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ARTÍCULO ORIGINAL

Viability of Bovine's Strongyloidea eggs in a System of Anaerobic Biodigestion

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ABSTRACT

*Cattle **Strongyloidea** egg-viability was studied in closed system of anaerobic biodigestion. Two thirds of a biodigestor were filled with liquified manure from eight Hosltein cows, nacturally infected. For 10 consecutive weeks of observation, the biodigestor internal temperature varied from 24ºC to 28ºC and external temperature, from 26ºC to 33ºC. All the effluent samples showed a constant pH of 7. The samples of the effluent were weekly collected, in a total of 10, and submitted to techniques to detect the presence and viability of the helminth eggs. It was detected a 35-day egg viability. The infective larvae of **Haemonchus, Cooperia** and **Oesophagostomum** genus were found until the 14th day of observation and the infective larvae of **Trichostrongylus** and **Ostertagia** genus, until the 35th day. Therefore, liquefied cattle manure containing **Strongyloidea** eggs must remain under anaerobic conditions for at least 35 days before its safe return to the environment.*

Key words: *Strongyloidea eggs, viability, anaerobic digestion, liquid manure.*

INTRODUCTION

One of the current practices in zootechnic explotation is that of liquefaction of animal manure and residues, facilitating their removal and consequently reducing the labor force. Such a technique and the crowding of animals by confinement of a same species increase the risk of dissemination of transmitted diseases, often of subclinical character, through the animal manure and secretions, wich ultimately affects the environment.

Furthermore, space limitation leads liquefied organic matter, under composting, to remain stored for a period of time insufficient for the sanitary control. In a varety of systems consisting of liquid composting of animal, vegetable and human residues, the digestion process frequently ocurs in anaerobiosys. This means that the caloric energy and the pH increase to alcalinity, what is crucial to the control of the transmissible agents and potentially present in composts do not manifest themselves spontaneously.

In Brazil, there are no studies on liquefied residues and on their consequence before and after biodigestion, for animal and human health, or to the risk involved when they are employed as fertilizers and soil conditioners. In this study,

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the viability of eggs from nematodes of Strongyloidea superfamily was evaluated in bovine feces. These parasites are very common in state of Rio Grande do Sul (RS), and are responsible for the disease known as gastrintestinal verminosis, one of the main causes of diminishing animal productivity. The investigation was carried out in a biodigestion system in wich the processes of fermentation and putrefaction occurs in anaerobiosys.

MATERIAL AND METHODS

The experiment was conducted at the laboratory of the Veterinary School - Federal University of Rio Grande do Sul, in the summer, in a medium air temperature of 28°C.

A 200 liter cylindric tank (87 cm high, 72 cm diameter) was used as a biodigestor. The equipment contains a valve for the control of gas pressure in the upper part of the tank, a faucet for the removal of the material in the digestion process (laterally situated and at 30 cm of the bottom) and a thermometer for the measurement of the system´s internal temperature (at the same point of the faucet). The digestor was installed and handled according to recomendations[1,2].

The digestor was filled with 83 Kg of manure, from eight cows of the Holland race in lactation and naturally infected with causal agents under study. The manure was collected and dissolved in water (1:1,w/w), without hypocloride treatment, in the same day of harvesting[3].

The resulting 166-liter load was kept under anaerobic biodigestion for 63 days.

From the seventh day of observation, a one-liter sample of the product, was collected and immediately subdivided into five subsamples of 200 ml each. The weekly collections were made through the digestor faucet, always after homogeneization by manual agitation of the tank for aproximately 2 minutes. External temperature of the laboratory and the internal temperature of the digestor were registered twice a day, at 8:00 AM. and at 6:00 PM. The internal measurement were always performed at the medium part of the load, using the digestor thermometer, and sample pH values were assessed by using na indicator paper (MERCK).

For the recovery of the Strongyloidea eggs in the subsamples, it was used the Willis technique[4]. The viability of the eggs was tested through a coproculture, for the detection of the infective larvae and also the counting and generic identification of these larvae were carried out[5,6].

For the quantitative analysis of the Strongyloidea eggs was used the mean, as a measurement of dispersion. The analysis of the infective larvae of Strongyloidea was made by the generic identification and expressed in percentages.

