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Effectiveness of Clinoptilolite Zeolite for *Mycobacterium avium* subsp. *Paratuberculosis* (MAP) Control in Dairy Slurry

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Abstract

The use of slurry as a fertilizer on grassland efficiently spreads infectious agents. Among those using the fecal–oral way of transmission, *Mycobacterium avium* subsp. *Para tuberculosis* (MAP) represents an interesting pathogen model to be studied in slurry management systems. In order to fulfill the requirements of a practical control implementation, we aimed to investigate the effect of natural zeolite on MAP viability and concentration in dairy cattle naturally contaminated slurry.

MAP viability and number in dairy cattle slurry following a physical separation treatment was estimated. A randomized block design constituted by four separation phases of treated slurry (solid supernatant, liquid supernatant, zeolite with slurry, liquid residue) plus one control (not filtered) was used. To assess MAP detection, each obtained sample was evaluated by culture sampling along with MAP quantification.

Zeolite+slurry treatment showed the lowest number of viable MAP. The zeolite-based treatments reduced significantly the survival of MAP in treated slurry.

The results suggest that zeolite treatments using the zeolite filter may be an interesting alternative of MAP control in slurry. The use of zeolite treatments to control MAP, and maybe other pathogens too, seems promising, however further research is needed to understand and clarify the mechanisms that explain these results in detail.

Keywords: Cattle; Slurry; MAP; Zeolite; Filter; Control

Introduction

Intensification of the dairy industry in Chile has resulted in fewer and larger herds. This creates challenges regarding the management of large volumes of dairy cattle slurry. The high content of organic matter and nutrients make slurry useful as a fertilizer [1]. However, slurry also harbors microbial pathogens making it a potential health risk for both animals and humans [2]. *Escherichia coli* O157: H7, *Listeria monocytogenes*, *Salmonella* spp., *Mycobacterium avium* subsp. paratuberculosis (MAP), *Cryptosporidium parvum* and *Giardia* spp., are the most common zoonotic pathogens found in cattle slurry [3]. In addition, some of these pathogens occur with a high infection rate as is the case of MAP, which, in southern Chile, the prevalence has been estimated as being high [4].

MAP is the causative agent of paratuberculosis in domestic and wild ruminants; a contagious, economically important, intestinal disease [5]. It is also linked to Crohn's disease in humans [6]. This obligate pathogen is notoriously resistant to harsh environmental conditions [7,8]. There are few published studies on the persistence of mycobacteria such as MAP in slurry [9,10]. Being among the hardiest of pathogens in slurry, MAP could be an excellent biomarker for pathogen control systems applied to dairy cattle slurry.

A wide range of potential slurry treatments exists [11], but many are too expensive for livestock farming. Separation systems tend to be the exception. A zeolite filtration method to purify ground water that removed 99% of viruses and 100% of *E. coli* was reported [12]. Since zeolite is inexpensive, we investigated the use of natural zeolite as a means of filtering slurry to remove or inactivate MAP.

Material and Methods

Experimental design

Slurry (4 L) was applied to the top of 10.5 cm dia. × 70 cm high PVC columns packed with 15.99 cm deep zeolite filters (density=0.92103 g/cm3) using a randomized block design. MAP viability was measured in each of the four blocks or slurry separation phases: 1) the uppermost solid floating matter, 2) the middle liquid phase, 3) the bottom zeolite filter matrix, and 4) the liquid filtrate (Figure 1). Control columns (5th block) had no zeolite filter. Thus there were a total of five blocks with four replicates for each block. Each replicate was destructively sampled 24 h post-treatment.

Sample origin and treatments

Slurry samples were collected from the storage lagoon of a commercial cattle dairy herd at INIA Remehue, Osorno, Chile (40°52'S, 73°04'W), which is a herd known to be MAP-infected. Slurry (200 L) was collected in a plastic container, homogenized, and then subdivided into 20 - four liter subsamples. This slurry was applied to the top of the columns previously packed with zeolite and allowed to separate by gravity for 24 h. Fluid passing through the column (filtrate) was collected in a plastic beaker (Figure 1).

Bacteriological analysis of the slurry samples

After slurry separation, each block (slurry layer and the liquid filtrate) was sampled and tested. Samples were processed and cultured using the BACTEC-MGIT 960 liquid culture system with MGIT ParaTB Medium™ according to the manufacturer's instructions for bovine fecal material (Becton Dickinson, Sparks, MD) and modified for slurry samples [13]. All signal-positive tubes were verified as having MAP by IS900 PCR [14]. The time to detection (TTD) values for all IS900 PCR-confirmed cultures, as reported by the MGIT 960 instrument, were converted to an estimated number of viable MAP per mL of tested material by a standard curve [14].

