Prevalence of intestinal parasites in pig manure and the potential for zoonotic transmission in urban/peri-urban areas of Morogoro municipality, Tanzania

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Abstract

Information on the prevalence and potential health consequences associated with the presence of parasites in livestock manures is lacking in many developing countries. In 2015, a cross-sectional study was conducted in 79 pig farms to estimate farm-level prevalence of helminth eggs using McMaster technique and protozoan parasite oocysts using the Modified Ziehl–Neelsen technique in Morogoro municipality, Tanzania. Farm-level representative samples of manure were collected from pig manure disposal sites in each farm. Pig farmers' knowledge and practices related to pig husbandry and manure handling were assessed using a structured questionnaire.

All pigs were reared indoors and their manures were handled in solid form. Farm-level prevalence of strongyle eggs was 7.6% (95% CI: 1.8, 13.4) and that of *Ascaris spp.* was 6.3% (95% CI: 0.9, 11.7). Prevalence of acid-fast protozoan oocysts was 6.3% (95% CI: 0.9, 11.7), including*Isospora spp.*, *Cryptosporidium spp.* and *Eimeria spp*. Approximately 27.8% (95% CI: 17.9, 37.7) of the samples had acid-fast objects that resembled microsporidia and other fungal spores. About 94.9% (95% CI: 90.0, 99.8) of the farmers reported to have experienced diseases in their pigs, commonly helminthosis. Only about 42.5% (95% CI: 31.3, 53.9) believed that pig manure could cause some human health problems. Approximately, 65.3% (54.3, 76.3) reported to clean their pig pens daily. About 61.1% (95% CI: 49.9, 72.3) reported to wear special clothing when removing pig manure. Handling of pig manure is associated with potential biological hazards with potential for zoonotic transmission in urban/peri-urban areas of Morogoro municipality, Tanzania. Further studies are needed to measure the actual risks associated with livestock manure handling and utilisation including parasite viability and characterization studies to guide implementation of appropriate bio-security and bio-safety measures in urban/peri-urban livestock farms.

Key words: *bio-security, peri-urban farming*

Introduction

Livestock manure is an organic matter derived from livestock faeces, which can be used as organic fertiliser, a source of bio-fuel or feed for livestock and fish (Müller 1980). Manure can either be handled in liquid form (slurry) or in solid form (farmyard manure). In many countries (Tanzania inclusive), most manure is applied directly to farm lands or only after some period of storage in pits or heaps. In semi-intensive and free range systems, livestock dispose of their faeces directly on farms. A recent global assessment revealed a wide variation of manure management practices, with a common trend on the difficulty in managing urine and liquid manure especially in non-mechanized situations and on smaller farms because of labour demand (Teenstra et al 2014). In a few areas where manures are treated before use, the main reasons for the treatment are odour control, energy recovery, reduction of manure volume, reduction of nutrient content and enhancement of the decomposition of manure (Teenstra et al 2014; Beaulieu 2004). Most of manure treatment methods also automatically reduce the number of microorganisms that are present. In general, the more treatment technologies used by a manure management system, the lower the microbial survival (Cole et al 2008). A simple storage of manure may not attain any significant reduction in the levels of microorganisms (Pourcher et al 2007), especially taking into account that new manures are continually added on the top and they may be applied to land without sufficient decomposition.

Globally, much concern on the use of manures has been on air pollution (e.g. by ammonia, nitrous oxide and methane gases), soil accumulation of minerals (e.g. P, Cu, and Zn) or water pollution through the mechanism of leaching of soil nitrates (Jongbloed and Lenis 1998). However, there is emerging evidence of potential microbial hazards that may be associated with manure (Jongbloed and Lenis 1998; Fayer et al 2000; Gajadhar and Allen 2004; Cacciò 2005). Manures can contain pathogenic bacteria, viruses, fungi and/or parasites that may cause infections in humans and animals. Diaz et al.

