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Survival of Salmonellas and *Ascaris Suum* Eggs in a Thermophilic Biogas Plant

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Plym-Forshell, L.: Survival of salmonellas and Ascaris suum eggs in a thermophilic biogas plant. Acta vet. scand. 1995, 36, 79-85. – In a continuous biogas plant, receiving manure from 200 dairy cows and 400 calves and young stock, survival of salmonellas and *Ascaris suum* eggs was studied. The bacteria and parasite eggs were kept in filter sacs in the manure that had a temperature of 55°C. No viable salmonellas or *Ascaris suum* eggs could be found after 24h in the digester.
Survival of salmonellas and *Ascaris suum* eggs was also studied in the manure pit where the manure was stored after digestion. The temperature in the manure pit varied between 22-27°C. Salmonellas survived 35 but not 42 days. On day 56, when the experiments had to be stopped, 60% of the Ascaris eggs were viable.

manure; anaerobic digestion; tenacity.

Introduction

It has been known for many years that grazing animals may be infected by salmonella bacteria if contaminated manure or sludge has been spread on the pasture *(Bicknell* 1972, *Breer* 1981, *Hage & Brest Nielsen* 1977, *Hess & Breer* 1975, *Jack & Hepper* 1969, *Linklater & Graham* 1985). It is also clear that salmonellas are transferred in infectious cycles in which waters, pastures, insects, birds and rodents play an important role in transmitting salmonellas from sludge or manure to domestic animals and man *(Edel et al.* 1972, 1976, 1978). Reduction or, better still, total elimination of viable salmonellas in manure and sludge is therefore important in order to break the infectious circles.

In conventionally stored liquid animal manure the survival time of salmonellas is considerable, especially in cattle manure *(Strauch* 1977). Liquid cattle manure must in summer be stored for 2 months and in winter for 5 months before most intestinal nematodes have died *(Enigk* 1980). In order to prevent salmonellas and parasite eggs from being spread with manure from infected herds to the environment, the manure must thus either be stored for a long time or be treated in some special way to obtain destruction of these organisms.

Anaerobic thermophilic digestion is an interesting method for treating manure. Apart from the production of biogas this treatment also gives a considerable odour-reduction when the manure is spread and may be a method to shorten the survival times of pathogenic microorganisms and hence lessen the

health hazards and the demands on storage capacity. The aim of the experiments described here was to study survival of salmonellas and *Ascaris suum* eggs in a full scale thermophilic biogas plant running in normal production.

Materials and methods

Description of the plant

The plant received manure from 200 dairy cows and 400 calves and young stock. Fig. 1 shows a schematic drawing of the reactor. Every 24h, manure was pumped into the antechamber, 20 m^3 in capacity, where it was heated up to process temperature, 55°C, with hot water. Heating up the manure took 7h and the manure was then pumped into the processing chamber. The volume of the processing chamber was 240 m^3. The contents in the processing chamber were mixed intermittently. Digested manure was pumped from the processing chamber into a manure pit every 24th h. Immediately there-after fresh manure was again pumped into the antechamber etc. The construction of the plant was such that fresh manure could not be pumped directly to the processing chamber. The shortest possible detention time in the processing chamber was thus 17h, since heating up in the antechamber took 7h.

Experiments in the processing chamber

Salmonella Senftenberg and *Salmonella Typhimurium* were used as test bacteria. The strains were streaked on Brilliant Green Agar (Oxoid, CM 329) and incubated at 37°C for 24h. Two colonies from each strain were then transferred to 20 ml of meat broth and incubated at 37°C for 24h. Following this, tenfold dilutions in sterile saline were made from the broths and from each dilution 1 ml of broth was added to TGE-agar (Oxoid, CM 127) using the pour plate method. The number of salmonellas per ml of broth was then determined by viable counts after 72h at 30°C. Controls from the broths, to check that no contamination had occurred, were made. Appropriate amounts of the broths to get approximately 5×10^7 salmonellas were then transferred to sacs made of nylon filter (Acropor®, Gelman Sciences, Inc.) with a pore size of 0.45 μ. The filters were heat sealed. The filter sacs, with a size of 10×2 cm, were placed in a tube made of iron wire netting. The diameter of the iron wire was 0.4 mm. The tube was 40 cm long, had a diameter of 5 cm and the holes in the net were 2×2 mm. A piece of stainless steel weighing 0.7 kg was put in the tube to secure that it did not float on top of the manure. The tube was closed in both ends by plastic lids.

