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Original Research Paper

INACTIVATION OF *MYCOBACTERIUM AVIUM PARATUBERCULOSIS* IN SHEEP MANURE

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Summary: Livestock manures and composts can be contaminated by Mycobacterium avium subsp. paratuberculosis (MAP) which can cause paratuberculosis – Johne's disease, an intestinal infection of domestic and wild ruminants. Therefore the inactivation of inoculated MAP ATCC 43015 in the compost and in the manure in storage was carried out. MAP (2.0×10^6/g) was inoculated into the compost in three actively ventilated and isolated vessels and in a conventional manure storage pile. Herrold's Egg Yolk medium with supplements for MAP growth, followed by IS900 PCR for isolate identification was used. Direct PCR assessment of the persistence of sequence IS900 in the compost and manure material in parallel was implemented. Moisture, ash and ammonia content in the compost and manure specimens were determined and pH was measured. Salmonella enterica serovar Senftenberg (S. Senftenberg) was introduced into the compost and manure materials to demonstrate the hygienisation process.

The presence of MAP on the culture media was confirmed in the samples 16 and 24 hours after exposure in compost and manure, respectively. No MAP was isolated on the medium after 24 hours of exposure. However, using the PCR assay of compost specimens, persistence of MAP was proved in the compost samples until day 7 and in the manure in storage even after 21 days of exposure. *S. Senftenberg* S-73/98 was not present 24 hours after exposure either in compost or manure storage.

Key words: *Mycobacterium avium subsp. paratuberculosis; Salmonella enterica* serovar Senftenberg; microbial inactivation; bacterial DNA - analysis; composting; sheep manure

Introduction

MAP infections can cause paratuberculosis and intestinal infections of domestic and wild ruminants and result in considerable economical losses in the livestock production (1). Feces, milk and semen are the main dissemination sources of MAP, and present an important epizootic risk (2, 3) depending on animal management, especially owing to overstocking or to group sizing of young animals (4, 5). The disease manifested in sub clinical forms even increases epidemiological risks, since MAP can be disseminated by infected animals for a long period without any specific clinical signs (6).

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MAP is highly resistant to physical and chemical factors owing to its special cell wall structure containing mycoside C, mycolic acid, peptidoglycans and lipopolysaccharides (7, 8). Most of the studies regarding the inactivation of MAP in complex environments, were conducted before 1985 (9). However, composting is the one of a most widely applied treatment methods used for bio waste biodegradation and hygienisation influenced by different factors which has an important role on microbiological inactivation (10, 11, 12, 13) (Watanabe et al. 1997; Böhm R 1998; Watanabe et al. 2002; Vinneras et al. 2003. Temperature, oxygen content, microorganisms competition and antagonisms, degradation of organic material, the increase of ammonium concentrations, pH and the composting time, were often selected to control and monitor the composting processes which are responsible for the effect of

proper decomposition and hygienisation processes (14, 15).

The objective of this study was to compare the persistence of inoculated MAP during two different manure treatment systems using commonly applied manure storage and composting of sheep manure. Composting is one of the most convenient and optimal manure hygienisation methods, and the application of in-vessel method enables good control of the method (16). Composting of livestock manure can be a method of choice in preventing MAP dissemination into the environment.

Materials and methods

Design of the study

Three ventilated composting vessels, each with the volume of 1m^3 (1m x 1m x 1m), and one experimental manure storage pile containing approximately 3 m^3 of material (2m × 2m × 1.5 m) were used for the study. Composting material was the mixture of sheep deep litter manure and pine bark with addition of water, so that the moisture content reaches approximately 65%. The composting mixture had been prepared fresh before experimental composting started. The compost temperature was controlled and limited up to 68 °C using radial fans which were also used to achieve aerobic composting process. (Fig. 1) The temperature of compost was controlled by PT 100 probes and by the "Visi DaQ"® computer program (Advantech, USA). The probes were placed in three vertical levels (16 cm, 50 cm and 66 cm above the vessel bottom) and inserted 30 cm deep into the material. The experimental manure storage pile, consisted of sheep deep litter manure only. The temperature was also measured by PT 100 probes at three vertical levels (25 cm, 75 cm and 125 cm above the ground), inserted 30 cm deep into the manure. (Fig. 1) The temperatures were measured every minute and recorded by data loggers (Testo 175 T3, Germany).

