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Transmission dynamics of *Ascaris suum* in organic pigs

Master's Thesis by

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Title: **Transmission dynamics of *Ascaris suum* in organic pigs**

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Front page photo: *Ascaris suum* in the small intestine of the pig. Photo by Helena Mejer.

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Summary

The transmission of *Ascaris suum* depends upon the type of swine production system. Organic farm with outdoor facilities provide favorable conditions for the development and survival of this soil transmitted helminth. The present study was carried out from late October 2013 to late February 2014 to study the infection dynamics of *A. suum* in pigs born and raised on two Danish organic farms (farm A and farm B). Contamination with infective *A. suum* eggs in farrowing paddock was estimated once by soil sampling on late October 2013. Pensample collection was performed twice on late October and late November 2013 from farm A and late October and early December 2013 from farm B. At three different time points, 45 pigs (15 pig/time) from each farm were necropsied at three different time points (n=15) when the pigs were 7-9 (weaners; on late October 2013 from both farms), 11-13 (growers; on late November 2013 from farm A and early December 2013 from farm B) and 23-25 weeks old (finishers; on mid-February: farm A and late February: farm B). Very young (≤1cm), young (>1- <12 cm: male and >1- <15 cm: female) and adult (Male: ≥12 cm and Female ≥15cm) *A. suum* were recovered from the small intestine. The lungs and superficial white spots on the livers were enumerated. Faeces of the necropsied pigs were examined for *A. suum* eggs.

The outdoor pastures and indoor pens of both farms were found to be contaminated with infective *A. suum* eggs. The prevalences of lung larvae and very young worm in the small intestine were higher in the weaners (lung larvae: 87%; white spots: 100%; very young worms: 93%) compared to the growers (lung larvae: 73%; very young worms: 87%) and the finishers (0% lung larva, very young worms: 47%). The prevalence of adult worms were higher in the finishers (87%) compared to the weaners (33%) and the growers (20%). Similarly, on farm B, 100% weaners had *A. suum* larva in the lungs. In total, 93% of them had very young worms and 33% had young worms. Of 15 growers, 13% had lung larva, 47% had very young and 27% had young worms. None of the finishers had lung larva, 40% had very young and 13% had young worms. The prevalence of adult worm was higher in the finishers (47%) followed by the weaners (0%) and the growers (13%).

Based on the results of the present study it can be concluded that all pigs could have acquired infection before weaning. After moving to the indoor pens, most of the pigs can

expel the worms and reinfected if the pens are contaminated with *A. suum* eggs. Over time, the intensity and prevalence of the infection decreases which is most likely due to development of immunity. It has also been shown that the environmental contamination with infective *A. suum* eggs is present in both indoor pens and outdoor pasture. Therefore, the level of infection on the farms could be minimized by combining anthelmintic treatment (after diagnosis) with proper cleaning and drying of the pens.

1 Introduction

Ascaris suum is the most common helminth in all pig production systems around the world. In Denmark, *A. suum* is present in the most intensive (specific pathogen free/SPF) systems, but it is especially the organic farms which are most affected (Roepstorff and Nansen, 1994). For organic pig production, certain mandatory rules (such as access to outdoor grazing, use of water sprinklers, etc) have been laid down by the International Federation of Organic Agricultural Movements (IFOAM, 2000). These regulations have provided better health and animal welfare but they have also increased the risk of parasitic transmission (Früh et al., 2011).

Pork production has been a major source of income in Denmark for more than 100 years. It has been estimated that in 2012 only, there were more than 12 million pigs in Denmark and the Danish pork industry produced 29.1 million pigs the same year of which 100,000 came from organic pig production (Danish Agriculture and Food Council, 2013). Since its early days, Danish swine industry has been changed from free-range farming system to an intensive indoor system which helped to eradicate some helminths such as *Hyostrongylus rubidus* (Roepstorff et al., 1998). However, re-introduction of outdoor grazing system in the pig farming practice has benefited the development and survival of certain helminths such as *Ascaris suum, Oesophagostomum* spp. and *Trichuris suis* (Carstensen et al., 2002; Haugegaard, 2010).

