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Effect of temperature and shear stress on the viability of *Ascaris suum*

J. E. Thomas, J. T. Podichetty, Y. Shi, D. Belcher, R. Dunlap, K. McNamara, M. V. Reichard, J. Smay, A. J. Johannes and G. L. Foutch

ABSTRACT

Ascaris eggs are commonly used as a bioindicator to test the success of waste treatment systems. The ability to inactivate this organism's eggs indicates probable destruction of a variety of pathogens. The intent of these experiments was to determine operational parameters for a conical-augur device to treat human solid waste in a time-efficient manner (seconds), without exogenous chemicals (e.g. lime, an alkalizing agent) or an external heat source. Literature reviews and independent viscometry experiments established a target temperature range and residence time within the device. The ability of applied shear force to inactivate *Ascaris suum,* directly or indirectly, was assessed using viscometry. Shear force alone, as friction, failed to inactivate *A. suum* eggs. However, shear force was used to generate sufficient heat in a human-fecal simulant to inactivate *A. suum* eggs under a variety of test conditions. Target operating conditions for the conical-augur device were 70 °C with a 6-s residence time; increasing the temperature allowed for reduced residence time to achieve *A. suum* inactivation.

Key words | *Ascaris suum*, rheology, viscous heating

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INTRODUCTION

Endoparasites, such as *Ascaris lumbricoides*, infect around 1 billion people worldwide and result in malnutrition, malabsorption, and in some cases obstruction or rupture of the intestinal wall (Cash & Glass 2010). Another parasite, *Ascaris suum*, is closely related to *A. lumbricoides* with identical life cycle and genetic similarity. *A. suum* can cause similar clinical signs in swine as *A. lumbricoides* causes in humans (Bowman 2014). Owing to similarities between the digestive systems of humans and swine (Swindle & Smith 2000), *A. suum* in pig feces is an ideal model for controlled studies to evaluate conditions for sterilization.

A. suum eggs are highly resistant to adverse environmental factors. *Ascaris* spp. eggs remain viable for years in the environment, given appropriate conditions (Feachem *et al.* 1983). In contrast, under similar conditions other pathogens may survive only for weeks (protozoa) to months (many bacteria, viruses). Compared with other gastrointestinal parasites, *Ascaris* spp. remain viable under more adverse conditions (e.g. other nematodes, cestodes, trematodes, protozoa). For these reasons, *Ascaris* eggs are commonly used as a bioindicator to test waste treatment systems, since inactivating this organism's eggs indicates probable destruction of a variety of pathogens, including other parasites, bacteria, and viruses (National Research Council 2002; Bowman *et al.* 2003).

Ascaris suum Goeze (1782) serves as a model for *Ascaris lumbricoides* Linnaeus (1758). *A. suum* and *A. lumbricoides* eggs are morphologically indistinguishable from one another, and some sources argue that they are the same species (Leles *et al.* 2012). However, most modern authors believe they are separate, but similar, species (Bowman 2014). The taxonomic status of these species remains

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uncertain, after both single and multilocus genetic comparison (Anderson *et al.* 1993; Peng *et al.* 1998, 2003; Zhu *et al.* 1999, 2000; Anderson 2001; Zhou *et al.* 2011) and proteomic evaluation (Xu *et al.* 2013). Owing to the similarities, *A. suum* is often used in the testing of waste and water treatment systems as this species exhibits seemingly identical biological behavior to *A. lumbricoides*, but is easier to obtain (National Research Council 2002; Bowman *et al.* 2003). Feces containing unembryonated eggs can be obtained in large volume from abattoirs, or eggs can be dissected from the uteri of female worms and used to spike waste or water samples for testing (Costello 1961).