Physical-chemical analyses of compost and sheep manure

Samples were analysed in two parallels. Moisture content was determined in the specimens as a loss upon drying at 105 °C – 110 °C after 24 h by weighing with the accuracy of 0.001 g (Exacta, Tehtnica Železniki, Slovenia).

The pH was determined in the liquid extract after the specimens had been oven dried (105 °C – 110 °C) (5 g). mixed with distilled water (25 g) and stabilized (10 minutes) using a calibrated pH meter (Iskra, Slovenia).

Ammonia was determined by titration, with 0.1 M NaOH, of 150 ml of distillate (acquired out of 10 g of the specimen, 250 ml H_2O and 3 g MgO) blended with 50 ml of 0.1 M H_2SO_4 and metal red dye.

The ash content was determined as solid residua after the samples had been incubated at 550 °C for 30 minutes and weighed with the accuracy of 0.0001 g.

Preparation of the samples for exposure and sampling

To apply the bacteria in compost and manure, we used diaphyses of bovine long bones as carriers. The carriers were filled up with approximately 5 grams of composting mixture or manure respectively and inoculated with the tested bacterial suspension (Fig. 2). Bone holes stand open, and therefore the

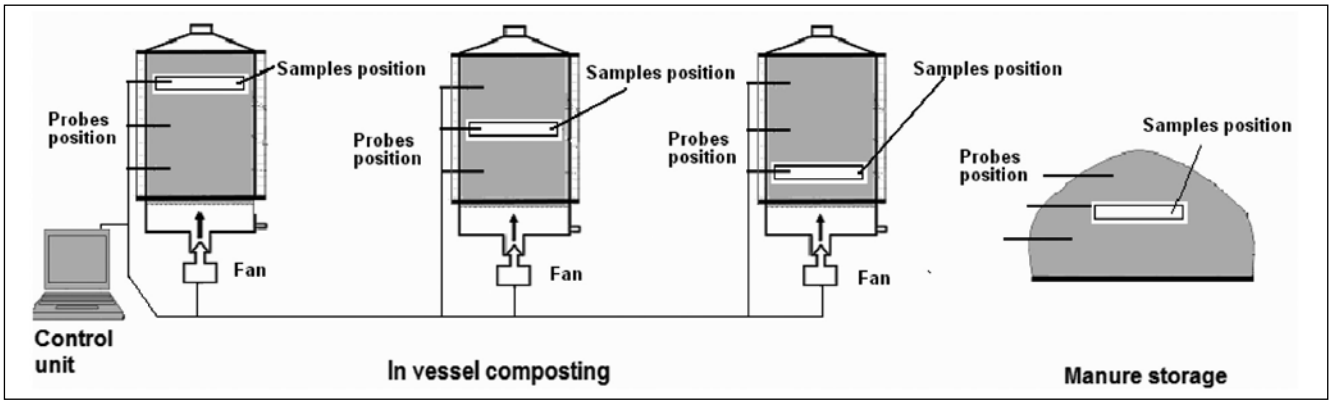

Figure 1: Design of the study, placing of the clusters of samples and temperature probes

material in carriers had direct contact with the surrounding material. The filled and inoculated carriers were placed in metal mesh baskets. Two holders with MAP inoculum and one carrier with *S. Senftenberg* inoculum were placed in each basket. The rest of the baskets' volume was filled up with composting material or manure respectively, and was also used for chemical analyses. Completely filled up baskets were placed in horizontal clusters (sample positions), each containing 9 mesh baskets at three different altitudes and at least 20 cm from the walls (Fig. 1). One filled up mesh basket was removed from the material at each sampling time according to the sampling scheme (Tab 1) covering the period of 21 days. So each sampling (N°) comprised six samples of MAP from the composting vessels (54 samples in 21 days) and two samples of MAP from the manure in storage (18 samples in 21 days). *S. Senftenberg* was sampled simultaneously, and comprised three samples from compost (27 samples in 21 days) and one sample from manure in storage (9 samples in 21 days). During transport to the laboratory, the mesh baskets were sealed in plastic bags and cooled below 6 °C.