Ascaris suum is responsible for economic losses to the swine production though it seldom causes any clinical disease (Eriksen et al., 1992a; Hale et al., 1985). The reduced feed efficiency, reduced weight gain and condemnation of the liver are the major causes of the economic losses (reviewed by Thamsborg et al., 2009). In addition, a study by Steenhard et al. (2009) indicated that *A. suum* infection reduces the efficacy of *Mycoplasma hyopneumoniae* vaccine. Nevertheless, the overall effect of *A. suum* depends upon various factors such as health status, age of the pigs, concurrent infections and level of exposure to the parasites. Apart from these, there are several evidences of zoonosis by *A. suum* (Anderson, 1995; Galvin, 1968; Nejsum et al., 2005). In a recent study by Nejsum et al. (2005), *A. suum* infection was seen in children exposed to pig manure in Viborg, Denmark, thus indicating a risk of transmission to the human population.

The maintenance of high level of hygiene (such as use of slatted floors in indoor pens without beddings on intensive production systems) (Roepstorff and Nilsson, 1991) is believed to decrease the transmission rate. There are certain rules and regulations regarding the management of organic pig production system which has set higher priorities for improved animal health and welfare. Some of those norms are: farrowing sows and piglets should be kept outdoors in pasture all year round with a provision of huts and natural shelter; the pregnant sows should be kept in pasture for minimum of 150 days; the piglets must be weaned at the age of minimum of seven weeks and thereafter they should be moved to the indoor pens with the provision of bedding material and an access to outdoor run (Früh et al., 2011). Furthermore, pigs should be given organic diet and anthelmintics use should be limited, and in order to avoid heat stress, the pens should have sprinklers (or wallows on pasture) (Früh et al., 2011).

The microclimatic condition of the outdoor environment favors the development of *A. suum* eggs (Larsen and Roepstorff, 1999; Mejer and Roepstorff, 2006). The bedding material that is provided to the pigs in the indoor pens further increases the survivability of the eggs (Roepstorff and Nilsson, 1991). Moreover, the restriction of chemicals in organic farms makes it difficult to remove the eggs from the contaminated pens.

It has been reported from several studies that pigs exposed continuously to infective *A. suum* eggs, eliminate the majority of the larvae from the small intestine (Eriksen et al., 1992a; Mejer and Roepstorff, 2006; Roepstorff and Murrell, 1997). The surviving population of the worms in the small intestine has an aggregated distribution within the host population (Mejer and Roepstorff, 2006). This phenomenon may be the sequel of the development of acquired resistance of the host (Urban et al., 1988). Moreover, in organic pig production, the prevalence of *A. suum* is higher in younger pigs (Carstensen et al., 2002; Roepstorff et al., 1998) while in intensive farming, higher prevalence can be seen in fatteners and sows (Roepstorff and Murrell, 1997; Roepstorff and Nansen, 1994). However, most of the studies were performed experimentally mimicking the natural environment which may partially relate to the real-life situations. Therefore, the infection dynamics may alter when the studies are carried out under natural conditions. It has become utmost important to control *A. suum* by the use of alternative control measures in organic farms. Therefore, understanding

transmission dynamics over time may provide insights to prevent the *A. suum* transmission in pigs.

The hypothesis of the present study was that piglets harbor infection in their early lives from the contaminated environment (farrowing pasture). It was also believed that the farmers clean the pens and allow them to dry after removing old batch of pigs so that there should be no or only a few number of infective eggs left in the pens thereby minimizing the risk of infections to the following (upcoming batch of the weaners).

Herein, this study attempted to investigate the transmission patterns of *A. suum* in different age group of pigs on two Danish organic farms and describe the sequential infection dynamics from the farrowing unit until the pigs are slaughtered. Moreover, this thesis also gives an overview of the environmental contamination in the empty pens where the pigs are moved to.

1.1 Objectives of the study

1.1.1 General objective

- To describe the transmission of *A. suum* in naturally exposed cohorts of pigs from farrowing to normal slaughter weight.

1.1.2 Specific objectives

- To investigate the pattern of *A. suum* infection in organic pigs of two farms at three different time points when the pigs are 7-9 weeks, 11-13 weeks and 23-25 weeks old.
- To determine the number of infective eggs in the outdoor farrowing pasture and in the indoor pens.

2 Literature review

2.1 *Ascaris suum*

Ascaris suum is a large roundworm of pigs, and has a worldwide distribution. The length of the adult male *A. suum* is around 15-25 cm and males tend to bend their posterior end (Fig 1) while the slender females can reach upto 20-35 cm (Roepstorff and Murrell, 1997) and can produce 0.2 -1.9 million eggs (Fig 2) per day (Olsen et al., 1958).