RESULTS AND DISCUSSION

During the observational period the internal temperatures were always lower than that of 35ºC detected in a similar anaerobic process and sufficient, according to the author, for the patogenic control[7]. The results are also in agreement with those relative to the fermentation process[8]. Accordingly, when the system is in anaerobiosys the process takes place without temperature elevation.

The pH value of the digestor product was found to be 7.0, in all weekly collections. This indicates that the biologic degradation of the mixture ocurred in anaerobiosys, since there was no alteration of the pH during the putrefaction process, as it was already noted in another study[9]. It was observed that a pH over 9.0 destroys certain bacterian agents[10]. In the case of parasites agents, the pH values between 5.0 and 8.5 do not affect the survival of the Strongyloidea infecting larvae, but interfere in the increase of the eclosion rate of this evolution phase[11-14].

Table 1 shows the results concerning the variation of the average number of Strongyloidea

Table 1. Weekly variation in the mean value and range of Strongyloidea eggs from five fecal samples of 8 g, collected from cattle manure kept in an anerobic biodigestor

Week	Mean value	Variation
0	4	2 - 5
1	3	1 - 6
2	1,4	0 - 4
3	1,6	0 - 3
4	0,8	0 - 2
5	0,2	0 - 1
6*	0,6	0 - 2
7*	0,2	0 - 1
8	0	0

*All eggs presented morphologycal alterations.

eggs in relation to the longevity in a closed system of anaerobic biodigestion in five weekly-taken subsamples of the mixture.

Concerning Strongyloidea eggs, the product of this system became negative on the 35th day of observation. These results differ from another experiments in wich even at lower temperatures (18ºC to 22ºC) it was obtained a lower survival time for eggs of the *Trichostrongylus colubriformes*, *Cooperia punctata*, *Ostertagia ostertagi* species and of the *Strongyloides* genus - between 7 and 28 days, in liquefied manure[15-17]. These authors managed to increase the longevity of the Strongyloidea eggs from 64 to 172 days respectively, only when the manure temperatures lowered to 8ºC and 3ºC.

An internal temperature of 27º C, so close to that observed in this study, also obtained different results, with only 25 days of survival for eggs of the *Cooperia curticei* species[18].

Increased liquefied manure temperature to 35ºC, through aerobic fermentation obtained a reduction in the survival time of the tricostrongilid s eggs to 10 days[19].

Other authors also observed a reduction in the survival time of parasite agents to 30 and zero days, respectively. In temperatures that ranged from 32ºC to 56ºC, but obtained only through aeration and forced movimentation of the organic material[20-25].

In this experiment the results concerning temperature and pH agree with those wich proved that the residues of liquefaction do not increase spontaneously the temperature of the mixture, thus promoting the preservation of the existing pathogens[26,10].

The results concerning the eggs longevity in a closed anaerobic digestion system of five weekly-taken subsamples of the liquefied manure are shown in Table 2.

In the present work, infecting larvae belonged to the *Haemonchus, Ostertagia, Trichostrongylus, Cooperia* and *Oesophagostomum* genera.

From the 35th day, there were no infecting larvae of any of the genera studied, even though the eggs had shown structural modifications only after the 42nd day of observation, with an avarage internal temperature of 26ºC.

The infecting larvae of the *Haemonchus* genus appeared in samples collected on the 7th day of observation. Concerning longevity a study obtained a larger survival in water of the infecting larvae of the *Haemonchus* genus (98 to 280 days), at temperature between 0ºC and 30ºC, respectively[27,28].

This includes the temperature range obtained in this experiment, wich varied from 24ºC to 28ºC. The discrepant results obtained by these authors could be related to the vehicle used - water.

In this study, the infecting larvae of *Cooperia* and *Ostertagia* genera were detected on the 7ht and 21th day, respectively. Different results were showned a lower temperature (20ºC), with a maximum survival of 28 days for the same genus and environment[16].

Still concerning the infecting larvae of the *Ostertagia* and *Trichostrongylus* genera, they were detected more frequently and recovered for a longer period of time. This coincides partially with other results in wich observed a larvae frequency of *O. ostertagi* and *Cooperia oncophora* species in similar conditions as this experiment[29]. In different environment, such as excremental matter and pasture the infecting larvae of *Cooperia* species had a larger frequency and resistance[30,31].

