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Original article

Survival of BPV and Aujeszky's disease viruses in meat wastes subjected to different sanitization processes

Z. Paluszak[1], A. Lipowski[2], A. Ligocka[1]

[1] Department of Microbiology, University of Technology and Life Science, Bernardyńska 6/8, 85-029 Bydgoszcz
[2] Państwowy Instytut Weterynaryjny, Zakład Chorób Świń, al. Partyzantów 57, 24-100 Puławy

Abstract

The effect of composting and anaerobic fermentations under meso- and thermophylic conditions (37° and 55°C) on the survival of bovine parvovirus (BPV) and Aujeszky's disease viruse (ADV) in meat wastes has been examined in this study. Viruses were adsorbed on filters and introduced into carriers which were made of meat fragments of different sizes and bones or in the form of suspension they were introduced into the biomass in the course of processes of waste treatment. Carriers were removed at appropriate time intervals and virus titres were determined. The thermoresistant parvovirus survived for the longest time during mesophylic fermentation (almost 70 days), slightly shorter during composting (7-9.5 days depending on the type of carrier) and for the shortest time – at 55°C (46-76 hours). Its inactivation rate was the fastest in a suspension, slower in meat and bone carriers. ADV inactivation proceeded considerably faster, as compared with BPV. Its active particles were not detected as early as in the 30th minute of thermophylic fermentation, the 6th hour of mesophylic fermentation and at the first sampling time during composting (at the 72nd hour). Total survival time ranged from 50 min to 13 hours. All the tested technologies enabled the effective elimination of ADV and on average twofold decrease in BPV titre. From the study conducted it follows that of both viruses, the BPV should be applied for validation processes of methods used in meat waste processing, particularly if this refers to methods where higher temperature is the factor inactivating pathogens.

Key words: Aujeszky's disease virus, bovine parvovirus, sanitization, meat wastes, composting, fermentation

Introduction

Meat waste management constitutes an important ecological problem in all the member states of the European Union. Rich in organic matter, which determines their high manurial value, meat wastes can at the same time contain numerous pathogenic micro-organisms, including viruses (Ligocka and Paluszak 2008, Nørrung and Buncic 2008). Bovine parvovirus (BPV), bovine enteroviruses (ECBO), herpes-, or adenoviruses are isolated the most frequently (Rutishauser et al. 1984, Biermann et al. 1989). For the epidemiological reasons, only meat wastes which have been previously subjected to sanitization, e.g. by

Correspondence to: Z. Paluszak, e-mail: paluszak@utp.edu.pl

means of composting or meso- or thermophylic fermentation, can be used for agricultural purposes. The necessary conditions which must be met in the course of processing are set up by EU Regulation No. 1774 (2002). In case those processes are disturbed, or there is no effective control, products obtained as a result of their treatment may contribute to the contamination of water, soil and plants. Viruses introduced into the environment along with organic fertilizers can be adsorbed on soil particles and then transferred in deeper layers where they can survive for months (Straub et. al. 1993). The survival of two viruses – Aujeszky's disease virus (ADV) and bovine parvovirus (BPV) – has been tested in this study in order to estimate which of them could serve as an indicator virus in validation processes of particular waste treatment technologies intended for their agricultural use. Both viruses are resistant to environmental factors, although their pathogenic properties are different (Lipowski and Pejsak 1996, Pejsak and Truszczyński 2006). Aujeszky's disease virus has an envelope and it retains its virulence in urine and liquid manure up to 3 weeks in summer and to 10-15 weeks in winter. It is resistant to drying. Bovine parvovirus, in turn, does not have an envelope and is characterized by a high resistance to high temperatures (it survives at 56°C for one hour), chloroform, natrium desoxycholate, or low pH values. Infectious properties of BPV under natural conditions and its role in pathogenesis of cattle diseases have not been fully explained. Nevertheless, due to its high thermoresistance, it is taken into account, besides ECBO viruses, calici- and circoviruses, as a model virus serving for determination of catering waste treatment effectiveness.