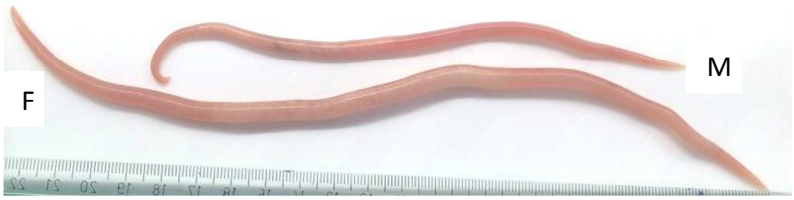

Fig 1. Male (M) and Female (F) *Ascaris suum*

Fig 2. A fresh *Ascaris suum* egg

2.2 Life cycle of *A. suum*

The life cycle of *A. suum* is direct in that it is transmitted via a faecal-oral route (Fig 3). Male and female *A. suum* reside in the upper small intestine and after mating female worm starts producing eggs. The eggs are then excreted in pig faeces. Under suitable environmental conditions (temperature and moisture), a larva develops inside the eggs where it undergoes two ecdyses producing a third stage larva (L3) (Seamster, 1950). In Denmark, it normally takes 4-6 weeks for complete embryonation of eggs in summer while it may take a year to develop for the eggs deposited in the autumn or winter (Larsen and Roepstorff, 1999; Roepstorff and Murrell, 1997). Pigs get infected upon ingestion of infective eggs containing L3 which hatches in the upper small intestine by secreting proteinases and chitinases that degrade the different layers of the eggshell (Geng, 2002; Hinck and Ivey, 1976). The larva then penetrates the mucosa in the caecum and colon within 24 hr (Murrell et al., 1997; Rhodes et al., 1977) and migrates to the liver within 3-4 days (Roepstorff and Murrell,

1997). The larva migrates within the liver parenchyma before it migrates to the lungs. At this stage the host immune response triggers the development of pathological lesions in the liver, which are referred to as white spots or milk spots (Ronéus, 1966). The formation of white spot in liver as suggested by Roepstorff (2003) is the result of immune responses to the mechanical injury associated with the parenchymal migration of the larvae. These white spots provide information on how recently the infection occurred in the host as these spots disappear 3-6 weeks after exposure (Eriksen et al., 1980). The larva then travels to the lungs via bloodstream within 6-8 days post infection where it gets trapped in the lung capillaries (Douvres et al., 1969) and enters the alveoli and goes to the upper respiratory tract (first to bronchi and then the trachea). The larva is coughed up and gets swallowed and reaches the small intestine around day 10 (Douvres et al., 1969). Most of the larvae are expelled at this stage and the remaining larvae moult to L4, and to L5 at around 23-24 p.i. and finally mature to adult by day 42-49 p.i. (Roepstorff and Murrell, 1997).