Single aliquots (200 µl) inoculated into carriers contained 2.0 x 10^6 of live MAP ATCC 43015 in suspension. The number of MAP was ascertained after 6 weeks of incubation at 37 °C by counting the colonies on Middlebrook 7H10 medium. Serial dilutions of suspension from 10^{-1} to 10^{-7} were used for inoculating (100 µl) the medium for enumeration.

The used suspension of *S. Senftenberg* (S-73/98) contained 11.7 x 10^9/ml of live bacteria. The suspension was inoculated into the compost/manure at the ratio of 1:10. The number of live *S. Senftenberg* was determined by bacteriological method in three different media (blood agar, Rambach, xylose lysine deoxycholate agar (XLD)), and in two parallels each. Serial dilutions of the suspension from 10^{-1} to 10^{-7} were used for inoculating on the media. The colonies were counted after 24 hours of incubation.

Bacteriological analyses of compost/manure samples

MAP

Two grams of the MAP inoculum was removed from each bone holder, mixed with water (50 ml), homogenized (for 30 sec.) in a stomacher (IUL, Spain), shaken for 30 minutes, and left at room temperature for 30 minutes to settle. Afterwards, 5 ml of supernatant was mixed with 25 ml of 0.9% hexadecylpyrimidum chloride (Sigma, USA) while 1 ml of supernatant was shaken again for 30 minutes and left at room temperature for 18 hours. The samples were centrifuged for 20 minutes at 1400 g (Heraeus 1.0 R, Germany). Meanwhile the supernatant was discarded, the sediment was diluted by 1500 µl of H_2O.

200 µl of diluted sediment was inoculated on the Herrold's Egg Yolk medium with supplements: 1. without any mycobactin; 2. with mycobactin, "3. with mycobactin and egg yolk, malachite green, penicillin and amfotericin, and on Bectom Dickinson medium. The inoculated media were incubated in 25 ml tubes in horizontal position (at 37 °C for two weeks) and vertical position (for 3 months).

S. Senftenberg

The number of S. Senftenberg was determined on Rambach and XLD (Xylose lysine deoxycholate agar) medium in three parallels in up to 24 hours of exposure. Portions of 1 g each of inoculated material from the carriers was mixed, i.e. diluted with 9 ml of sterile physiological solution, and the specimen was further diluted to up to 10-5. 0.1 ml of each dilution was inoculated on the medium. After 24 hours of incubation at 37 oC, the colonies of bacteria were counted. Results with 15 - 300 colonies

Bacterial holders | Inoculation of bacteria to holders | Placing of the mash basket with holders | Order of sampling

Figure 2: Application of bacteria to the compost, placing of filled-up baskets and sampling order

per plate were considered as adequate for counting analysis.

The presence of *S. Senftenberg* in specimens was determined on the basis of pre- enrichment incubation of 1 g of specimens in buffered peptone medium - BPW at 37 °C for 16 – 20 hours. After pre-enrichment, aliquot of 0.1 ml of medium with colonies was transferred to selective enrichment media - Rappaport Vassiliadis Broth (RVS) and Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn). The specimens were incubated on RVS at 41.5 °C for up to 27 hours and on MKTTn at 37 °C also for 21 – 27 hours. After incubation, *S. Senftenberg* was determined on Rambach and XLD media by counting typical colonies after incubation at 37 °C for 21 to 27 hours.

DNA extraction and direct molecular determination of MAP in compost material

After extraction by QIAamp DNA Stool Mini Kit (Qiagen, Germany), DNA was amplified for IS*900* using P90 (5' GTT CGG GGC CGT CGC TTA GG 3') and P91 (5' GAG GTC GAT CGC CCA CGT GA 3') primers, respectively. After the amplification, 400-bp PCR-products were separated using electrophoresis (Power Pac 300, BioRad) on 2% agar gel stained by ethidium bromide and analyzed by scanning and a visualization system (Gel Doc 1000, BioRad, USA).