Ascaris spp. eggs are highly resistant to mechanical disruption, which is one of many reasons why they remain so persistent in the environment. Rupturing *Ascaris* spp. eggs requires 110–206 MPa, when passing them through a French pressure cell (Costello & Brown 1962; Ward & Fairbairn 1972; Goldstein & Straus 1978). Though this system is useful for obtaining DNA for molecular analysis, egg destruction by this method is impractical for large-scale processing, such as waste treatment. The pressure sufficient to inactivate *Ascaris* spp. eggs may not alter the morphological appearance of the eggs (Rosypal *et al.* 2007). The use of high hydrostatic pressure, as available in commercial food high pressure processing units, has been investigated for the inactivation of *Ascaris* sp. eggs. Though the data are limited, pressures $\geq$300 MPa for 60 s were sufficient for inactivation, and treatment with 207–250 MPa delayed embryonation by 4–16 days (Rosypal *et al.* 2007, 2011). Treatment with 241–250 MPa for 60 s resulted in >95% inactivation of *A. suum* eggs (Rosypal *et al.* 2011). To the authors' knowledge, the ability of shear force to disrupt or inactivate *Ascaris* spp. eggs mechanically has not been tested, and literature references regarding this method of *Ascaris* spp. egg treatment could not be found.

Numerous studies have evaluated the impact of temperature on the inactivation of *Ascaris* spp. (Seamster 1950; Brannen *et al.* 1975; Feachem *et al.* 1983; Pecson & Nelson 2003; Capizzi-Banas *et al.* 2004; Pecson *et al.* 2007; Hawksworth *et al.* 2010; Maya *et al.* 2012). Many of these studies consider other factors (e.g. pH, moisture content) in addition to temperature. However, waste treatment processes common in industrialized regions (i.e. liming) are impractical for many regions of the world where ascariasis is most common (Crompton 2001). In addition, these studies investigate heat treatment over longer time periods (days to months). The use of heat treatment for short periods of time (seconds to minutes) is of greatest interest for the development of new human waste sanitation methods.

We have extended viscous heating technology to sanitize fecal sludge and present experimental data of the inactivation of *A. suum* in a human-fecal simulant (HFS). The purpose of this study was to develop a treatment system which works in seconds, allowing for the rapid processing of human waste into a safer substrate. This paper examines the effect of the following conditions on *A. suum* embryonation rates: (1) shear force alone; (2) heat alone, at short time intervals; and (3) shear force and heat combined. Target temperature range and processing times were established, and the most efficient combination of shear force and thermal parameters for *Ascaris* spp. egg inactivation was determined. The results of initial experiments were used to define the viscosity of the HFS that allows shear force to generate heat by molecular friction sufficient for *Ascaris* spp. inactivation. These parameters were validated in a modified version of a previously described conical-augur device for human fecal sanitization (Podichetty *et al.* 2014).

MATERIALS AND METHODS

All studies were conducted at Oklahoma State University (Oklahoma, USA), in the School of Chemical Engineering and the Department of Veterinary Pathobiology, Center for Veterinary Health Sciences.

Determination of initial target temperatures using a comprehensive literature review

A literature review was conducted, compiling experiments testing the heat and time required to inactivate *Ascaris* spp. eggs in waste treatment systems. Both peer-reviewed and non-peer-reviewed literature were considered. Data extracted from these sources were plotted along with the Vinnerås *et al.* (2003) equation utilized by the United States Environmental Protection Agency for solid waste treatment systems. These results were used to establish initial target temperatures for use in the short-contact time studies detailed below.

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Parasite material

A. suum eggs were purchased from a commercial vendor who collected porcine gastrointestinal contents from an abattoir and isolated the *A. suum* eggs using a proprietary water filtration method (Excelsior Sentinel, Inc., Trumansburg, NY). Eggs were shipped in a temperature-controlled container by overnight courier and stored at $4 \pm 0.5\,^{\circ}$C. Upon arrival, a small aliquot of *A. suum* eggs were examined by light microscopy (Olympus BX51, Olympus, Melville, NY) to ensure embryonation had not begun and that unfertilized eggs represented $\leq$0.01% of the population. Eggs with and without the outer proteinaceous coat were present.