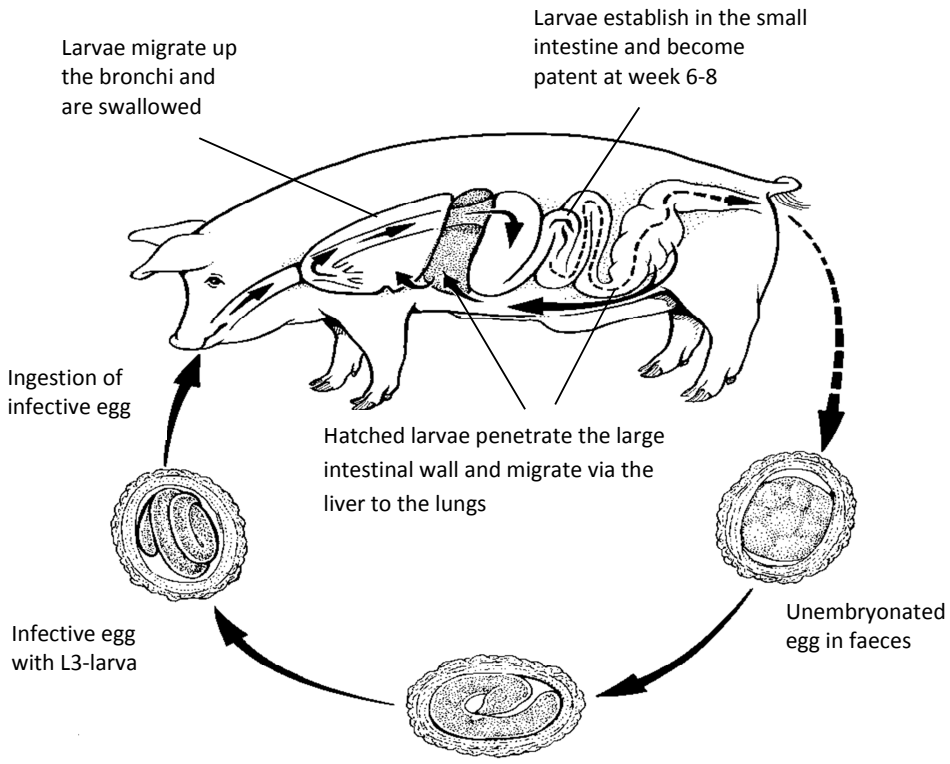

Fig 3. The life cycle of *Ascaris suum* as illustrated by William P Hamilton (Roepstorff and Nansen, 1998).

2.3 Structure of *A. suum* egg shells

The complex thick-shelled eggs of these nematodes can resist harsh environmental conditions. The eggs have four-layered shell consisting of an inner lipid layer (ascaroside), a middle chitinous layer, a lipoprotein vitelline layer and an outermost mucopolysaccharide and a proteinaceous uterine layer (Wharton, 1980). The lipid layer protects the egg from desiccation by minimizing the loss of water (Wharton, 1980). This impermeable lipid layer also protects the eggs from various chemicals such as strong acids, bases and surface-acting agents (Barrett, 1976). The thickest chitinous layer is a protein/chitin complex that provides structural strength to the eggs (Wharton, 1980). The vitelline layer is derived from the vitelline membrane of the fertilized oocyte (Wharton, 1980), but the function of this layer has not been reported yet. The uterine layer is an acid mucopolysachharide-protein complex derived from the cells of the worm uterus and has ridges and depressions on the surface (Wharton, 1980).

2.4 Epidemiology

The transmission of *A. suum* depends upon the development and survival of eggs in the environment, management system of the farms, and age, behaviour, general body condition and the genetics of the host.

2.4.1 Survival of eggs in the environment

Eggs of *Ascaris* spp. are resistant to freezing and can survive upto 15 years in the soil (Kransnonos, 1978) but the development of the eggs take place only when the environmental temperature is in between 16-34°C (Oksanen et al., 1990; Seamster, 1950). Under optimal conditions when the temperature of the environment ranges between 16.7°C-34.4°C, the eggs require at least 2-5 weeks (Seamster, 1950) to develop into infective eggs.

The development and survival of the eggs depends not only upon the temperature but also to the exposure to the direct sunlight (due to which eggs could dry out), vegetation and precipitation (Kraglund, 1999; Larsen and Roepstorff, 1999; Roepstorff et al., 2001). Seamster (1950) observed that the embryonation of *Ascaris* eggs did not take place when the relative humidity was below 100% at 31°C. Similarly, the anaerobic condition or a very low concentration of oxygen in the surrounding can also affect the embryonation of *A. suum* eggs

(Passey and Fairbairn, 1955). The main reason for the low level of egg survivability inside the pen, as suggested by Katakam (2014), can be due to low levels of the oxygen present in the bedding materials present in litter areas in the indoor environment compared to outdoor pastures. The other factor that plays role in the development and survival of the *A. suum* eggs are the presence of ammonia (which has detrimental effect on *A. suum* eggs) (Katakam et al., 2013) and pH of the environment (Gantzer et al., 2001). Hence, the embryonation of the eggs can only take place if the microclimatic conditions of the environment are suitable.

2.4.2 *Ascaris suum* transmission in different management system

Transmission of *A. suum* infection varies with the type of management system. Organic pig production systems having outdoor farrowing pastures can provide suitable environment for the transmission of *A. suum* (Borgsteede and Jongbloed, 2001; Mejer and Roepstorff, 2006). In an outdoor environment of organic farms, the eggs can escape from dehydration and exposure to direct sunlight by getting protection from the vegetation and from being buried in the soil (Kraglund, 1999; Kraglund et al., 2001; Larsen and Roepstorff, 1999; Rose and Small, 1981) which favors the long-term survival of the eggs. Katakam (2014) found that the transmission of *A. suum* can take place in indoor fattening pens on organic farms as well. Roepstorff and Nilsson (1991) found that the use of slatted or concreted floors without bedding materials in indoor pens on intensive pig production systems reduces the risk of the transmission of *A. suum*. Similarly, the use of anthelmintic, disinfectant also minimizes the transmission in intensive pig production (Roepstorff and Nilsson, 1991).