Sampling was done after 24, 48 and 72h. After sampling the filters were brought to the laboratory within 1h. At the laboratory the filter sacs were cut open and let down in 20 ml of buffered peptone water. After incubation at 37°C for 18h, 5 ml of this preenrichment medium was transferred to 45 ml of selenite broth (Oxoid CM 395, Oxoid L121). After incubation at 43°C for 24 and 48h 10 μl was streaked on the surface of a Brilliant Green Agar plate which was then incubated at 37°C for 24h. Colonies suspected to be salmonella were tested biochemically and serologically and for sensitivity against O-1-bacteriophage.

Ascaris suum eggs were isolated by dissection of sexually mature worms. The contents of the distal part of 1 uterus horn were added to 5 ml of tap water and then transferred to nylon filter sacs (Acropore®, Gelman Sciences, Inc.) with a pore size of 3.0μ. The contents of the distal part of the other uterus horn were kept as controls in tap water at 30°C for 30 days. The filters were heat sealed and placed in the same iron wire tube as the salmonella filter sacs. Sampling was done after 24, 48 and 72h.

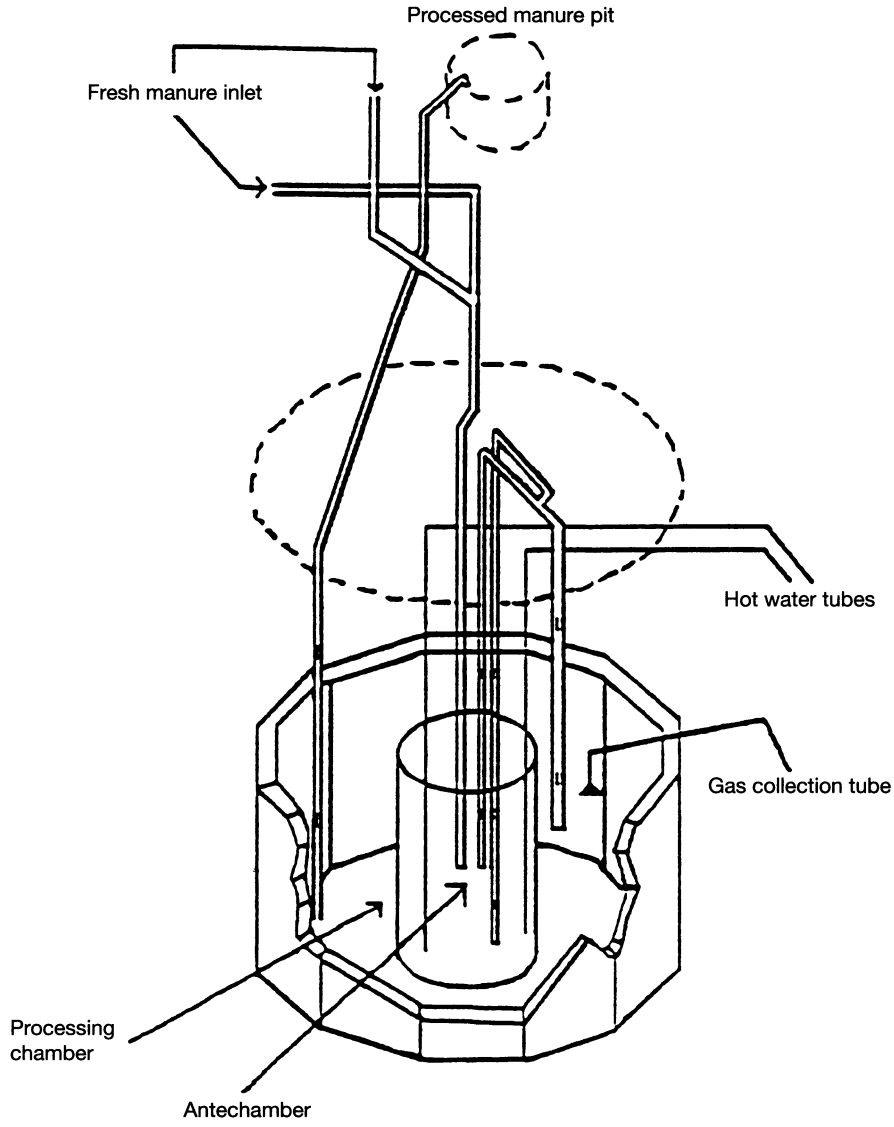

Figure 1. Schematic drawing of the reactor.

After sampling, the contents of the sacs were incubated in tap water at 30°C for 30 days whereupon 200 eggs were studied and the number of embryonated eggs was recorded. The experiments were repeated once.

Manure pit experiments

Similar experiments were performed in the manure pit where the digested manure was stored. Salmonella Dublin and Salmonella Typhimurium were used as test bacterias and

Ascaris suum as test parasite. Sampling was done once a week. The temperature in the manure was recorded daily by permanent temperature sensors. Otherwise, the methods used were the same as in the processing chamber experiments.

Results

Processing chamber experiments

The manure had a dry matter content of 8.3% and a pH of 7.4.