Preparation of HFS

A HFS was prepared to ensure that *A. suum* was the only pathogen present in the test material. Since pigs are monogastrics, like humans, and both can produce feces of similar consistency, feces from Class A pigs fed a commercially available diet (Teklad Lab Animal Diet, Harlan, Madison, WI) were collected and homogenized. For every 2.5 kg of feces collected, a fecal flotation using Sheather's sugar solution (Zajac & Conboy 2012) was performed to confirm lack of *A. suum* eggs in the starting material.

Homogeneous, small particle fecal samples were prepared by mixing pig feces 1:1 (v/v) with water and processing in a standard household blender until smooth. The blended solution was strained through a coarse mesh strainer and the flow-through aliquoted into 50 mL centrifuge tubes. Samples were centrifuged at $1{,}950 \times g$ (approximately 3,700 rpm) for 15 min and all material with particle size <600 µm pooled. The pooled material was mixed on a stir plate, weighed to the nearest gram, and heated using a standard laboratory hot plate. Once boiling, powdered instant mashed potatoes were added at 4:1 (w/w) feces to potatoes and mixed thoroughly to achieve the desired viscosity. Potatoes prepared as described in Podichetty *et al.* (2014) approximate closely the viscous behavior of human feces. Processed fecal material was placed in a sealed container at $4\,^{\circ}$C until use. The resultant HFS approximated a type 4 fecal sample using the Bristol stool form scale (Lewis & Heaton 1997). No additional viscosifier (e.g. methylcellulose) was necessary. The HFS was spiked with *A. suum* eggs at concentrations of 500–1,000 eggs/gram. The viscosity of the prepared HFS was determined prior to testing in the shear force study and the viscous heater runs. In addition, this analysis was repeated on samples after processing through the viscous heater to determine the effects of the equipment on material composition.

Effect of varying temperature (only) on *Ascaris suum* embryonation

HFS (0.6 g) spiked with approximately 500 *A. suum* eggs was thoroughly mixed inside a 7 mL polyethylene (PE) scintillation vial. The HFS was spread as a thin layer around the inside of the vial to ensure equal temperature distribution throughout the sample. The layer of HFS was approximately 1 mm thick. Samples were sealed, wrapped in Parafilm to ensure water did not enter the scintillation tubes, and submerged fully in the water bath for 60 s at 10 degree intervals from 40 to $100\,^{\circ}$C. Temperature was verified using three NIST-traceable thermometers placed at varying points within the water bath. The scintillation vials were manually moved through the water for greater confidence that the desired temperature was in contact with the exterior of the tube at all times. After 60 s submergence, the tubes were removed and placed immediately in a $27\,^{\circ}$C water bath for 60 s to return the sample to ambient temperature quickly. Samples were processed and cultured as described below.

Ascaris suum culturing and analysis

A. suum was cultured according to the methods established by the United States Environmental Protection Agency (USEPA 2003). Incubation was extended to 28 days as it takes a minimum of 27 days for the infective stage (L3) to develop (Geenen *et al.* 1999). Final analysis of samples was performed using Sheather's sugar solution (SP = 1.2) to allow for analysis at higher levels of magnification than can be performed using a Sedgewick rafter cell. Early analyses using zinc sulfate fecal flotation (SP = 1.2) and the coverslip hatching method (Eriksen 1981 as described in Geenen *et al.* 1999) were quickly abandoned as the larval deformation and salt crystallization decreased the ability

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to provide accurate counts. Distinction between L2 and L3 stage larvae was not performed, as differentiation requires use of interference or phase-contrast microscopy and would have required additional incubation time (Geenen *et al.* 1999). The presence or lack of formed *Ascaris* spp. L2/L3 in an egg was used to determine percentage embryonation. Morphologic appearance of the L2/L3, including vacuolated or granular, was not considered as there are no sources which demonstrate differences in viability based on these internal structures. In addition, even L2/L3 with vacuolated or granular appearances were stimulated to move within the egg by the light and heat provided by the microscope, as described in Bowman *et al.* (2003). Eggs which contained embryos arrested at a stage of development prior to L2/L3 (i.e. 2-cell stage, 8-cell stage, etc.) were considered inactivated given the extended culturing parameters.