2.4.3 Age-wise prevalence of *A. suum*

Based on faecal examination of intensive swine herds, Roepstorff et al. (1998) had found higher prevalence of *A. suum* in gilts (25%) and large fatteners (21%) followed by small fatteners (14%) and dry sows (13%). More recently, Haugegaard (2010) reported 23% prevalence in gilts and 21% in sows from 79 modern indoor farms which was also based on faecal examination. Carstensen et al. (2002) reported prevalence of 28% in the weaners, 33% in fatteners, 4% in dry sows and 10% in lactating sows on Danish organic herds. These data show that *A. suum* provides limited age resistance as old fatteners and sows above two years of age can be susceptible to infection in commercial SPF herds if they were not previously exposed (Eriksen et al., 1992a).

2.4.4 Host behaviour on transmission of *A. suum*

Rooting is a common behavioural characteristic shown by pigs (Thomsen et al., 2001). Pigs are not selective grazers and do not avoid fouled herbage and faecal material and can thus be continuously exposed to the parasite eggs. On a two-year study Thomsen et al. (2001) found a little relationship between host population density and *A. suum* transmission between pig herds having high stocking density and another with low stocking density. Therefore, the authors assumed that the difference in transmission could be due to the difference in behaviour of the animals.

2.4.5 Genetics of the host

On a repeated experimental exposure to *A. suum* in 195 pigs, Nejsum et al. (2009a) found a strong relation between *A. suum* infection and host genetics. Similarly, on a study conducted in Jirel community in Nepal, Williams-Blangero et al. (2012) observed variation in susceptibility to soil transmitted helminth transmission due to variation in host genetics within-population. Therefore, host genetics can influence the transmission of *A. suum* infection within the host population.

2.5 Overdispersion within the host population

Within the pig population *A. suum* is highly aggregated which means only a few pigs harbour most of the worms while most of the pigs harbour only a few or no worms (Boes et al., 1998; Eriksen et al., 1992b; Nejsum et al., 2009b; Roepstorff and Murrell, 1997). On a continuous exposure (for 10-11 weeks), Boes et al. (1998) found that 10% of the animals had 80% *A. suum* and on an experimental study, Nejsum et al. (2009b) found 20% of pigs had 80% of the worms. In contrast, a less aggregated distribution was observed by Mejer and Roepstorff (2006) when the pigs were exposed naturally on contaminated paddocks. Though the mechanism behind the aggregated pattern of distribution is very complex, the study of Boes et al. (1998) revealed that the host intrinsic factors and environmental factors play an important role in distribution of parasites between the hosts.

2.6 Impact of *A. suum* infections

The impact of *A. suum* depends on multiple factors such as level of exposure, stage of infection, builds up or maintenance of immunity, nutritional and physiological status (reviewed by Thamsborg et al., 2013).

Despite its chronic establishment in the host, morbidity associated with *A. suum* is subclinical. The clinical manifestations occur due to the migrating larvae and the presence of adult worms in the small intestine (reviewed by Thamsborg et al., 2013). During lung penetration by the migrating larvae, the animal suffers from respiratory distress which is reflected in higher breathing rate, dyspnoea and dry coughing (reviewed by Thamsborg et al., 2013). Similarly, pigs that are heavily infected with the adult worms can have reduced feed utilization and weight gain (Forsum et al., 1981). *Ascaris suum* is also responsible for production losses due to condemnation of liver and reduced weight gain (reviewed by Thamsborg et al., 2013). Economic losses as a consequence of reduced feed conversion and weight gains and the condemnation of affected organs in the *A. suum* infected pigs have been well established all over the world (Permin et al., 1999; Roepstorff et al., 1998; Weng et al., 2005). In addition, it has been shown from several studies that the concurrent *A. suum* infection in pigs have shown negative effect against *Mycoplasma hyopneumoniae* vaccine (Steenhard et al., 2009).