Results

Physico-chemical analysis

During the first 16 hours, average hourly temperatures exceeded 50 °C in the upper and lower positions, and in the next 8 hours reached the limited maximum of 68 °C in the upper positions of composts (Fig. 3). Mean hourly temperatures (43.5 °C ± 5.2 as the 95% confidence interval of the difference, S.D. 6.8) in composts were higher than the average temperatures (23.1°C±14.2, S.D. 5.7) of the sheep manure with no correlation to outdoor temperatures in the first 24 hours (Fig. 3). In the upper positions of the composts in vessels, the temperatures over 60 °C lasted from day 2 to day 7 (148 hours), while they persisted in the range of 50 to 60 °C from day 2 to day 6 on the upper (135 hours), middle (108 hours) and lower (62 hours) positions, respectively (Fig. 4). Sheep manure was heated to more than 60 °C in the first 3 to 9 days of the test (120 hours), but after this period, the temperatures did not exceed 50°C (Fig. 2). Mean daily temperatures (47.4°C) were significantly (P=0.00) higher in sheep manure than in the composts at the middle (38.8°C±4.27, S.D. 9,3) and lower (33.2°C±4,8, S.D. 10,5) positions; however, the temperatures were lower (44.1°C±7.39, S.D. 16.3) at the upper position of compost as well (Fig. 4).

Mean hourly and daily temperatures of the composts and in the sheep manure are presented in Fig. 3 and Fig. 4.

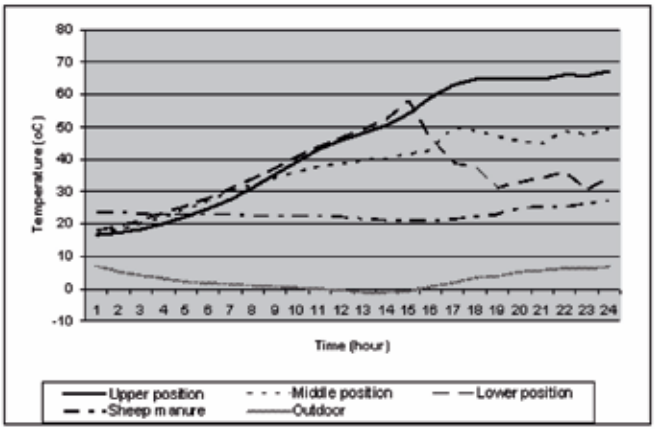

Figure 3: Mean hourly temperatures (°C) in composts at different sample positions and in the sheep manure in storage during the first 24 hours of the experiment

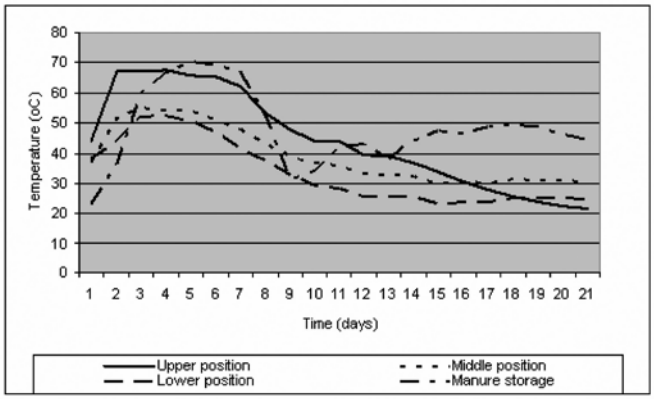

Figure 4: Average daily temperatures (°C) in composts at different sample positions and in the sheep manure in storage during the period of 21 days

The average moisture content of the composting mix reached approximately 65% at the beginning of the study. During the experiment, the moisture content in composts decreased (most significantly in the lower position of composts) as was not the case in the sheep manure (Tab. 1).

During the experiment, the pH values in composts varied from slightly acid to neutral (Tab. 1). The values of pH increased during the first 24 hours and persisted in nearly neutral range to the end of the study (Tab. 1). A slight trend to basification was no-

Table 1: Physicochemical properties of compost samples and sheep manure in storage