Viscometry operation and data processing

To determine the effects of shear alone on *Ascaris* eggs, rheometer studies were performed using *A. suum* eggs in water and *A. suum* eggs in HFS. Temperatures were chosen based on the literature review. *A. suum* eggs were diluted in distilled water to obtain a solution of approximately 200 eggs/mL. The solution was sheared using a high-precision stress controlled rheometer (Bohlin C-VOR 200, Malvern Instruments, Westborough, MA) under isothermal conditions at $1.5 \pm 0.2\,^{\circ}$C. The measuring system used was CP 4°/40, which consists of a 40 mm diameter stainless steel cone with a 4° angle and 60 mm diameter stainless steel plate. The minimum gap between cone and plate is 0.15 mm. The shear rates applied were 10, 100, and 1,000 s^{-1}. A maximum shear rate of 1,000 s^{-1} was applied to the water-based samples due to the low viscosity and resultant splashing above this rate. The applied time intervals were 60, 300, and 600 s. A solvent cover was used to prevent sample drying when temperatures were increased above ambient conditions.

HFS with a viscosity greater than 5 Pa.s was used as the default sample condition. The mixture had a viscosity of around 20–40 Pa.s at $25 \pm 0.5\,^{\circ}$C and shear rate of 1 s^{-1}. The measurement was performed using both CP 4°/40 and C-14 measuring systems. Water was used as a temperature control fluid and circulated in the jacket surrounding the system. A constant shear rate of 1,500 s^{-1} was applied to samples for 300 s each at four different temperatures. A heated jacket circulated water at 47, 51, 55 and 60 °C. The loading time for each sample was approximately 120–180 s, followed by the constant shear rate time; then sample removal from the testing cup to collecting tube took 120 s.

The C-14 concentric measuring system was used which consists of a rotating bob with a diameter of 14.0 mm inside a fixed cup with a diameter of 15.4 mm. The portion of the sample undergoing applied shear is contained in the annular gap between the bob and inner walls of the fixed cup. Some portion of the sample is located below the bob and experiences the applied temperature but no significant shear force. A second set of shear/temperature samples were processed due to a conductive heat transfer delay on temperature. The target temperatures were set to 60, 70 and 75 °C. After loading the HFS, a 1,200-s waiting time was introduced to allow the sample to achieve approximately 90% of the target temperature. Constant shear rate of 1,500 s^{-1} was applied for 300 s. This time, samples within the annular gap were collected as the treated group with both shear and temperature. Separately, samples were collected from between the bob bottom and cup bottom that experienced the same temperature treatment but without significant shear. These samples were used as the control group.

Conical viscous heater setup and operation

Podichetty *et al.* (2014) discussed the technology required to use viscous heating caused by molecular friction to generate sufficient heat to deactivate infectious organisms. By passing a high viscosity mass through a high shear zone significant heat is observed. An example is polymer injection molding processes where temperatures exceed the melting point. Podichetty *et al.* (2014) describe a conical-augur device that generated heat in simulant materials. This device – essentially a small-scale reactor – was used as part of this study to supply confirming data for the calculated parameters before a field-test device and subsequent commercial-scale viscous heaters were designed.

The viscous heater was set up and operated as in Podichetty *et al.* (2014). The viscous heater was modified to

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prevent any leakage or pressure loss, a groove was incorporated and a rubber O-ring was placed close to the outlet. An O-ring was added around the lever that controls gap variation, which allowed for smooth gap changes. A nut was added on the motor end of the inner rotating core to improve system alignment. The ball valve was replaced with a gate valve to prevent clogging of the outlet pipe. Insulation was added to the entire outer surface, including the outlet, to reduce heat loss to the surroundings. During operation, a constant gap space of 0.75 mm was used, the rpm was set to 1,800 and temperature was monitored at specific flow rates. The residence time was calculated from the total mass through the device in a measured amount of time while maintaining constant flow rate. Temperature was measured at both the terminal end of the viscous heater and at the outlet by direct contact with the HFS and a thermocouple. The temperatures listed for the core are nominal (T_c).

