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Characterisation of a new, highly effective method for detecting nematode eggs (*Ascaris* spp., *Toxocara* spp., *Trichuris* spp.) in sewage sludge containing flocculants

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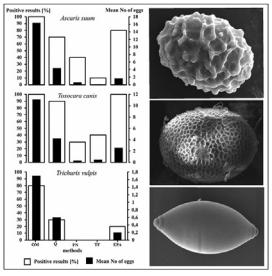
HIGHLIGHTS

- Standarization of new method for nematode eggs detection in sludge with flocculants.
- This method is many times more sensitive, than other compared methods.
- The method allowed to recover 10–36% of eggs from samples of sewage sludge.
- LOD_{50} was calculated as 3–10 and LOQ as 50-200egg/50 g depending on the eggs type.

GRAPHICAL ABSTRACT



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ABSTRACT

Because traditional methods used for sewage sludge parasitological examinations have low sensitivity, a new, highly effective method (own method – OM) was devised. The principle of this method is to eliminate the flocculent effect on the structure of sewage sludge by mechanically damaging floccules in the presence of surfactants and to increase the effectiveness of egg isolation processes in large volumes of liquids. The objective of this study was to estimate the effectiveness of the OM in detecting nematode eggs in sewage sludge samples containing flocculants. In the first stage, the effectiveness of the OM was compared to 4 other methods routinely used in parasitological examinations of dehydrated sewage sludge. Next, method standardisation was performed using sewage sludge samples supplemented with eggs from 3 parasite species (*Ascaris suum*, *Toxocara canis* and *Trichuris vulpis*).

The study demonstrated that OM efficiency was 6–65 times greater than other methods, depending on the method and type of detected eggs. Limit of detection (LOD) calculations for the OM were performed on samples supplemented with a known number of parasite eggs resulting in 10, 5 and 3 eggs/50 g of sample for *A. suum*, *T. vulpis* and *T. canis* eggs, respectively. The limits of quantification (LOQ) of the OM were established as 200 eggs/50 g of sample for *A. suum* and *T. vulpis* eggs and 50 eggs/50 g of sample for *T. canis* eggs. The rectilinear regression functions, which determined the relationship between the number of eggs detected in OM measurements and the number of eggs contained in the samples, were characterised by high and statistically significant coefficients of determination (r^2). The slopes of the trend lines were 0.3188, 0.3821 and 0.3276, and the intercepts were −11.223, -9.0261 and −23.15 for *A. suum*, *T. canis* and *T. vulpis* eggs, respectively. Method sensitivity, calculated as the slope coefficient of the regression function and expressed as a percentage, ranged from 32% to 38% depending on egg type.

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The study confirmed that the OM may be applied to quantify parasite eggs in dehydrated sewage sludge containing polyelectrolytes.

1. Introduction

The obligation to perform a parasitological examination of sewage sludge results from Polish and EU legal regulations (Directive 86/278/EEC, 1986; WHO, 2006; Final Report, 2008; Gantzer et al., 2001). According to these regulations, sewage sludge designed for agricultural use cannot contain live *Ascaris*, *Toxocara* or *Trichuris* intestinal parasite eggs. Sewage sludge used in the re-cultivation of green areas and plant production designed to produce compost should not contain more than 300 live parasite eggs in 1 kg d.m. (Regulation of the Minister of Environment, 2010). The presence of the above-mentioned parasite eggs is considered an indicator to evaluate the hygienic status of sewage sludge (Pike et al., 1998; Strauch, 1998) because these eggs have an extremely hazardous effect on human health (Toze, 2006; Crompton and Nesheim, 2002; WHO, 1987; Stephenson et al., 2000), show considerable resistance (Zdybel et al., 2015: Gaspard et al., 1995) and persistence in the environment (Melvin et al., 2001; Nelson and Darby, 2001), which could result in long-term environmental contamination (Johnson et al., 1998). Parasitological studies of sewage sludge have been conducted in Poland since the mid-1990s and have shown that these pathogens are common in sludge—parasite eggs were observed in 28.3–45.1% samples of sewage sludge (Kłapeć et al., 1999). However, it is notable that these studies were conducted preceding the common use of polyacrylamides in sewage sludge production in Poland. More recently, parasitic nematodes in municipal sewage sludge have been detected at much lower levels (Jadczyszyn and Stachyra 2005; Bojarska et al., 2007). This decreased detection appears to have occurred because research methods did not adjust to technological changes that had occurred in wastewater treatment processes, primarily the dehydration of sludge. These processes included the application of flocculants, which turn fine organic particles suspended in wastewater into permanent floccules, facilitate its dehydration and consequently, considerably hinder the detection of parasite eggs in sewage sludge samples.