(1991) noted that the most frequently found parasites in intensified pig farming are *Ascaris suum*, *Oesophagostomum dentatum*,*Trichuris suis*, *Strongyloides ransomi*, *Balantidium coli* and *Hyostrongylus rubidus*. Some studies have found that *A. suum* and *T. suis* are transmitted by eggs that are long-lived and highly resistant to environmental factors. For example, *A. suum* eggs have been demonstrated to remain alive in soil for up to 14 years (Strauch 1991). *Ascaris* is one of the most common worms infecting humans worldwide, with approximately 25% of the global population infected with this parasite (O'Lorcain and Holland 2000). *Cryptosporidium* oocysts have been found to remain viable for about 18 months in a cool damp or wet environment (Anonymous 2008). Despite the fact that cryptosporidiosis in pigs does not always result in clinical signs, human cases infected with *C. suis* and *C. scrofarum* suggest that the two pig-adapted *Cryptosporidium* species are potentially zoonotic (Carciò 2005).

While a few studies have estimated prevalence of parasites in faecal materials collected directly from pigs in Tanzania (Esrony et al 1996; Esrony et al 1997; Ngowi et al 2014), to date there is not any study that has estimated prevalence of parasites in manures after they have come out of the pig environment. This information is necessary to guide appropriate measures to prevent transmission of infectious agents between animals and humans and amongst animals. The overall objective of this study was to estimate prevalence of helminths and protozoan parasites in pig manure and assess potential health consequences associated with pig and pig manure handling in urban and peri-urban pig farms in Morogoro municipality, Morogoro region, Tanzania.

Materials and methods

Study area

This study was conducted in urban and peri-urban areas of Morogoro municipality located between latitude 5.7-10°S and longitude 35.6-39°E with elevation of 500-600 m above sea level. The annual average rainfall ranges from 500 to 1800 mm with temperatures between 18°C and 28°C. Morogoro municipality is sub-divided into 29 administrative wards and 272 streets (Anonymous 2012). This study included eight of 13 wards that were keeping pigs based on the municipal livestock statistics.

Study design

A cross-sectional study was conducted between February and May 2015 to estimate farm-level prevalence of helminth eggs and protozoan parasite oocysts in stored pig manures in urban and peri-urban areas of Morogoro municipality, Tanzania.

Study population, sample size and sampling design

A pig farm was the unit of data collection and analysis. Because of lack of previous study that had reported farm-level prevalence of any pig internal parasite in Tanzania, sample size estimation in this study was based on information from pig-level studies. Esrony et al. (1997), reported pig level prevalence of various pig helminths, including *A. suum* (prevalence of 12%) and *T. suis* (5%). A recent study by Ngowi et al. (2014) in a nearby district of Kongwa in Dodoma region reported prevalence of 3.9% *A. suum* and 3.2% *T. suis*. In the present study we assumed a farm-level prevalence of 5% *A. suum* or *T. suis*, being parasites of our interest because of their potential zoonotic implications. Based on the current Morogoro municipal livestock statistics it was estimated that Morogoro urban and peri-urban areas would have about 500 pig farmers. Being a finite population, the sample size was adjusted to take this into account. First, the sample size was calculated at pig level using the formula for simple random sampling (Martin et al 1987):

$n=z^2pq/d^2$ where: n= sample size, z=standard normal deviate (1.96 for 95% confidence level), p=known or estimated proportion of the parameter in the target population estimate, q=1-p, d=desired precision of estimation,

Thus for this study, $n= 1.96^2 \times 0.05 \times (1.0- 0.05)/ 0.05^2$, approximately 73 pig farms.

This sample size was first adjusted for multistage sampling by raising it by 25%, which resulted to approximately 92 pig farms. Finally, the sample size was adjusted for the finite population (N) using the formula $n_2= nN/(n + (N-1))$ (Martin et al 1987), where N = 500 pig farms. Thus at least 78 pig farms were required for this study.