RESULTS AND DISCUSSION

Comparative literature data

Numerous studies have evaluated the impact of temperature on the deactivation of *Ascaris* spp. Many of these studies considered additional factors such as moisture content and alkaline concentration. Though the authors' main focus was on temperature, analysis and comparison of literature data notes secondary effects as well. All the results extracted from the discussed literature are presented in Figure 1.

Seamster (1950) reviewed the viability of *A. suum* and *A. lumbricoides* near the optimal 33 °C growth temperature. The effects of numerous chemical agents and humidity in addition to temperature were included. While temperature is not discussed in detail, a single data point indicates that 8 days at 37.8 °C 'proved lethal'. Numerous publications discuss the effects on *Ascaris* species at lower temperatures – typical of an ambient local environment. Examples include the work of Berggren *et al.* (2004) and Kim *et al.* (2012). We are interested in a shorter contact time than experimentation defining fate in the environment or composting conditions so much of these data are not included in this discussion.

Hawksworth *et al.* (2010) present data for *A. lumbricoides* over a temperature range from 10 to 60 °C and in conditions of high and low relative humidity (RH). Though the authors state the two relative humidities tested to be 0 and 100%, the experimental conditions described cannot achieve those parameters. At 60 °C die-off occurred within 24 h, irrespective of humidity. At 50 °C and 40 °C, a significant variation existed with humidity. For complete die-off

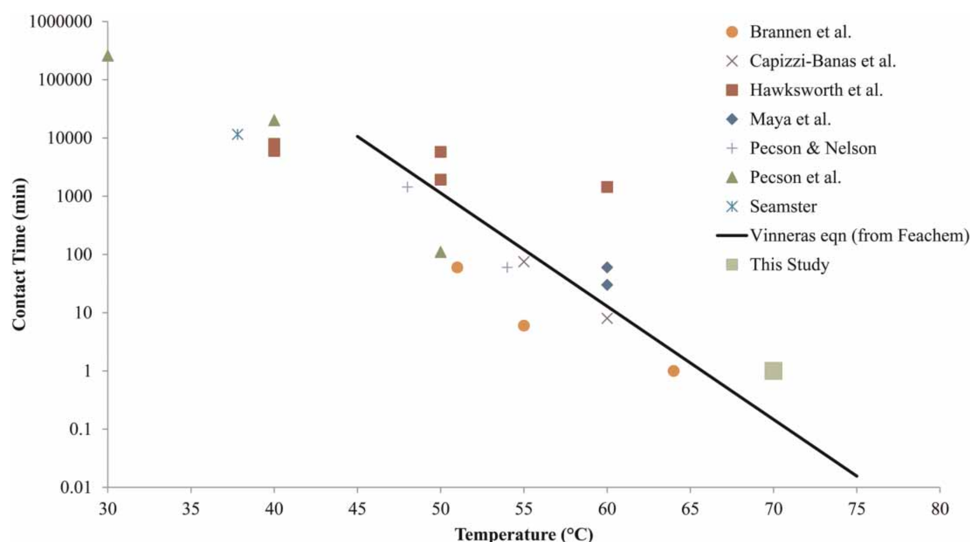

Figure 1 | Selected literature data of complete *Ascaris* spp. egg inactivation plotted with cited temperature only.

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at 50 °C, 96 and 32 h were required at the tested high and low RH, respectively. At 40 °C, 132 h and 100 h were observed at high and low RH, respectively.