Considering the absence of parasitological research methods adjusted to this type of matrix, the Department of Parasitology and Diseases developed a method to examine parasites in dehydrated sewage sludge. The main principles of this method are to eliminate the effect of flocculants by mechanically damaging floccules in the presence of surfactants and isolate eggs in large volumes of floating and sedimentation liquids.

The present study aimed to validate the developed method, primarily to determine its parameters, including sensitivity, determination limits, quantification limits, coefficients to calculate the actual number of eggs in a sample based on the number of eggs isolated, and to compare the efficiency of the method with the methods most frequently applied in Poland and worldwide.

2. Materials and methods

2.1. Ascaris, Toxocara and Trichuris parasite eggs

Parasite eggs were obtained from the uteri of adult female nematodes isolated from animal intestines naturally infected with these parasites. *Ascaris* spp. eggs were obtained from nematodes isolated from the intestines of pigs killed in a slaughterhouse (*Ascaris suum*). *Trichuris* spp. eggs were isolated from nematodes isolated from a dead, naturally infested dog from the Puławy Province (*Trichuris vulpis*). *Toxocara canis* eggs were obtained from nematodes excreted with stool from intensely infested dogs. The eggs obtained from the isolated parasites (conserved in 1% formalin solution) were placed in an ultrasonic cleaner and subjected to ultrasound for 15 min to break down the forming agglomerations of adhering eggs. Subsequently, the suspension was mixed with a magnetic stirrer for 10 min and stained with 2% eosine solution. After mixing, the eggs were mixed with a magnetic stirrer for 10 min, remained at room temperature for 24 h, and subsequently stored at approximately 4 °C.

2.2. Sewage sludge

The study utilised sewage sludge samples from 10 mechanical-biological municipal water treatment plants, which differed in the size of agglomeration and localisation (various regions of Poland). All water treatment plants applied polyelectrolytes (flocculants) to dehydrate sewage sludge. Sludge was dehydrated using a press at each treatment plant. A 3-kg sample of hydrated sludge was directly obtained from the press at each treatment plant. In addition, at 1 water treatment plant, a sample of hydrated sludge was obtained before the flocculation stage, i.e., prior to the addition of polyelectrolytes. This sludge was used to prepare the samples with an admixture of a known number of parasites. In all samples, dry mass was determined using a moisture analyser (according to the manufacturer's instructions).

2.3. Flocculant

A 0.2% solution of PRAESTOL 644 BC—a multi-particle polyelectrolyte based on a cationic derivative of strong cationic acrylamide (Stockhausen GmbH AG, Krefeld, Germany)—was applied. A granulate flocculant applied during water treatment plant processing was prepared in accordance with the manufacturer's instructions, i.e., 10 kg of flocculating agent per 1 m^3 water from the water supply system, mixed for approx. 1 h. The prepared 0.2% solution was added at 2000 l/hour, i.e., approx. 20 m^3 of sewage suspension. The 0.2% flocculant solution used in this study was obtained directly after mixing it with water from the water supply system. Further dilutions were performed in the laboratory during sewage sludge sample preparation with a known number of eggs.

2.4. Obtaining samples of dehydrated sludge with known numbers of parasite eggs

Sewage sludge samples obtained from the wastewater treatment plant before dehydration were used to prepare supplemented samples to make the experimental and plant conditions nearly identical. Parasite eggs were added to these sewage samples, and subsequently, the samples were subjected to flocculation and dehydration under laboratory conditions. A specified number of eosine-stained *Ascaris, Trichuris* and *Toxocara* eggs were added to a 300-ml hydrated sewage sludge suspension without well-mixed flocculant. Approximately 100 μl of a previously well-mixed