Selection of study wards and farms

A random sample of eight out of 13 wards that were keeping pigs was selected. This is approximately 62% of the pig-keeping wards and was considered representative of the pig-keeping population. A ward is an administrative division consisting of several streets. In each ward, approximately ten pig farms were visited using a snowball sampling method because of lack of official registers of pig farmers.

Assessment of pig and manure handling practices

Prior to pig manure sample collection, a questionnaire was administered to the farmer to gather information related to pig management and manure handling practices, including number of pigs owned, husbandry practices, previous experience with pig diseases on the farm, disease control measures and manure handling practices. Other information included knowledge on health hazards associated with pig manure handling.

Collection and preservation of manure samples

A gloved hand was used to collect about ten faecal material of approximately 1 g each from different locations outside each pig pen and pooled to one sample for the farm. The sample included manure of various ages (fresh and old) if present at the sampling points. The samples were kept cool in a cool box while in the field and refrigerated upon arrival to the laboratory at Sokoine University of Agriculture (SUA), Morogoro, Tanzania

Sample processing and examination for helminth eggs and protozoan oocysts

All the laboratory protocols were performed as described by the World Organisation for Animal Health (OIE 2008). Manure samples were first concentrated using sucrose solution of 1.18 specific gravity and centrifuged at 3000 revolutions per minute for 5 minutes. The two-chamber McMaster slide technique was used to identify eggs of various helminth species under the microscope at a magnification of 100. For the identification of protozoan oocysts, the Modified Ziehl-Neelsen stain (MZN) technique was used. Confirmation of oocysts was done under microscope at 1000 magnification where the sizes of characteristic oocysts were measured. Micrographs were taken for records and further interpretation. The micrographs of the various oocysts and other acid-fast objects were further assessed by two to three analysts to strengthen the identification.

Data analysis

Prevalence of a parasite species or questionnaire response was calculated as the number of positive samples or responses divided by the total number of samples examined for the parasite or persons responded to the question. Associations between prevalence of the parasites and farm-level factors were assessed using Chi-square tests in 2 x 2 tables and considered significant if $p<0.05$.

Research ethics

Permission to carry out this study was granted by the municipal agricultural/livestock officer and all executive officers of the study wards. All questionnaire respondents verbally consented to participate in the study after clear information regarding the objective of the study was provided by the researchers who also allowed voluntary participation. All individual responses and information were kept confidential.

Results

General results

In total, 79 farms were visited, all of which were interviewed and manure samples collected. The total number of farms finally included in the study was based on the variability of number of pig farmers in the eight wards studied. All questionnaire respondents were adults. Of the 79 respondents, 38 were females, 35 males and six had their sex information missing because of an oversight by the recorder. Number of adult pigs owned by the farmers ranged from 1 - 62 (median 6) while piglets ranged from 0 - 30 (median 0). About 82.2% of the pig farmers were also keeping other livestock including cattle, sheep, goats, hare, dogs, cats, ducks, chicken, turkeys and pigeons.

Pig and pig manure handling practices

All pigs in all 79 pig farms studied were kept indoors during the time of visit to the farms and all farmers reported to permanently confine their pigs. Most farmers reported to have experienced different diseases in their pigs and received veterinary services (Table 1). Common disease conditions which necessitated frequent medication of pigs were helminthosis, diarrhoea and itching based on farmers' reports. All farmers were managing pig manure in solid form and most reported to remove pig manure from pig pens daily (Table 1). About 14.1% of the respondents (n = 71) reported to store pig manure together with manure from other livestock they were rearing. While all of 73 participants who responded to a question on use of tools when handling pig manure reported to use certain tools, only 61.1% (95% CI: 49.9, 72.3) reported to wear special clothes such as tracksuits, aprons, gloves or gumboots. Most farmers reported to store pig manure in a pit dug near the pig pen for some time before transporting them to farms. Nevertheless, disposal of manure on land surfaces were also commonly observed during the visits (Figure 1). One farmer who was keeping pig manure in bags outside his house pending transportation for disposal reported that previously the municipal council was collecting pig manure when collecting other household wastes but pig manure collection was later on excluded after the service was privatised.