Sampling interval (Nº)	Samplig day	Compost* I / Manure** II	Dry matter (g/kg)	pH	Ash (g/kg dm)	Total N (g/kg dm)	Ammonia (mg/kg)
1	1	I	356,8±9,1	6,66±0,29	164,7±29,2	24,7±2,3	3814,8±2924,5
		II	369,1	6,57	191,0	26,0	2961,3
2		I	348,5±13,8	6,96±0,14	/	/	/
		II	328,3	6,71	/	/	/
3		I	359,3±29,4	6,96±0,14	/	/	/
		II	368,8	7,01	/	/	/
4		I	353,7±33,1	7,23±0,52	/	/	/
		II	339,8	6,34	/	/	/
5	2	I	382,0±74,5	6,9±0,25	/	/	/
		II	387,8	6,92	/	/	/
6	3	I	395,6±93,0	6,8±0,44	/	/	/
		II	394,8	6,9	/	/	/
7	4	I	458,66±154,5	6,83±0,29	/	/	/
		II	426,6	6,92	/	/	/
8	7	I	437,8±92,53	6,83±0,63	165,4±64,7	20,1±0,75	3594,3±1692,4
		II	388,0	7,67	207,5	22,2	3605,9
9	21	I	720,7±149,4	6,73±0,38	194,5±112,0	22,1±6,13	602,9±612,2
		II	348,5	7,57	175,0	31,3	3752,4

*Values are: one sample T test means resulting from biochemical analyses of composts at the upper, middle and lower position ± 95% confidence interval of the difference

** Values are: the results of biochemical analyses of the sheep manure in storage

ticed in the sheep manure on days 7 and 21, respectively (Tab. 1). Ash content increased for 18% from day 1 (164.7 g/kg dm) to the day 21 (194.5 g/kg dm) in composts; however an 8.4% decrease was noticed in the sheep manure. From the first day (24.7 g/kg dm) to day 21 (22.1 g/kg dm) the total N decreased for 10.6% in composts, but the N values increased for 20.3% in sheep manure. Ammonia content was high in raw material – sheep manure, and in compost samples (Tab. 1). Average ammonia values in compost samples recorded on day 21 (602.9 mg/kg) were 84% lower than the values recorded on day 1 (3814.8 mg/kg), while the ammonia content recorded in manure in storage even rose for 21% during the same period of time (Tab. 1).

Microbiological analysis

Persistence of MAP determined by culture method showed that MAP was isolated in 3 samples of 24 extracted MAP inoculums from the upper and lower position of composts and in 5 of 8 samples from the sheep manure in storage during the first 24 hours after exposure to the composting process. After day 1, MAP was not isolated by culture method in any sample either in compost or in manure in storage (Tab. 2).

Using PCR-hybridization assay IS*900* by day 7, 46 of 48 samples (95.8%) were positive on MAP in all positions of samples in the compost. None of the 6 compost samples tested on day 21 were MAP positive. However, data show that 17 of 18 samples (94.4%) of the manure in storage taken during this period of 21 days were positive using IS*900* (Tab. 2).

Eight hours after exposure, the presence of *S. Senftenberg* in the compost and manure samples was between 15.8 x 10^7 and 1.1 x 10^7/g, while after 16 hours, *S. Senftenberg* could be isolated only in the lower position. In the period between 16 and 24 hours, the persistence of *S. Senftenberg* was not con-

Table 2: MAP and *S. Senftenberg* inactivation in compost and manure in storage

MAP						*S. Senftenberg*		
Sampling interval (N°)	Sampling day	Sample exposition (hours)	Diagnostic method	Compost (n=6)	Manure storage (n=2)	Culture medium	Compost (n=3)	Manure storage (n=1)
1	1	4	PCR	6	2	Rambach	4.0×10^7	2.9×10^7
			Culture	0	1	XLD	3.3×10^7	*
2		8	PCR	6	2	Rambach	1.1×10^7	0.19×10^7
			Culture	1	1	XLD	15.8×10^7	13.0×10^7
3		16	PCR	6	2	Rambach	0.2×10^7	<1000
			Culture	2	1	XLD	0.6×10^7	<1000
4		24	PCR	6	2	Rambach	<100	<100
			Culture	0	2	XLD	<100	<100
5	2	48	PCR	6	2	Rambach	0	0
			Culture	0	0	XLD	0	0
6	3	72	PCR	6	2	Rambach	0	0
			Culture	0	0	XLD	0	0
7	4	96	PCR	5	2	Rambach	0	0
			Culture	0	0	XLD	0	0
8	7	168	PCR	5	2	Rambach	0	0
			Culture	0	0	XLD	0	0
9	21	504	PCR	0	1	Rambach	0	0
			Culture	0	0	XLD	0	0
			PCR	Σ n=54	Σ n=18	Rambach	Σ n=27	Σ n=9
			Culture	Σ n=54	Σ n=18	XLD	Σ n=27	Σ n=9

n = number of samples

firmed; the limits used for detection are stated (Tab. 2). In samples examined 24 hour after exposure the persistence of *S. Senftenberg* was excluded.