Therefore, the survival time of infecting larvae of the *O. ostertagi* and *C. oncophora* species was reduced by increasing the temperature of the

Table 2. Comparison of variation in the percentage of Strongyloidea infective larvae from bovines in 5 subsamples of 80 g from the efluent and the time spent in a closed anaerobic biodigestion system

	Day of observation									
Genus / L2i	0	7	14	21	28	35	42	49	56	63
Haemonchus	4	2	0	0	0	0	0	0	0	0
Ostertagia	0	12	23	33	6	0	0	0	0	0
Trichostrongylus	58	83	77	67	20	0	0	0	0	0
Cooperia	5	1	0	0	0	0	0	0	0	0
Oesophagostomum	13	2	0	0	0	0	0	0	0	0

liquefied manure to 56ºC, through the utilization of the Licom moving system and forced aeration, since the temperature elevation in the liquefied manure did not occur spontaneously[22].

In this study, the infecting larvae of *Trichostrongylus* species were found on the 28th day of observation, with an avarage internal temperature of 26ºC. This agrees with other experiment wich considers the temperature between 20ºC and 30ºC as ideal for the "in vitro" development of the *Trichostrongylus axei* and *T. colubriformis* species[32].

In this study, the infecting larvae of the *Oesophagostomum* genus were found on the 7th day of observation, with an avarage temperature of 26ºC. Different results were obtained in another study with a survival time of 43 days in a temperature of 22ºC in an anaerobic environment. 16 It should be emphasized that here, although the temperature difference had been of only 4ºC, the survival time was reduced.

Finally, all the eggs found in this experiment, begining on the 35th day of observation, were non-viable, since they presented structural modifications, such as morula alteration and one third reduction of its size. This partially explains, the lack viability of the eggs. Such findings contradict the results obtained in another experiment wich registered the lack viability of the parasites stating at the seventh day, in temperatures between 18ºC and 26ºC[33]. The authors related these findings to the hypotheses of the presence of the putrefactives bacteria and oxygen reduction in the liquefied manure in anaerobiosys.

The results concerning the viability of the studied parasites agents coincide with the hypothesis that the processes of fermentation and putrefaction in the system of liquid compostation were directly proportionate to anaerobiosys and inversely proportional to the pathogenes[7].

There are suggest that the liquefied animal residues do not produce, by themselves, temperatures capable of controlling pathogens during their storages[9].

The results obtained through experimentation allow the conclusion that the viability of Strongyloidea eggs was of 35 days. The infecting larvae of the *Haemonchus, Cooperia and Oesophagostomum* genera were controllable after 14 days of storage and those of *Ostertagia* and *Trichostrongylus* genera, after 35 days.

The most resistant infecting larvae in the studied environment were, in a decreasing order, of helmints of the *Trichostrongylus, Ostertagia*, *Haemonchus, Cooperia* and *Oesophagostomum* genera.

Hence, in order to allow the utilization of manure from Strongyloidea helminthus superfamily - parasited bovines without risks to animals health, composting should not be made "in natura" over the soil or pasture. The retention period of the anaerobic digestão product should be of at least 35 days before it be returned to the environment.

RESUMEN

El objectivo deste estudio fué evaluar la viabilidad de huevos de Strongyloidea de bovinos en un sistema cerrado de biodigestión anaerobia. Dos partes del biodigestor fueran completadas con estiércol liquido de ocho vacas Holstein Breed, naturalmente infectadas con los parásitos estudiados. Durante 10 semanas consecutivas de observación, la temperatura interna del tanque varió de 24 º hasta 28º C y, externamente, de 26º hasta 33º C. El pH medido fué siempre7. Se examinaran las muestras del efluente semanalmente, en un total de 10, las cuales fueran submetidas a técnicas para detección de la existencia y viabilidad de huevos de helmintos. Se observó que los huevos de Strongyloidea tuvieran viabilidad por 35 días. Se encontraran las larvas infectivas de los generos *Haemonchus*, *Cooperia* y *Oesophagostomum* hasta el 14º día de observación y las del *Trichostrongylus* y *Ostertagia* hasta el 35º día. Los datos obtenidos muestran que el estiércol líquido bovino, infectado con huevos de Strongyloidea, deverán ser mantenidos en biodigestión anaerobia por 35 días, por lo menos, antes de su retorno al medio ambiente.

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