The content of viruses in organic wastes is determined considerably less frequently than other pathogens due to the lack of simple methods for their isolation. In most cases, virus suspension is introduced into the waste biomass, and then a decrease in virus titre is determined. The process, however, is technically complex (Lund and Nissen 1983). That is why in this study we used either filters with adsorbed viruses or their suspension in Eppendorf tubes, from which the isolation of viruses was simpler than from all the biomass infected.

The aim of this study was to compare the inactivation rate of ADV and BPV introduced into meat wastes subjected to different sanitization methods: composting and fermentation under meso- and thermophylic conditions. In case of properly conducted technological processes, the products obtained will not pose an environmental hazard and could be applied in agriculture.

Materials and Methods

Three organic waste treatment technologies were tested in terms of the effectiveness of inactivation of BPV and ADV: composting in a closed rotation bioreactor, mesophylic fermentation (37°C) in an agricultural biogas-works and thermophylic fermentation (55°C) in an experimental mini-reactor on a semi-technical scale. In presented study, different kinds of waste biomass were used. For composting it consisted of the stomach content, fat, trims and blood of pigs (60%) and of sawdust (40%). For anaerobic fermentation either under mesophylic or thermophylic conditions the waste biomass used consisted of pig slurry (70%), pig stomach content and trims (8%), and corn silage (22%). Prior to introduction into the waste biomass, the viruses were adsorbed on special filters (the Filter-Sandwich method). One milliliter of suspension of BPV and ADV with a titre of, respectively, 5.8 log $TCID_{50}$/ml and 3.55 $TCID_{50}$/ml in phosphate buffered saline (PBS) with pH 6.5 were placed on the nylon membrane Zetapor (Cuno Inc.) 500 µm thick and with a pore diameter of 0.45 µm. The membranes were placed in polycarbon sacks with a pore diameter of 0.015 µm (Infiltec GmbH), which prevented virus particles from penetrating outside the carrier. Carriers were placed in meat and bone fragments imitating animal wastes of different degree of fragmentation. Minced meat consisted of pork was applied, as well as small and large meat dice of 3 and 5 cm, respectively, and a fragment of thigh bone shaft. Such carriers were introduced into the biomass of a drum composter and reactors in which fermentation occurred. The study was also based on inactivation of virus suspension placed in Eppendorf tubes, which were introduced into the biomass. Carriers were removed at proper time intervals and virus titres were determined, which enabled the estimation of their elimination rate during these processes. The technique described by Traub et al. (1986) was applied in this study. Titre analyses were carried out on cell lines: BEL for BPV and SK-6 for ADV. The titres were calculated with the Kärber method (1931) and presented as log $TCID_{50}$/ml. The results obtained characterizing the number of active particles of viruses were analyzed statistically using the Statistica programme. The theoretical time needed for microorganism inactivation was calculated on the basis of regression lines.

Results

The results of the study are presented in Tables 1-6 and Fig. 1. The maximum temperature of the biomass during composting was 57.2°C and took place in the 108^{th} hour of the process (Fig. 1).

Table 1 shows that ADV underwent faster inactivation during meat waste composting, since as early as in the 72^{nd} hour of the process active particles were not detected in any carriers (Table 1). In case of the thermoresistant BPV, a gradual decrease in titre at success-

Table 1. Mean titres of Aujeszky disease virus (AD) and BPV [logTCID$_{50}$/ml] in various types of carriers during composting process.

Type of carrier	Sampling time [hours]							
	0		72		102		126	
	AD	BPV	AD	BPV	AD	BPV	AD	BPV
Filter in a small meat carrier	3.55	5.8	n.d.	4.8	n.d.	3.3	n.d.	1.8
Filter in a bone carrier			n.d.	4.8	n.d.	3.3	n.d.	2.3
Eppendorf tube in the biomass			n.d.	4.55	n.d.	2.3	n.d.	1.3

n.d. – not detected

Table 2. Regression lines describing survival of BPV viruses during composting process.