Farmers' perceptions on health hazards associated with pig manure handling

Less than half of the farmers agreed that pig manure could cause some health problems to humans while an important proportion reported to have no idea (Table 1).

Table 1. Pig farmers' perceptions on health hazards associated with pig and pig manure handling in urban and peri-urban areas of Morogoro Municipality, Tanzania, 2015.

Factor	Answer	Number	Percentage (95% CI)
Has experienced pig diseases on his/her farm (n=79)	Yes	75	94.9 (90.0, 99.8)
Common treated problems (n=77)	Helminthosis	55	71.4 (61.3, 81.5)
	Others (including diarrhoea)	20	26.0 (16.2, 35.8)
	None	2	2.6 (-1.0, 6.1)
	No	17	23.6 (14.1, 33.1)
	Others	11	15.3 (7.3, 23.3)
Has an idea on potential problems to human caused by pig manure (n=73)	Yes	31	42.5 (31.3, 53.9)
	No problem	16	21.9 (12.4, 31.4)
	Don't know	24	32.9 (22.1, 43.7)
	Others	2	2.7 (-1.0, 6.4)
Frequency of manure removal from the pig pen (n=72)	More than once per day	1	1.4 (-1.3, 4.1)
	Daily	47	65.3 (54.3, 76.3)
	After several days to two months	19	26.4 (16.2, 36.6)
	Others (e.g. when manure is full)	5	6.9 (1.1, 12.8)

Fig 1. Common (A and B) and rare (C) methods of keeping pig manure pending their disposal or transportation to farm land as observed in Morogoro municipality, Tanzania, 2015.

Prevalence of helminth eggs in pig manure

Of the 79 pig manure samples analysed for helminth eggs, six (7.6% [95% confidence interval (CI): 1.8, 13.4]) had strongyle helminth eggs and five (6.3% [95% CI: 0.9, 11.7]) had *Ascaris* spp. eggs (Figure 2). Mixed Strongyle and Ascariid eggs were found in two (2.5% [95% CI: -0.9, 5.9]) of the samples. The helminth eggs were detected in five of the eight wards studied, suggesting wide distribution.

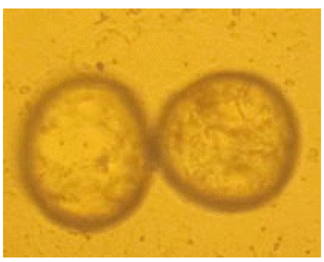

Fig 2. *Ascaris* species eggs isolated in one of pig manure samples in Morogoro municipality, Tanzania, 2015. Original micrograph under 100 magnification.

Prevalence of protozoan parasite oocysts and fungal spores in pig manure

Five of the 79 farms (6.3% [95% CI: 0.9, 11.7]) were contaminated with acid-fast protozoan oocysts of sizes ≥ 4 µm. On the other hand 22 (27.8% [95% CI: 17.9, 37.7]) had acid-fast objects measuring 1-3 µm in size, most likely microsporidia and other fungal spores. Nevertheless, mixed infections were common. The oocysts measuring ≥ 4 µm were probably those of *Isospora* , *Cryptosporidium* and *Eimeria* species as described in Figure 3.

Identification of protozoan parasite oocysts

Several guidelines were consulted during the identification of the protozoan oocysts isolated from the pig manure samples (OIE 2008; Lindsay et al 1997; Cuomo et al 2009). In addition, expert consultations were made. Figure 3 presents the different protozoan oocysts isolated and their possible identifications to genus level. Confirmation of the protozoan species would require specific monoclonal antibody techniques or molecular methods such as polymerase chain reaction.

