

## Evolution of indicator and pathogenic microorganisms in dairy manure treated with biological methods.

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### Introduction

Environmental problems related to manure management include health risk caused by the presence of zoonotic pathogens in manure. Therefore, adequate control of pathogens may require management interventions to achieve reduction of zoonotic agents in manure. The reduction and controlling of microorganisms in manure can be done by several methods but all of them require investments and operational costs thus the selection becomes dependent of the economic possibilities of each dairy farmer. In this study different methods have been tested in dairy manure from Cantabria (Northern Spain) a region where less than 200 cows are found in each dairy. Therefore, low cost manure treatment systems must be studied.

**Aim:** The purpose of the present study was to evaluate the efficiency of biological methods to reduce culturable counts of indicator microorganisms (faecal coliforms) and indigenous pathogens (*Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Mycobacterium paratuberculosis* and *Cryptosporidium parvum*) in dairy manure.

### Material and methods

**Experimental systems:** 50 litres' plastic cylindrical containers were filled with 50 l of raw manure from 4 different dairy farms and maintained at room temperature without lid. Three biological methods were tested during 6 weeks: anaerobic storage, mechanical uninterrupted aeration (pumping 2 litre air per min during 24 h) and mechanical intermittent aeration (pumping 2 litre air per min, 10 min every 3 h). Furthermore, the role of a cover of commercial peat in the anaerobic storage system was evaluated. Mechanical aeration systems were fed with 2.5 l of fresh manure weekly. Fresh manure was fed at different loading rates in the anaerobic storage systems (0, 0.05, 0.65 liter/dm<sup>3</sup> container manure volume/week). In all systems 2.5 l of sample for pathogens determination were removed every week with a multisampler (*Eijelkamp*).

**Determination of pathogens levels:** *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes* were detected following UNE-EN ISO instructions. The observation of protozoan cysts was performed by a modification of Ziehl-Neelsen acid-fast method and for *M. paratuberculosis* determination 3 different agars were used: Lowenstein Jensen, Herrod Egg Yolk and Coletsos agar.

### Results

#### Pathogens survival.

1) *Salmonella* spp was detected in all 4 types of manure assayed. *Listeria monocytogenes*, *C. parvum* and *M. paratuberculosis* each was detected in a different type of manure. No presence of *Campylobacter* spp. was found in any manure sample.

2) Mechanical aeration vs. anaerobic storage. Effect in the survival of pathogens.

- Pathogenic bacteria survival: No differences were found between the two biological treatments.
- Pathogenic protozoan survival: *Cryptosporidium parvum* was detected in one of the four type of manure assayed. A faster elimination of cysts was observed in the systems with mechanical aeration.

### 3) Manure loading rates. Effect in the survival of pathogens. (Fig. 1)

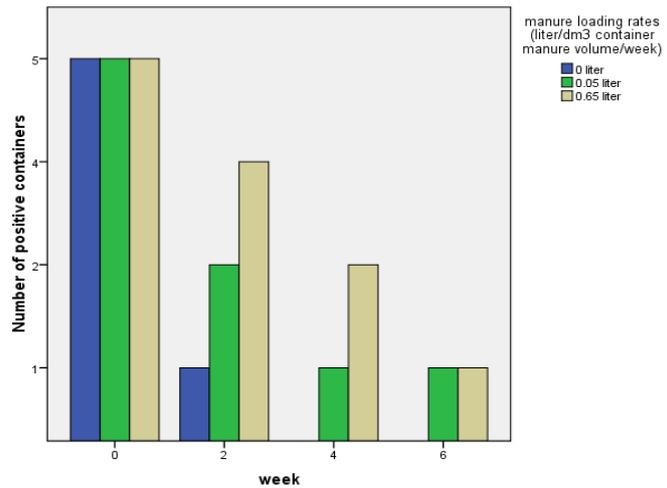


Fig. 1: Number of containers positive for presence of any of the pathogenic bacteria investigated in samples removed from anaerobic storage systems 2, 4 and 6 weeks after the beginning of the experiment

Higher number of pathogenic bacteria was determined in containers where the initial manure volume was lower and the manure loading volume was higher than in the rest of the systems.

### Faecal coliforms evolution

A decrease in the levels of focal coliforms was observed in all the containers. Significant differences ( $p < 0.001$ ) were observed between the systems with a different loading rate. No differences were found between systems with mechanical aeration and anaerobic storage at the same manure loading rate. (Fig. 2)

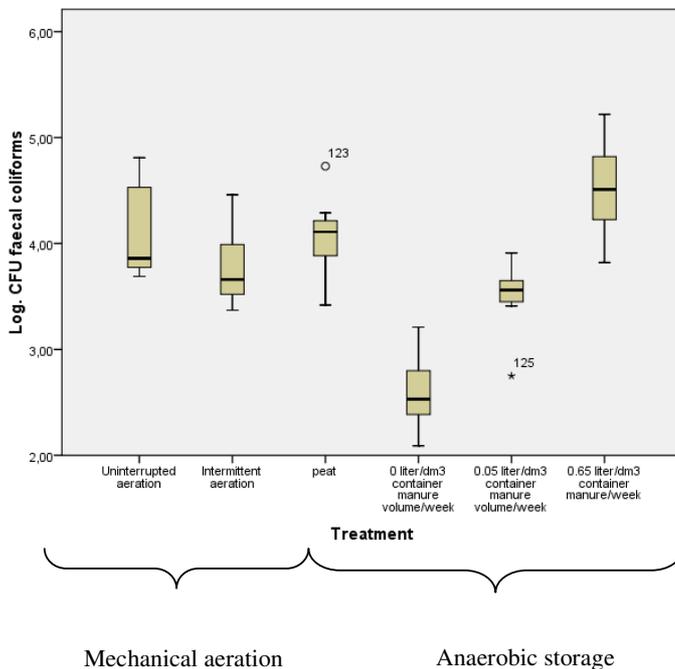


Fig 2: Box plot showing the distribution of the logarithm of colony forming units (log. CFU) faecal coliforms 6 weeks after the beginning of each treatment

### Conclusions

- Mechanical aeration system was more effective to eliminate *Cryptosporidium parvum* cysts.
- No differences in the presence of pathogenic bacteria were found between anaerobic storage and mechanical aeration systems.

- Manure loading rate influenced in the levels of zoonotic agents. A higher survival time of pathogenic bacteria was observed in containers with a low volume of manure stored and a high volume of fresh manure fed.
- Faecal coliform indicator levels decreased in all treatments. Differences in the decrease speed were observed between systems with different manure fresh loading rate.

### **Bibliography**

J.R. Bicudo, S.M. Goyal. *Pathogens and Manure Management Systems: A Review*. Environ. Tech., 24, 115-130. (2003)

Caroline Coté, Daniel I. Massé, Sylvain Quessy. *Reduction of Indicator and Pathogenic Microorganisms by Psychrophilic Anaerobic Digestion in Swine Slurries*. Bioresource Technology. 97, 686-691. (2006)

Helvi Heinonen-Tanski, Mohammed Mohaibes, Päivi Karinen, Jari Koivunen. *Methods to Reduce Pathogen Microorganism*. Livestock Science, 102, 248-255. (2006)

Mindy Spiehs, Sagar Goyal. *Best Management Practices for Pathogen Control in Manure Management Systems*. University of Minnesota. (2007)