Type of carrier	Regression equation	Total time of virus survival [hours]	Decrease in virus titre [logTCID$_{50}$/hour]
Filter in a small meat carrier	y = -0.03x + 6.17	205.6 (8.5 days)	0.03
Filter in a bone carrier	y = -0.027x + 6.08	225.2 (9.4 days)	0.027
Eppendorf tube in the biomass	y= -0.036x + 6.16	171.1 (7.1 days)	0.036

Table 3. Average titres of AD and BPV viruses during mesophylic fermentation and regression lines.

AD virus			BPV		
Time [hours]	Titre [logTCID$_{50}$ /ml]	Regression line	Time [days]	Titre [logTCID$_{50}$ /ml]	Regression line
0	3.55	y = -0.26x + 3.42 x = 13.15 hours – total time of survival	0	5.8	y = -0.085x + 5.9 x = 69.4 days – total time of survival
1	3.05		7	5.55	
2	2.8		14	4.55	
4	2.55		21	4.05	
5	2.05		28	3.55	
6	n.d.				

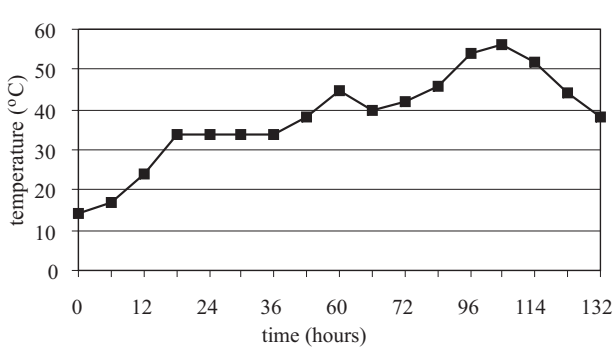

Fig. 1. Course of temperature during composting process.

ive study times was observed. A decrease in the virus titre was fastest in the suspension, which may have been caused by small protective effect of tube walls from the action of high temperature. The carrier made from meat and bone definitely best protected introduced viruses from the effect of a higher temperature. The inactivation of viruses was the slowest in the bone carrier.

On the base of regression lines it can be established that a relatively faster decrease in the BPV titre occurred in the suspension and the meat carrier than in the bone. Microorganism survival ranged from 7.1 – 9.4 days (Table 2).

During anaerobic fermentation under mesophylic conditions only the effect of temperature on viruses was analyzed. The viruses studied were in the form of suspension placed in Eppendorf tubes. The total inhibition of the ADV activity occurred between the 5th and 6th hour of fermentation. From the course of regression line it follows that the total time of survival of ADV was about 13 hours, and a decrease in its titre – 0.26 log TCID$_{50}$/hour. Inactivation of BPV proceeded very slowly. Samples were analyzed weekly for one

month. It appeared that a decrease in titre by about 2 log $TCID_{50}$ occurred at that time, which resulted in an average daily decrease of 0.085log (Table 3).

Due to various properties of the viruses tested, different times of exposure to the temperature 55°C were applied in experiments during thermophylic fermentation. Suspensions of the ADV were removed from the bioreactor at intervals of several minutes, whereas the BPV every several hours. The last specimen from which active particles of the ADV was identified, was exposed to high temperature of the biomass for 10 min. After 30 and 60 minutes no living virus was detected in the suspensions (Table 4).

In case of the BPV, a dependence between the type of a carrier and a decrease in titre was illustrated (Tables 5 and 6). In carriers with larger sizes (meat, bone), best protecting viral particles from the effect of high temperature, the decrease was the slowest, at the order of 2log after 24 hours. The inactivation of BPV was the fastest in suspension in the Eppendorf tube placed in the biomass. The total time of survival ranged from 46.9 to 76.0 hours and was the shortest for the suspension and the longest for viruses in the bone.

Table 4. Average titres of AD virus in suspension during thermophylic fermentation and regression line.