Oocyst **A** (Figure 3) resembles *Isospora suis* in size and shape, which can range from 17-25 x 16-21 (Lindsay et al 1997). In addition, *I. belli* of human and certain *Isospora* spp. of dogs and cats fall within the observed size (Lindsay et al 1997). The size of oocyst **B** falls within *Eimeria* species, especially those from avian. For example, *E. tenella* of chicken and *E. innocua* of turkeys. Oocyst **C** resembles *Cryptosporidium* spp. by size, shape and presence of a vacuole at the centre. *C. suis* and *C. parvum* can be considered in this case. Similarly, oocyst **D** has features of *Cryptosporidium* species. A vacuole was evident under the microscope. Based on the size of this oocyst, it is most likely *C. galli* of chicken.

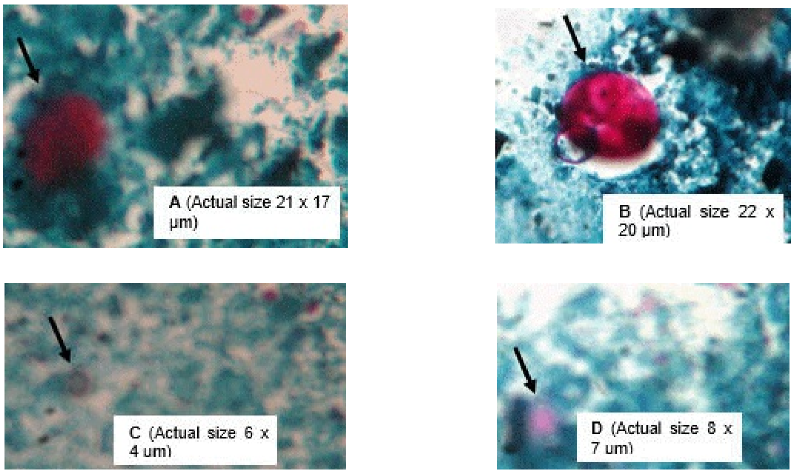

Fig 3. Different types of protozoan oocysts detected by the modified Ziehl-Neelsen stain in pig manure samples in Morogoro municipality, Tanzania, 2015 and their tentative genera. 1000x magnification.

Association between prevalence of helminth eggs and farm-level variables

As all pig farmers were confining their pigs during the study, association between parasite prevalence and pig husbandry system could not be assessed. Factors assessed were presence of piglets on the farm, number of adult pigs present (farms with < 10 pigs versus those with ≥ 10 pigs), presence of other livestock species, storage of pig manure together with those from other livestock species and farmers' knowledge on pig manure as a cause of health problems in humans. Nevertheless, there was not any significant association between overall prevalence of helminth or *Ascaris* spp. eggs and any of the variables assessed ($p > 0.05$).

Discussion

This study has for the first time estimated the prevalence and potential zoonotic implications of intestinal parasites in pig farmyard manure in an area of Tanzania. The estimated prevalence of between 6 and 8 percent of potentially zoonotic helminths and protozoan parasites merit further attention to safeguard pig manure handlers and consumers of foods and water that may come into contact with pig manure. The observed prevalence of helminth eggs in this study should be considered possibly an underestimation of the actual prevalence as the McMaster technique used has a sensitivity of detecting samples that have at least 50 eggs per gram of manure. On the other hand, several studies have found that the MZN stain can be 100% sensitive and specific in detecting *Cryptosporidium* and *Isospora* spp., especially after faecal concentration followed by centrifugation (El

Naggar et al 1999; Rigo and Franco 2002). This suggests that the observed prevalence of the protozoan parasite oocysts is closer estimate of their prevalence in the study area. Being the first investigation and geographically limited, one should be careful not to over interpret the results of the current study.