The temperature in the processing chamber varied between 53,4-55,8°C during the experiments. No salmonellas survived for 24h in the chamber. No embryonated Ascaris eggs could be found in any experimental culture. 75-80% of the eggs kept as controls were embryonated.

Manure pit experiments

The temperature in the manure varied between 22-27°C during the experiments. *Salmonella Dublin* and *Salmonella Typhimurium* survived 35 but not 42 days. 75-80% of the Ascaris eggs were embryonated up to the 42nd day. The number of embryonated eggs then declined and at day 56 about 60% were embryonated. 75-80% of the control eggs were embryonated. The experiment had to be stopped at day 56 because the pit had to be emptied.

Discussion

The results of the present experiments confirm previous reports on these topics. Salmonella bacteria are known to be relatively sensitive to moist heat. The D-value (= the heating time required to kill 90% of the bacteria at specified temperature) for *Salmonella Senftenberg* 775W, considered to be the most heat-tolerant salmonella, is 80 min at 55°C *(Burge* 1983). The D-value for *Salmonella Senftenberg* 775 W at 55°C in Heart Infusion Broth is 36.2 min and for a heat resistant strain of *Salmonella Typhimurium* 18.8 min *(Baird Parker et al.* 1970). In phosphate buffer at a pH of 7.0 *Liu et al.* (1968) reported D-values of *Salmonella Senftenberg* 775 W at 54.4°C to vary between 13 and 20 min. *Corry & Roberts* (1970) found D-values at 55°C in phosphate buffer for *Salmonella Typhimurium* and *Salmonella Dublin* to be 4 min. In nutrient broth the D-value for *Salmonella Typhi* and *Salmonella Paratyphi* at 55°C is 5 min and for *Salmonella Enteritidis* 5.5 min *(Lee & Riemann* 1971). However, it must be kept in mind that the thermoresistance of pure cultures of microorganisms in broth might be, and of course often is, quite different from what is found in complex material such as manure and sludge. Many factors, biological, chemical and physical, will have a strong influence on the results.