2.7 Immunity to *A. suum*

The acquired immunity is believed to downregulate the prevalence of *A. suum* infection by the development of immune mediated pre-hepatic barrier which may partially or almost completely inhibit the migration of the newly hatched larvae (Eriksen et al., 1992a; Eriksen et al., 1992b; Lunney et al., 1986; Urban et al., 1988).

Several studies have shown that the number of white spots in liver significantly reduces with time by the development of the immunity (Mejer and Roepstorff, 2006; Urban et al., 1988). In a trickle infection, Eriksen et al. (1992b) found a maximum number of liver spots in week 6 which gradually decreased over time despite repeated infection. Similar findings were observed by Mejer and Roepstorff (2006) where the number of liver spots was maximum in week 7 post-partum (pp) which was significantly reduced when the pigs were slaughtered at week 19 pp.

In a single infection Roepstorff et al. (1997) found that majority of the larvae which had completed the migration are expelled from the intestine between day 17 and 21 by self-cure mechanism regardless of the inoculation dose. A similar finding was reported by Miquel et al. (2005). Recently, Masure et al. (2013) explained that the expulsion of worms from the intestine is due to increased gut movement which is associated with increased eosinophils and intra-epithelial T cells in the jejunum.

2.8 Diagnosis of *A. suum*

The infection caused by *A. suum* can be diagnosed by faecal examination, post mortem technique for counting adult worms, larvae and white spots in the liver and by serological analysis.

Diagnosis of *A. suum* infection by faecal examination is normally expressed as numbers of eggs per gram pig faeces (epg) (Roepstorff and Nansen, 1998). This method is a quantitative method and is relatively cheap and an easy. However, there are chances of getting both false-positive and false-negative results. False-positive results may occur due to ingestion of unembryonated eggs which pass unhatched in the faeces. Boes et al. (1997) found 4-36% false positive pigs depending on housing system. False-negative result can occur before the infection becomes patent (i.e. presence of very young worms) or in a single sex infections.

Using post mortem technique, large worms from the small intestine are recovered directly performing necropsy procedure and the small migrating larvae can be isolated using various techniques such as agar-gel technique (Slotved et al., 1997), digestion of lungs and liver and macrobaermannization (Eriksen et al., 1992b). In Denmark, Slotved et al. (1997) introduced an agar-gel technique to extract larvae that have returned to the small intestine from migration. Since the larvae reaching the liver are about 229 µm (Douvres et al., 1969), these are difficult to extract from any of the above mentioned procedures so the easiest method for diagnosing the recently migrating *A. suum* larvae is examining the liver for superficial white spots.

Serological analyses for the detection of *A. suum* infection can be performed by using different serological tools such as enzyme linked immunosorbent assay (ELISA) which can detect the anti-*A. suum* IgG (Roepstorff, 1998)

3 Materials and methods

3.1 Study design

The present study was carried out on two Danish organic pig farms (farm A and farm B) from late October 2013 to late February 2014. On each farm, one cohort of pigs was followed over time and necropsied at three time points when the pigs were weaners (7-9 weeks of age), growers (11-13 weeks of age) and finishers (23-25 weeks of age). Superficial white spots on livers were enumerated. The larvae from lungs were recovered through digestion and sedimentation. The adult worms from the small intestine were collected using forceps and the very young worms were harvested by an agar gel technique. Rectal faecal samples of the slaughtered animals were collected and examined by a McMaster Technique. Furthermore, soil samples from the pasture and the samples from the pen floor and walls were collected and examined for *A. suum* eggs.

3.2 Selection of the farms

The selection criteria of farms were as follow:

- History of helminth infection.
- Examination of faecal samples from the growers and the finishers using a concentration McMaster technique revealed a large number of *A. suum* positive animals on both farms.
- Willingness of the farmers to participate.

3.3 Farm and pen description

Farm A had 190 sows and produced 3000 pigs per year. The farm had pigs of all age groups in indoor pens and outdoor pastures. Pasture rotation scheme of 9 months had been practiced between the two areas. The pregnant and nursing sows were kept on large farrowing pastures having smaller paddocks for 1-5 sows separated by a single wire electrical fence. Newborn piglets had free access to the entire farrowing area and they were kept on the pastures until weaning. At this point, they were brought to the weaner-grower (WG) pen at a minimum seven weeks of age. The pigs were shifted to grower-finisher (GF) pen at 11-13 weeks of age at the time of the study. The layout of the WG/ GF pen is illustrated on Appendix 4a. The WG pens had accommodated 40-62 weaners per pen and had an area of 48m^2. Similarly, the