Discussion

The method used to introduce the test microorganisms into the composting material and manure in our study followed the intention of the Opinion (17) which describes typical procedures for exposing test organisms in composting. So a typical exposure method includes contamination of raw material by the tested microorganisms and placing of the contaminated material into a carrier (*e.g.,* textile sack) protected from mechanical destruction by a perforated metal basket and recovered at the end of the process. In our study, textile sacks were replaced by natural carriers, which stayed stable during exposure to composting and provided contact with the rest of the compost/manure environment.

The purpose of the *S. Senftenberg* inoculation was a MAP independent assessment of hygienisation process of the tested materials. Bacterial species involved in biodegradation, which are more active in temperatures ranging from approx. 15°- 40°C, are termed mesophilic bacteria. We failed to define any of the mesophiles in our study, however we believe in their significant influence on the laboratory MAP diagnostics from the first samples.

In the experimental composting vessels, the MAP ATCC 43015 test microorganism was exposed to rapidly growing temperatures, high ammonia concentrations and to intensive bacterial activity. In the present study, suitable conditions for optimal microbial biodegradability processes in experimental composting mixtures were achieved. Appropri-

ate homogenization, optimization of the composition and aeration of the composting material are responsible for favourable conditions for advantageous composting (18). Temperature rise of the material during the composting process is a result of microbial oxidative degradation of organic matter, and indicates the intensity of the process outcome (19). Temperatures in composts increase very rapidly (over 50 °C in 14 hours) since the sheep manure, as a part of composting mix, originates directly from the paddock's deep litter, where biodegradation has already started.

In the laboratory diagnostics, MAP cultures on the medium can be overgrown by the fast-growing mesophile microorganisms (20, 21, 22, 23). Owing to that, we believe that the initial composting conditions during the first four hours mainly influenced the possibility of the MAP culture diagnostics in our study. The temperatures of the compost and manure did not exceed 20 - 30 °C, and therefore mesophile microorganisms survived in the samples from that time. In laboratory tests mesophiles could thus overgrow and restrict MAP growth on the culture medium, even though MAP was present in the sample (24, 10, 25).

Temperature-sensitive mesophile microorganisms were reduced with the rising of temperature over the next 16 hours, thus this situation improved the possibilities for MAP isolation until the critical temperatures reached lethal values for MAP. Therefore, only 3 samples of MAP out of 18 were positive on the medium in the period of 16 hours after the composting started and utill the compost temperatures exceeded 50 °C. After the first 24 hours of exposure no MAP was isolated on the medium in compost samples, although the temperatures did not exceed 50 °C utill the end of the study. It is obvious that in our study the starting temperatures in composts were MAP lethal. Similar results to ours were obtained by the study of Fiesinger and Harrison (26). They did not find any viable MAP in the naturally MAP contaminated substrate after the first 24 hours of composting. Olsen et al. reported that MAP survived for 21 days in mesophile conditions as compared to only few hours in termophile conditions (27). After 16 hours, positive *S. Senftenberg* isolation was still determined only at the lower compost position where temperatures fell below 50°C, while no positive samples were determined after 24 hours, above all owing to the *S. Senftenberg* lethal temperatures of the compost reaching up to 55 °C (25, 10).

Although sheep manure from paddock was transported by front loader and loaded in a heap –this manipulation stimulates oxygenation and consequently biodegradation – the process resulted in temperature increase, but not earlier than on day 3. Slow temperature changes in experimental sheep manure in storage indicate weak mesophile bacterial activity in the first 24 hours from the start of the study, and for this reason the persistence of MAP was proven on the media in almost on half of samples during this period. However *S. Senftenberg* was significantly reduced in the first 16 hours after inoculation, irrespective of the moderate starting temperatures of manure (20 °C - 30 °C). Other influencing factors for *S. Senftenberg* inactivation can be the competitive and antagonistic growth of other microorganisms' species, organic matter decomposition, ammonia concentration, pH, and the exposition time (15).