Statistical analysis TTD was used as a proxy variable to assess differences in viable MAP concentration between the blocks using a two-way ANOVA test, followed by the Tukey multiple comparisons test when significant ($p<0.05$) differences were observed. Normality and homoscedasticity assumptions of the two-way ANOVA test were evaluated using the Shapiro-Wilk and Barlett test, respectively. All data analyses were performed with the R 3.1.2 software (R core team, 2014).

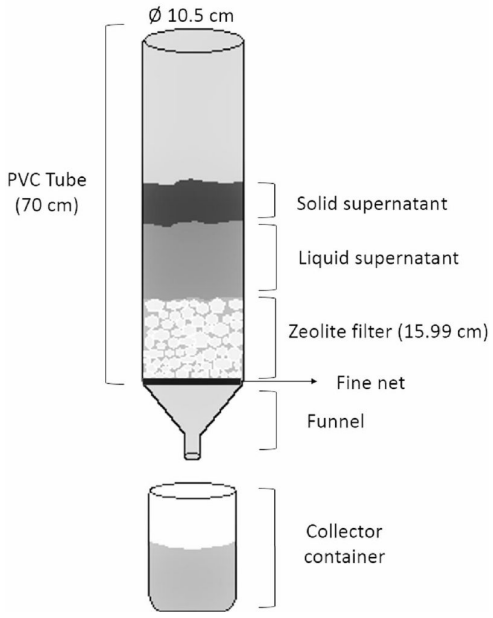

Figure 1: Zeolites filter experimental design. Diagram of the cylinder with key components labeled.

Results

The block 1 had the highest concentration of viable MAP. Block 3 had the lowest number of viable MAP (Table 1). The ANOVA test showed significant differences in TTD (p=0.0003) between the blocks. The post-hoc analysis indicates significant differences between the following treatments: block 3 *vs.* block 5 (p=0.003), block 1 *vs.* block 4 (p=0.004), block 3 *vs.* block 2 (p=0.005), and block 3 *vs.* block 1 (p=0.0003). No deviations of normality or homoscedasticity were observed for the TTD variable.

Treatment	Culture result	TTD	No. MAP/mL
Uppermost solid floating matter	+	19.33	348
	+	22.49	111
	+	27.53	32
	+	29.53	23
	Mean (SD)	24.72 (4.66)	128.30 (151.69)
Middle liquid phase	+	30.95	18
	+	29.16	24
	+	30.24	20
	+	30.32	20
	Mean (SD)	30.17 (0.74)	20.69 (2.38)
Bottom Zeolite + slurry layer	+	45.45	6
	+	39.41	8
	+	45.37	6
	-	-	-
	Mean (SD)	43.41 (3.46)	6.96 (1.16)
Liquid filtrate	+	44.74	6
	+	35.45	11
	+	34.16	13
	+	34.66	12
	Mean (SD)	37.25 (5.02)	10.53 (2.78)
Control	+	27.45	33
	+	30.41	20
	+	29.87	21
	+	29.24	24
	Mean (SD)	29.24 (1.29)	24.37 (5.66)

Table 1: Descriptive information on type of treatment and estimation number.

Discussion

A wide range of slurry treatments already exists to help sanitize and reduce offensive odors [9,15]. Expense limits the utility of many of

these slurry treatments throughout the world. Physical treatments such as separation tend to be the exception [11].

Slurry filtration through zeolite significantly decreases the number of viable MAP load in both the zeolite material and the liquid residue passing through the filter. The interaction between zeolite and MAP may have killed MAP or at least negatively affected the ability of MAP to replicate.

Zeolites are minerals with a unique structure allowing them to entrap or release various substances by cation-exchange reactions and adsorption [16]. The structure of clinoptilolite, the most abundant and frequently used natural zeolite, consists of interlinked four- and five-tetrahedral rings, which allows it to lose and gain water reversibly, acting as a molecule sieve and exchanging cations which significantly changes its structure [16].

Natural zeolites, originally discovered in Sweden by Cronsted in 1776 [17], exist in large mineable deposits found in rocks near active or extinct volcanoes [18]. Most published studies on the use of zeolites in the treatment of animal waste focus on the chemical aspects, particularly on adsorption and release of ammonia nitrogen [19]. They have also been studied as growth-promoting feed additives.

The zeolite clinoptilolites have been shown to be bactericidal for *E. coli* and able to adsorb its toxins [20,21]. Water adsorption by dry zeolite is highly exothermic, reaching temperatures of roughly 50°C. This heating could negatively affect MAP viability. Zeolites could also adsorb water from lipoprotein structures in the cell wall, thus rendering them unstable [22].

Under experimental conditions, this study provides preliminary evidence of MAP control in dairy slurry, through the use of natural zeolites; a simple, low-cost treatment method with limited negative environmental impact. The use of zeolite treatment to control MAP and maybe other pathogens seems promising, however further research is needed to understand and clarify the mechanisms involved as well as to develop practical recommendations from the shown evidence

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Conflict of interest statement

The authors declare no conflict of interest.

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