area of the GF pen was also 48m^2 and it had 7-24 growers per pen. The semi-open pens of farm A had shallow litter areas (LiA), watering areas (WA), feeding areas (FA), running areas (RA), slatted floors (SF) and latrine areas (LA). The bedding material was removed from pens as per the need and sometimes after removal/transfer of pigs to other pens. New bedding material was added from the top when the pigs were present. The LiA was covered by the roof which had covered two third of the pen and was semi-open for all age group of pigs. There was no clear separation between the indoor and outdoor areas. Separate automatic feeding and watering areas in all pens were kept in the either side of the pens. Automatic sprinklers were provided in each pen so as to provide a water shower to the pigs. The slurry and solid manure of the pens were used as fertilizer for agricultural purpose but not on pig pastures.

Farm B was comparatively larger than farm A with approximately 400 sows producing 6000 pigs per year. The pregnant, nursing and dry sows were kept on a large farrowing pasture which was sub-divided into smaller paddocks for 5 sows separated by a single wire electrical fence. A three year strip pasture grazing scheme was practiced on this farm. Newborn piglets had free access to the entire farrowing area and were kept there until weaning. The number of pigs weaned (at minimum 7 weeks of age) at any given time was high compared to farm A, and the pigs were kept into five WG pens (semi-open type, 20-30 cm litter) and were moved to five GF pens (closed type) when they were 11-13 weeks of age. In total 40-62 weaners were present in each pen, which had an area of 39 m^2. The layout of the WG and GF pens are illustrated on Appendix 4b and 4c, respectively. The GF pens (40-80 cm litter) had an area of 39 m^2 and accommodated 35-40 growers per pen. Both WG and GF pens of farm B were divided into litter area (LiA), watering/feeding area (WAFA), running area (RA), slatted floor (SF) and latrine area (LA). The LiA of the GF pens was connected to the semi-closed outdoor runs by a small opening covered with rubber sheets (in GF pens only) that reduce influx of cold air into the indoor stables. There were automatic sprinklers for pigs in each outdoor run. Bedding material was removed when it had reached the height of approximately 80-100 cm in the GF pens. Clean dry bedding materials were added in the moist part of the LiA once or twice a week when it got wet with the pig faeces and urine. This resulted in the greater buildup of litter material in the moist part compared to the resting parts. The feeding and watering areas were outside in the outdoor run in close proximity and

were automated as well. Like farm A, the slurry and solid manure from the pens were used for agricultural purpose as organic fertilizers.

3.4 Selection of the pigs

A total of 45 pigs from the same cohort from each of the farms were picked at three different time points and at each time point there were 15 pigs. At the first time point, the weaners were to be transferred from farrowing paddocks to the indoor WG pens. Similarly at the second time point, the growers were to be transferred from the WG pen to the GF pen and finally at the third, the finishers were to be brought for necropsy. Therefore, the time points were selected to find out how many worms they had acquired from previous pens and to determine the number of infective eggs in the following pens, which would subsequently infect the new pigs.

3.5 Collection of soil sample

The entire paddock areas of both farms were walked in “W” shape by two persons taking two alternate routes and approximately 50 soil subsamples (depending upon the area of the paddock) were collected (Roepstorff and Nansen, 1998). A long curved scoop was used as a tool for collecting soil samples from the top five centimeters. The manure and grasses on the route were avoided while sampling. These samples were collected in plastic bags, labeled and stored in refrigerator at 5°C until further processing.

3.5.1 Isolation and examination of eggs from soil samples

The soil samples were transferred into bucket and homogenized for 30-45 minutes until big lumps were disintegrated. After mixing, 20 subsamples were taken from different areas of the soil in the bucket. A 5 g subsample was transferred to a 50 ml tube. The eggs in 5 gm soil was isolated and counted as described by Roepstorff and Nansen (1998). Another 5 g soil subsample from the same bucket was collected and dried in oven at 105°C for 24 hours to calculate the dry weight of the sample. The isolated eggs were counted and examined microscopically (200×). The eggs with diffuse dark contents to compact one or more multicellular content were categorized as developing eggs. Eggs having slender larvae inside were categorized into infective (fully embryonated). The eggs with irregular or vacuolated contents were categorized as dead or non-viable.