Cryptosporidiosis has previously been reported in pigs and other animal species and humans in Tanzania. A pig level study in small-scale and semi-intensive management systems, estimated prevalence of *Eimeria* spp. and *Cryptosporidium* spp. of 36% and 7.8%, respectively (Esrony et al 1996). A previous study in Morogoro municipality and the nearby Mikumi national park reported prevalence of 5.3% in 486 cattle, 22% in 36 buffaloes, 28% in 25 zebras and 27% in 26 wildebeests based on MZN stain followed by confirmation by *Cryptosporidium* spp. specific monoclonal antibody ELISA (Mtambo et al 1997). A study by Cegielski et al (1999) reported prevalence of *Cryptosporidium* spp. infections in paediatric and adult patients with diarrhoea in Tanzania.

Molecular studies have provided evidence that *A. lumbricoides*, *A. suum* as well as several strongyle species can infect both humans and pigs (Steenhard et al 2000; Bendall et al 2011; Nejsum et al 2012). European legislations require that manure be sanitised to make them hygienically safe before they can be applied to farm lands or marketed (Strauch 1991). One of the four indicators of hygienically safe manure is assurance that indigenous or seeded eggs of *Ascaris* are rendered non-infectious (Strauch et al 1991). The intestinal coccidians, *Cryptosporidium parvum* and *Isospora belli,* are associated with gastrointestinal disease in humans (Ribes et al 2004), which causes serious symptoms especially in immunocompromised individuals (Amatya et al 2011). Microsporidia (recently re-classified from protozoans to fungi) are considered a cause of emerging and opportunistic infections in immunocompetent and immunodeficient humans and can also infect a wide range of animals (Didier et al 2004). Thus public health implication should be considered as one of important justification for controlling manure handling and disposal. As observed in this study, it was difficult to ascertain absence or presence of parasites originating from other livestock species than pigs as other animals such as chickens were found feeding on pig manure disposal sites. In addition, some farmers reported to store pig manure together with manure from other livestock species. Detection of *Eimeria* spp., most likely from avian hosts further confirms this interaction. Feeding of animals on contaminated manure from different animal species increases the risk of transmission of manure parasites between animal species as several parasites are less host specific.

The global assessment by Teenstra et al. (2014) identified four key barriers to proper manure management at farm level. The first and most important was the limited awareness by farmers, local extension staff and policy-makers on the importance of integrated manure management. The second was low knowledge of small-scale farmers coupled with lack of knowledge infrastructure to support farmers in improving manure management. The third barrier was limited access to financial credit and other incentives to farmers. The fourth was ineffective manure or related policies and legislation to support sound manure management. In the present study, the level of education of pig farmers was not assessed. However, the observed low knowledge of respondents on potential health hazards associated with manure highlights on their limited awareness. The Tanzania Environmental Management Act contains clear guidelines for the management of different types of domestic, institutional and industrial wastes. Major power is delegated to local authorities to prescribe for different kinds of waste management practices, including sorting of the wastes, types of waste receptacles and disposal mechanisms. In addition, every person is required at all times to provide and maintain a place where litter is likely to be deposited and prevent any escape of the litter onto a public place. The local authorities can contract private service providers to help with waste management (Anonymous 2004). In the present study there was not enough evidence on the involvement of local government in the management of pig manure in the study area, probably because the study did not specifically inquire about this. However, the information from one farmer who reported abandonment of his pig manure by a contracted private company is an indication of previous involvement by the local government in disposing pig manure with other wastes upon farmer's request. It is important for local government authorities to clearly state the terms of reference with contractors of waste management services and monitor their compliance to ensure adequate waste management.

Conclusion

- Findings of this study suggest that handling and utilisation of untreated pig manure is associated with risks of acquiring and transmitting parasitic diseases by humans and animals in urban and peri-urban areas in developing countries such as Tanzania.
- Control of pig manure handling and utilisation is needed to safeguard human and animal health.
- Further studies are needed to measure the actual risks associated with livestock manure handling and utilisation, including parasite viability and characterisation studies. Such information would guide implementation of appropriate bio-security and bio-safety measures in urban/peri-urban livestock farming in developing countries.

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