Maya *et al.* (2012) included *A. suum* and *A. lumbricoides* in a study of the impact of temperature, pH and dryness for both eggs which were placed in the testing system with larvae developed and fertile, undeveloped eggs, which the author calls larval and non-larval eggs, respectively. They inoculated eggs into sterilized sludge and exposed samples to defined conditions. Tables presented conditions where 100% inactivation was observed. At 80 °C, fertile, undeveloped *Ascaris* spp. eggs were inactivated after treatment for 3 h in samples with 90 and 95% total dry solids and were inactivated after 2 h in sludge composed of 80% total dry solids. Eggs which had already undergone development to larval forms were inactivated at 60 °C for 1 h in 80, 90 and 95% total dry solid sludge samples. Additional data were presented in plots of inactivation versus temperature and inactivation versus time. Times for both *Ascaris* species were similar and the authors state the differences were negligible, but no statistical analysis was provided. Only 35 eggs of each species were examined for each sample, so statistical power is highly limited.

Aitken *et al.* (2005) reported limited data for *A. suum*. At 50 °C, sludge treatment time was 5 days while 1 day was sufficient at 55 °C. Samples were for sludge with less than 7% solids. Pecson *et al.* (2007) presented data on sewage sludge within a temperature range from 20 to 50 °C and pH from 7 to 12. At 50 °C pH effects were negligible and temperature dominated with 1.83 h for 99% inactivation at pH 7. Inactivation (99%) at 40 °C was 14 days at pH 7. Capizzi-Banas *et al.* (2004) performed experiments at 50, 55 and 60 °C with varying concentrations of lime and quick lime. Data ranged significantly with treatment levels; however, the maximum times at each temperature that yielded complete inactivation were reported as 1.25 h at 55 °C and 480 s at 60 °C. More severe lime treatment reduced these times to 300 s and 60 s, respectively. Popat *et al.* (2010) performed experiments with *A. suum* at three temperatures up to 55.5 °C. Sludge with 2% solids was sampled at 30-minute intervals for 2 h with larger intervals after that. The authors concluded a 2-log deactivation within 2 h at the highest temperature. Examination of the plotted data indicates that the 2-log deactivation occurs at some point between the 1.5 and 2.0 h samples. Pecson & Nelson (2003) presented inactivation data for *Ascaris* eggs as a function of temperature, pH and ammonia concentration. They do show a baseline case for temperature only that has complete deactivation at 48 °C for 24 h contact time. They also cite unpublished data of 1 hour at 54 °C.

Sandia Laboratories published two reports (Brannen *et al.* 1975; Brandon 1978) and presented data for *A. lumbricoides* deactivation over a somewhat higher temperature range. Data included compost, water, and sludge with heat and/or radiation. Sample size and replications were not included in either report. For heat only, near-complete deactivation they cite 1 h at 51 °C, 360 s at 55 °C and 60 s at 64 °C. Samples exposed to these temperatures were 50% sludge in water. Data from plots for compost and in water are challenging to read, but inactivation times at 60 °C are approximately 120 s in water and 300 s in compost.

Some publications give data that are significantly different. Steer & Windt (1978) show laboratory incubator data of 5 days until complete deactivation at 50 °C. They also present pilot plant data showing 7 days for inactivation. However, for this case the temperature was not constant throughout the experiment ranging from a low of 36 °C to a high of 60 °C with an approximate average of 48 °C.

To examine temperature effects on *Ascaris* spp. egg inactivation, an accumulation of literature data was plotted. We have included one data point from our water bath experiment above. In most cases, the data included in Figure 1 examines secondary influences as well; for example, the impact of caustic chemicals or anaerobic conditions. So for any two separate data points, a direct comparison may not be valid without a discussion of these secondary effects. Some of the literature data defines complete inactivation as 99 + % and this figure does not separate those data points. The line in Figure 1 is the equation developed by Vinnerås *et al.* (2003) based on the collected data of Feachem *et al.* (1983) which are not duplicated in the accumulated data points. Their correlation begins at 45 °C and does not extrapolate lower.

In comparing the accumulated data, only a very general trend can be interpreted from Figure 1 because data have wide ranges of variation due to significant experimental variability among all researchers. In addition, time scales become overstated. For example, our data point in

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Figure 1 at 70 °C (discussed below) may, in fact, occur at lower temperature or at lesser time. This is because there was no data collection between 60 and 70 °C and contact time was limited to only 60 s. However, in general, all of the collected data appear reasonably consistent with the Vinnerås *et al.* (2003) equation of the Feachem *et al.* (1983) data.