Time [min]	Titre [log$TCID_{50}$ /ml]	Regression line
0	3.55	y = -0.058x + 2.92 x = 50.3 min – total time of survival; 0.058 log/min – decrease in virus titre
1	2.8	
3	2.8	
6	2.05	
10	2.8	
30	–	
60	–	

Discussion

Mistakes made in the course of treatment of meat wastes containing viruses for agricultural purposes may lead to the contamination of soil, ground waters and plants (Olszewska et al. 2008). The temperature generated during composting processes and anaerobic fermentations, particularly in thermophylic conditions, is the main factor which causes the inactivation of virus particles. At higher temperature, both capsomers and viral envelopes undergo denaturation. The effectiveness of denaturation is determined by the temperature (Wekerle and Albrecht 1983). In the present study, two viral species were tested – Aujeszky's disease virus having an envelope and the thermoresistant BPV virus without an envelope. Their survival during various sanitization processes were analyzed, including composting and fermentation at 37° and 55°C. The study revealed that the ADV introduced into carriers during composting process was not detected as early as after 72 hours, whereas the BPV was still detected in the 126th hour of the process, and its titre decreased by 3.5-4.5 log. The type of carrier had no considerable effect on the elimination rate of the virus mentioned, since it amounted to about 0.03 log/hour in each case.

Table 5. Average titres of bovine parvovirus in various carriers during thermophylic fermentation.

Type of carrier	Sampling time [hours]		
	0	5	24
Filter in minced meat	5.8	3.55	3.3
Filter in a small meat carrier		3.8	3.55
Filter in a large meat carrier		5.3	3.8
Filter in a bone shaft		4.55	3.8
Eppendorf tube in biomass		2.8	2.55

Table 6. Regression lines illustrating survival of BPV viruses during thermophylic fermentation.

Type of carrier	Regression equation	Total time of virus survival [hours]	Decrease in virus titre [log$TCID_{50}$/hours]
Filter in minced meat	y = -0.079x + 4.98	63.0	0.079
Filter in a small meat carrier	y = -0.071x + 5.07	71.4	0.071
Filter in a large meat carrier	y = -0.082x + 5.76	70.2	0.082
Filter in a bone shaft	y = -0.071x + 5.4	76.0	0.071
Eppendorf tube in biomass	y = -0.10x + 4.69	46.9	0.10

Results of virological tests also indicate a considerably shorter survival of the ADV, as compared with BPV during anaerobic fermentation under mesophylic conditions. The former were no longer detected as early as in the 6th hour of the process, whereas a decrease in the BPV titre after 4 weeks amounted to only 2 log, that is to 0.085 log during a day. Similar results, which evidenced a long survival of BPV, were obtained by Hoferer (2001), who analyzed viruses in pig slurry subjected to fermentation under mesophylic conditions. The results of studies on survival of ADV obtained by other authors are also very similar to those obtained in the present study. Bøtner (1991) introduced Aujeszky;s disease virus into slurry and subjected them to the effect of different temperatures. It appeared that under temperature conditions similar to those applied in the present study (35°C) the viruses survived for 5 hours, whereas at the temperature higher by 5°C – 2 hours.

During fermentation under thermophylic conditions, both tested viruses were exposed to high temperature for different times, due to their different thermoresistance. And thus, ADV was analyzed every several minutes during one hour, whereas BPV – during one day. The total survival time of ADV was 50 min, whereas that of BPV, depending on the type of carrier, remind within the range of 47-70 hours. Bøtner (1991), investigating the effect of the temperature 55°C on the survival of ADV in pig slurry (anaerobic conditions) obtained even faster inactivation than that observed in the present study, i.e. as early as after 10 min. Monteith and Shannon (1986) have reported that the BPV introduced in the form of suspension into cattle slurry subjected to fermentation at 55°C was still detected after 24 hours. In the present study, it survived two times longer, which was probably caused by the protective effect of meat, bones or tube walls from the direct action of physico-chemical parameters. Turner et al. (2000), compared the survival of Aujeszky's disease viruses on media and in slurry at different temperatures. It appeared that he detected the virus last time at 55°C at the 5th minute of the fermentation process, whereas in a medium they survived longer – up to 15 minutes, and its titre decreased by 2 log. The results of this study indicate a great importance of physico-chemical conditions in virus elimination generated during the fermentation process, apart from the temperature.

From the study conducted it follows that of both the viruses tested the BPV should be applied for validation processes of methods used in meat waste processing, particularly if this refers to methods where higher temperature is the factor inactivating pathogens.

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