3 log was observed, whereas at the bottom layer the elimination by the same order took place after 24 hours. Viruses at the middle layer survived for the longest time.

During the process of composting some differences occurred in the course of temperatures in individual places of the pile (Table 3). However, the full thermophylic phase did not develope in any of them, since the highest recorded temperature amounted to 48°C. In spite of that, a decrease in the virus titre was similar and amounted on average 0.1 $log_{10}TCID_{50}$ during an hour and the total time of their survival fluctuated between 34.35 and 44.5 hours (Table 4).

Discussion

The main factor eliminating viruses in the environment is an increased temperature. It can affect the survival rate of viruses through various mechanisms, including protein denaturation, nucleic acid damage or dissociation of a virus capsid (Yeager and O'Brien 1979). In the present study, a visible effect of an increased temperature on the rate of a decrease in Suid Herpesvirus titre was observed under laboratory conditions. At 30°C, daily decrease in titre amounted to 0.43 log_{10}, whereas at 40°C within an hour a decrease in titre by 0.11 log was observed. At 50°C, in turn, a decrease in titre by 9.5 log_{10} occurred after 1 hour.

A similar relationship between a decrease in the survival rate of Suid Herpesvirus and an increase in temperature occurs under anaerobic conditions. The study by Bierman et al. (1990) indicated a decrease in the virus titre by more than 5 log after 16 weeks in cattle slurry at 20°C and only by 3.25 log_{10} after 26 weeks at 4°C. Bøtner (1991) still isolated the virus from pig slurry stored at 5°C after 15 weeks, whereas at 20°C only after about 2 weeks. Under mesophylic conditions the survival rate was shortened to 5 hours, whereas at 55°C the virus was not isolated as early as after 10 min. Fast elimination of the virus under mesophylic conditions was also observed by Winter (2002). In slurry subjected to anaerobic fermentation at 51.5°C a decrease in virus titre after 20 minutes amounted to 3 log_{10}, whereas its complete elimination occurred after 30 minutes. Under mesophylic conditions the survival rate of the virus was longer. After

18 hours its titre fell by 4.3 $\log_{10}$. The survival rate of Suid Herpesvirus in composted carcases of dead piglets was studied by Garcia-Siera et al. (2001). Piglets with a weight of 10-15 kg infected nasally with the SuHV-1 were slaughtered and placed in a compost piles. On 7th, 14th and 35th day of composting animals brains were analysed for the presence of virus particles. The temperature of the pile on 8-9 day of composting fluctuated within the range 51.7-52.2°C, remained as such 14th day and then it started to decrease. The virus was not isolated as early as after 7 days of the process. These findings are in accordance with the observations by Morrow et al. (1995) and Paluszak et al. (2010), who reported that the temperature during composting effectively and promptly eliminates PRV from the environment. Davies and Beran (1981) indicated that at 37°C in a pH of 6-8 a decrease in the virus titre amounts to 0.6 $\log_{10}$ daily.

Besides an increased temperature, microorganisms of the biomass can also negatively affect the viruses in the compost pile. Some bacteria of the genera *Klebsiella*, *Vibrio* and *Flavobacterium* isolated from natural water exhibit antiviral properties (Girones et al. 1989). The study by Deng and Cliver (1995) showed that the survival rate of viruses in crude sewage was considerably shorter than in those treated in an autoclave, which suggests that biological activity is also an important factor limiting the virus survival rate in the environment. These observations are in accordance with the results of the present study, where a faster elimination of SuHV-1 was shown in the compost pile than in the suspension closed in the Eppendorf and subjected to the action of the increased temperature alone. During 24 hours, depending on placing of the carrier, the virus titre decreased by 2.45-2.75 $\log_{10}$ in the middle and lower layers and by 3 $\log_{10}$ in the upper layer with the highest temperature. From the calculated regression lines it comes that the survival rate of the virus ranged from 34.35 to 44.5 hours.

The results of the study show that the process of composting during which the thermophylic phase did not fully develop is also an effective method for the inactivation of Suid Herpesvirus. This may indicate the impact of factors other than the temperature of the composting process contributing to SuHV-1 inactivation.

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