Effect of varying temperature (only) on *Ascaris suum* embryonation

An initial set of experiments with *A. suum* eggs was performed to evaluate the impact on embryonation using temperature alone. The data presented in Figure 2 are restricted to exposure at 60 s. Rates of embryonation at 47 °C, 51 °C, and 55 °C were 94.7%, 91.1%, and 89.7%, respectively. Two independent samples were tested and evaluated at 60 °C that showed a statistically discernible difference ($X^2 = 71.032$, df = 1, $P = <0.001$) between each other, but consistent with the overall trend of decreasing embryonation with increase in temperature. The data indicate a significant reduction ($X^2 = 42.866$, df = 1, $P = <0.001$) in embryonation between 60 and 70 °C. The 70 °C data point in Figure 2 has been included for comparison with the literature data in Figure 1.

Temperature rise due to viscous heating

When operating the viscous heater with a 5 Pa.s fluid the effluent temperature increased for 150 s typically and approached steady state. Figure 3 shows core and effluent

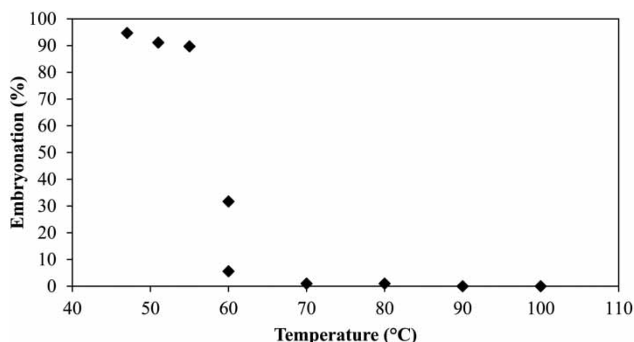

Figure 2 | *Ascaris suum* embryonation percentage in samples treated at constant temperature for 60 s. Sample sizes were in the range 91–399 *Ascaris suum* eggs per culture condition.

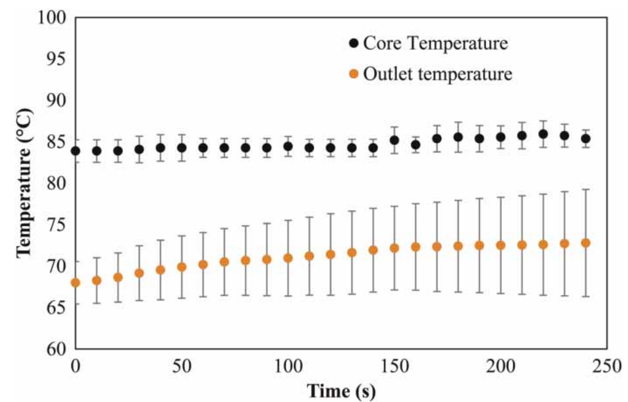

Figure 3 | Core and outlet temperature for 240 s run.

temperatures for a 240 s run with pig feces. The temperature of the feces in the equipment increases until the viscosity is decreased, resulting in reduced intermolecular friction and no further rise in temperature. The core temperature remained nearly constant throughout the run. Run lengths were limited to the volume of HFS contained in the feed reservoir.

Initial viscometry experiments supported literature studies indicating that a target temperature range for the device effluent of 50–85 °C should encompass the condition that would inactivate *A. suum* in pig feces. Experiments above an outlet temperature of 85 °C and with residence time greater than 240 s are not discussed as embryonation rates in effluent samples near zero were observed.