Regarding MAP determination, previous research has demonstrated that molecular methods have better sensitivity than the classical medium cultivation (22). Blocking of persistent inhibitors and optimization of DNA extraction can contribute to considerably better sensitivity of tests used (28,29). However, molecular methods did not enable us to prove viable MAP. In our study, negative MAP results determined by culture methods indicate the destruction or strong reduction of MAP in composts, but most of samples taken up to day 7 still contained intact sequence IS*900*. Data show that untill day 21 almost all samples of sheep manure were MAP positive using IS*900*. At this point of the study the question of DNA persistence in compost and manure, after the destruction of MAP cells still remains unresolved, owing to the fact that only limited data about DNA destruction during composting are available in the literature. Previous studies (30) confirmed the persistence of DNA in compost even six days after cell destruction.

At the start of our experiment, composts had relatively high average ammonia content compared to day 21 when ammonia content was more than 84% lower. This fall could be the result of active ventilation, which was not the case with manure storage process (31), where ammonia even increased by 21%. The role of ammonia influence on MAP in the compost is not clear as yet. Katayama et al. described negative influences of 3% of ammonium concentration on MAP destruction in hay, and greater influence on MAP persistence in materials with low humidity content (32). However, in our study we found

high values of ammonia in the composts and in manure (2.9 – 3.8 g/kg of dry matter), which can be an important factor in MAP inactivation.

Conclusions

We believe that manure composting can eradicate or at least drastically reduce the number of pathogenic microorganisms, and therefore it can be used as a preventive measure against spreading of MAP into the environment. The impact of particular hygienisation factors during composting on MAP still is not clear. Although in the present study temperatures in manure were higher than in compost, DNA material of MAP in manure did not decompose even to the 21st day of experiment, while DNA were not determinate in composts to that time. At present, the factors influencing bacterial DNA degradation owing to the composting processes are poorly known and require additional scientific explanation.

The main advantages of the in-vessel composting include a more efficient composting process, and may also ensure conditions requested for suitable hygienisation of the organic material. The composting procedure in vessels can provide for a rapid decomposition process regardless of external ambient conditions. Therefore, composting in vessels can be thermophilic, thus being most convenient for compost hygienisation, and recommended for the treatment of manures intended for pathogen-sensitive environments such as those for vegetable production, residential gardening, or application to rapidly draining fields (33).

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INAKTIVACIJA BAKTERIJ VRSTE *MYCOBACTERIUM AVIUM PARATUBERCULOSIS* V OVČJEM GNOJU

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Povzetek: *Mycobacterium avium ssp. paratuberculosis* (MAP) povzroča paratuberkulozo – Johnovo bolezen, črevesno nalezljivo bolezen pri domačih in divjih prežvekovalcih. V raziskavi smo uporabili laboratorijski sev bakterije MAP ATCC 43015. Bakterije MAP smo vnesli v kompostno mešanico v kocentraciji 2.0 x 10^6/g v nosilcih, in sicer v tri aktivno zračene in izolirane kompostne sode in v kup ovčjega gnoja. Preživetje bakterij MAP med kompostiranjem smo ugotavljali na Heroldovem gojišču z dodatki in z molekularno metodo PCR in insercijsko sekvenco - IS900, ki je služila za določitev izolatov. V kompostnem materialu smo sekvenco IS900 določali tudi neposredno. Med kompostiranjem smo v kompostnem materialu ugotavljali temperaturo, pH vsebnost vlage, dušika, amonijaka in pepela. *S. senftenberg* je bila vnesena v kompostni material in gnoj za prikaz higienizacijskih procesov. Bakterije MAP smo iz kompostnega materiala izolirali še 16 ur po pričetku kompostiranja, ne pa tudi po 24 urah. V kompostih smo z direktno metodo PCR ugotovili prisotnost MAP IS900 v vzorcih, odvzetih od začetka do 7. dneva raziskave, v nasprotju z gnojem, kjer smo ugotovili sekvence v vzorcih, odvzetih tudi po 21. dnevu. Vzorci *S. senftenberg* S-73/98 v kompostnem materialu in ovčjem gnoju niso bili pozitivni po 24 urah.

Ključne besede: *Mycobacterium avium subsp. paratuberculosis; Salmonella enterica* serovar Senftenberg; mikrobna inaktivacija; bakterijska DNK - analize; kompostiranje; ovčji gnoj