In cattle and in pig slurry digested anaerobically at 56°C D-values for *Salmonella Typhimurium* of 10-50 min are found *(Munch & Schlundt* 1983). *Schlundt* (1982) could not detect *Salmonella Typhimurium* after 6h in sewage sludge digested anaerobically at 53°C. The original concentration of *Salmonella Typhimurium* in his study was 10^6/ml sludge and the detection limit was 10 bacteria/ml. The D-value for salmonella varied between 0.68-1.89h with a mean of 1.07h. *Ginnivan et al* (1980) could not isolate *Salmonella Dublin* after 4h at 55°C in aerobically treated pig slurry. The original concentration was 10^6 Salmonella Dublin/ml slurry. The decimal decay rate was 1.8^{10}log/hour.

Errebo Larsen & Munch (1983) investigated the number of salmonellas in slurry from randomly selected Danish herds with unknown disease status and from herds with a known salmonella infection. The salmonella concen-

tration was generally less than 10 cells/ml slurry with 2.8×10^4/ml slurry being the highest recorded number. In slurry from randomly selected British pig and cattle herds occurrence of salmonellas was surprisingly frequent, but the concentrations found were low. In pig slurry 2×10^3 salmonella/ml was the highest observed concentration while in cattle slurry the concentrations were usually less than 1/g *(Jones et al.* 1976, *Jones & Matthews* 1975). It follows that with D-values such as these mentioned above, salmonella will be reduced to undectectable numbers in 5-6h in slurry digested anaerobically at 53-56°C provided that the concentrations of salmonellas found in the Danish and British surveys are of general validity.

Ascaris eggs are known to be extraordinarily resistant to environmental influence due to the complex structure of the egg shell *(Keller* 1951a). *Jettmar & Exner* (1951) stated that Ascaris eggs were killed in 5 min at 55°C. In a water bath with a temperature of 55°C Ascaris suum eggs were killed in 10 min *(Rudolfs et al.* 1951). *Schaffert & Strauch* (1976) studied rotating aeration (system Fuchs) of pig slurry and found that at 52.5-53°C irreversible lethal changes occurred in Ascaris eggs and they concluded that after reaching 55°C the slurry was acceptable from a parasitological point of view. Ascaris lumbricoides was killed after 2h at 54°C in raw sludge and in digested sludge *(Keller* 1951b). In pig slurry treated aerobically at 55° unembryonated *Ascaris suum* eggs did not survive 30 min and embryonated eggs did not survive 15 min *(Burden & Ginnivan* 1978).

Since Ascaris eggs are more resistant to environmental influence than eggs of gastro-intestinal nematodes of cattle *(Persson* 1974) it must be assumed that anaerobic thermophilic digestion of cattle slurry will rapidly kill eggs and larvae of trichostrongyle nematodes.

The biogas plant in which the experiments were done has a somewhat special technical design. The fresh slurry is pumped to an antechamber and not, as is usually the case, directly to the processing chamber. The vital aspect with this design from the hygienic point of view is that, even though the process is continuous, all of the slurry will be digested at 55°C for at least 17h.

On the basis of these experiments and of the present knowledge of survival of salmonellas and Ascaris eggs in slurry and sludge in high temperatures it can be concluded that thermophilic digestion of manure in a plant giving a temperature of at least 55°C and a detention time of 17h will give a hygienically acceptable end product with regard to salmonellas and eggs of intestinal parasites.

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Sammanfattning

Överlevnad av salmonellabakterier och Ascaris suum ägg i en termofil biogasanläggning.

Anläggningen mottog gödsel från 200 mjölkkor och 400 ungdjur. Bakterierna och parasitäggen förvarades i filterpåsar i gödseln, vilken hade en temperatur på 55°C. Inga viabla salmonellabakterier eller *Ascaris suum* ägg kunde påvisas efter 24 timmars uppehåll i rötningskammaren. Överlevnaden av salmonellabakterier och *A. suum* ägg undersöktes även i slutförvaringsbassängen till vilken gödseln pumpades efter rötningen. Gödseltemperaturen i slutförvaringsbassängen varierade mellan 22 och 27°C. Salmonellabakterierna överlevde 35 men inte 42 dygn. 60% av *A. suum* äggen var viabla på dag 56 vid vilken försöket avbröts.

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