Pre-run and post-run viscosity analysis of HFS

Samples of material collected after processing in the viscous heater showed a reduction in viscosity compared with the feed material. The effect is minor and not outside experimental error bars. With some loss of mass attributable to the vaporization of water as a result of heat generation, this may appear unexpected. However, contributing factors include the following: the high temperature and pressure inside the viscous heater can degrade organic components into lower molecular weights; and there is a significant mixing effect that homogenizes the components in the feed material and allows for greater flowability. A smoother texture is observed at the outlet. Figure 4 presents the

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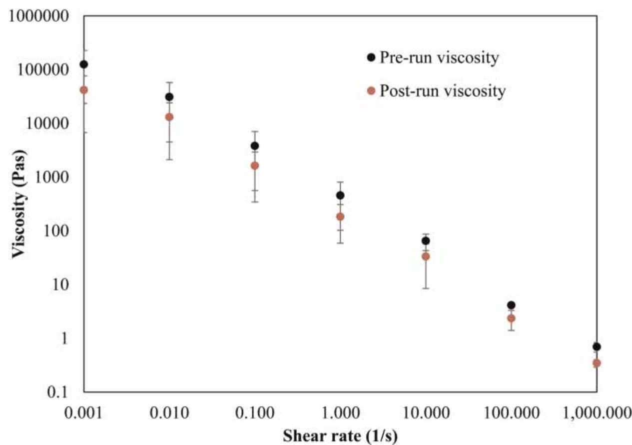

Figure 4 | Pre-run and post-run viscosity of pig feces.

observed viscosity differences in pre- and post-run samples at the same shear rate.

Shear with temperature

As mentioned previously, when the temperature was below 60 °C, there was no significant difference in embryonation between the sample exposed to shear (1,500 s^{-1}) plus a given temperature and exposure to that same temperature alone. The results of the temperature-only studies above support these findings. The rheometer experiments were repeated at higher temperature for confirmation. Based on placing multiple thermometers to measure temperature in the C14 sample well, three temperatures above 70 °C were tested. No embryonation was observed in either the shear plus temperature or temperature controls from the rheometer ($n = 1,759$). Controls for this study had an 87.2% embryonation rate. The lack of development in these samples (all over 70 °C) is supported by the temperature-only studies above.

Analysis of HFS for parasite destruction

The *A. suum* embryonation data from these experiments are presented in Table 1. Embryonation rates were at or near zero at temperatures above 70 °C. Comparison of the rates of embryonation for all temperatures and residence time were statistically discernible (Table 1).

CONCLUSIONS

The three experiments presented – water bath, viscometer and viscous heater – along with the literature review, point to a target temperature of 70 °C as the minimum required to deactivate *A. suum* for short contact times. Using the line of Vinnerås *et al.* (2003) at 70 °C requires a contact or residence time of 6 s. Operating at higher temperatures can reduce the required residence time. The Vinnerås *et al.* plot extends to 75 °C, indicating a 1.2 s residence time requirement. The goal of any viscous heating device would be to operate at these or higher temperatures while ensuring the corresponding residence time. There is no indication that shear alone has an impact on deactivation of *A. suum* over the range of conditions tested. Temperature increase should be the sole mechanism considered for the design of viscous heating equipment to deactivate *Ascaris* spp.

Table 1 | Numbers of *Ascaris suum* eggs observed at 28 days from samples of pig feces processed through a conical viscous heater

Core temp (°C)	Residence time (s)	Embryonated (n = # of eggs)	Not embryonated (n = # of eggs)	Embryonation (%)	X^2 results
70	80	3	7,269	0.04	$X^2 = 8{,}103.5$, df = 1, $P = <0.001$
70	30	0	6,842	0	$X^2 = 7{,}740.9$, df = 1, $P = <0.001$
80–81	43	0	9,127	0	$X^2 = 9{,}745.8$, df = 1, $P = <0.001$
80	25	4	7,448	0.05	$X^2 = 8{,}256.2$, df = 1, $P = <0.001$
69–82[a]	6	4	17,677	0.02	$X^2 = 17{,}167.0$, df = 1, $P = <0.001$
100 +	2	0	14,135	0	$X^2 = 14{,}116.6$, df = 1, $P = <0.001$
Control	–	2,174	330	87	

[a]Most of time at the lower temperature with higher spikes.

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