3.6 Pen sample collection

A total of five samples were collected from the LiA, WA, FA, RA, SF and LA from farm A at two different time points (Appendix 4a). The first sample collection was performed in late October 2013 when the weaners from the outdoor pasture were transferred to the WG pen. The bedding material had been removed but the pen had not been cleaned. During collection, large faecal particles were easily removed from the SF and the LA whereas the dried up material stuck in the floor of other areas and the walls had to be scrapped off. Since the layouts of WG and GF pen were similar, samples from the same part of the aforementioned areas were taken for the second period of collection in late November 2013. At second collection time, the old bedding material was not removed; therefore, the dry samples were collected by removing the old bedding material. The large faecal particles were removed from the pen but the dry materials were still found on the floor and walls.

Samples were collected from the LiA, RA, SFLA and WAFA farm B. The first sample collection was performed in late October 2013 on the WG pens when the pigs were brought from the pasture to the GF pens. At that time, the pens of farm B were emptied. Collection of the samples on the GF pens were done in early December 2013 but at that time the pigs had already been transferred into the finisher's pen so there were bedding materials present on the LiA. The pigs had used the corners of these areas into defaecation areas which were wetter than the rest. Dry samples from those pens were collected from the LiA so as to avoid fresh faecal materials which might have been voided by the new pigs.

3.6.1 Collection procedure of pen sample

On both farms, the dry samples were scrapped from the wall and floor using a scalpel blade and collected in 50 ml tubes. Wet samples around the LA were moister than the rest of the areas so they were collected by hand using plastic gloves. The samples were brought to the laboratory and were kept at 5°C until further processing.

3.6.2 Isolation and examination of eggs from pens samples

The samples collected were transferred into a large Petri dish and mixed for five minutes. The larger particles were cut to 2-5 cm pieces using scissors. A total of two subsamples (5 g each) were taken and eggs were then isolated using a flotation and sieving technique

(Katakam, 2014) as described for soil. The eggs were examined microscopically (200×) and categorized into developing, infective or non-viable/dead eggs as described above. The total number of eggs in each sample was estimated for samples having low number of eggs and 10-20% samples were examined to estimate the total number of eggs for samples containing large quantities of eggs.

Another 5 g subsample was taken to estimate the dry weight of the sample.

3.7 Necropsy procedure

At the day of necropsy, the farmers selected pigs randomly and the pigs were transported to the University (weaners and growers) or the abattoir (finishers). The sex and the weight of the animals were recorded. The weaners and the growers were stunned using a captive bolt pistol on their forehead and then exsanguinated, before the organs were removed. From finishers, as they were slaughtered in the abattoir using electric stunners, only viscera were brought to the University and the weight and sex of these pigs were taken on farm.

3.7.1 Faecal sample

Rectal faecal samples from the weaners and the growers were collected before slaughter. In finishers, faecal samples were collected from the rectum after necropsy (i.e. after removing the gastrointestinal tract from the carcass). All faecal samples were stored in the refrigerator at 5°C until examination. A concentration McMaster Technique with a lower detection limit of 20 eggs per gram of faeces using flotation fluid of saturated NaCl solution with 500 g glucose/L (specific gravity 1.27 g/mL) was used for examining the faeces (Roepstorff and Nansen, 1998).

3.7.2 Parasite recovery from the lungs

The lungs were weighed and finely chopped into 1 cm (approx.) pieces. It was then minced using a blender until 1-2 mm pieces were obtained. To each 100 gm of the blended tissue digestion fluid (12 ml of 30% HCl; 30 ml pepsin and 40°C tap water to make a total volume of 1000 ml) was added to a total volume of 1000 ml in a beaker. The beakers were placed on a magnetic stirrer and allowed to digest for 2 hrs at 37°C. Digested tissues were sieved using a large mesh sieve and poured into 4-5 sedimentation cones (Fig 4). Cold tap water was added to each glass to reduce the temperature and stop the digestion process. The samples

were then allowed to settle for 30 min. The supernatant was removed; sediments were combined in 2-3 sedimentation cones, rinsing cones with tap water and again allowed to sediment. This process was repeated three to four times until clear sediment was obtained. Finally all the sediments were combined to 1 sedimentation cone. The sediments from the last cone were transferred to a 50 ml tube and preserved in 70% ethanol until examination. Later, larvae were counted under a stereomicroscope.

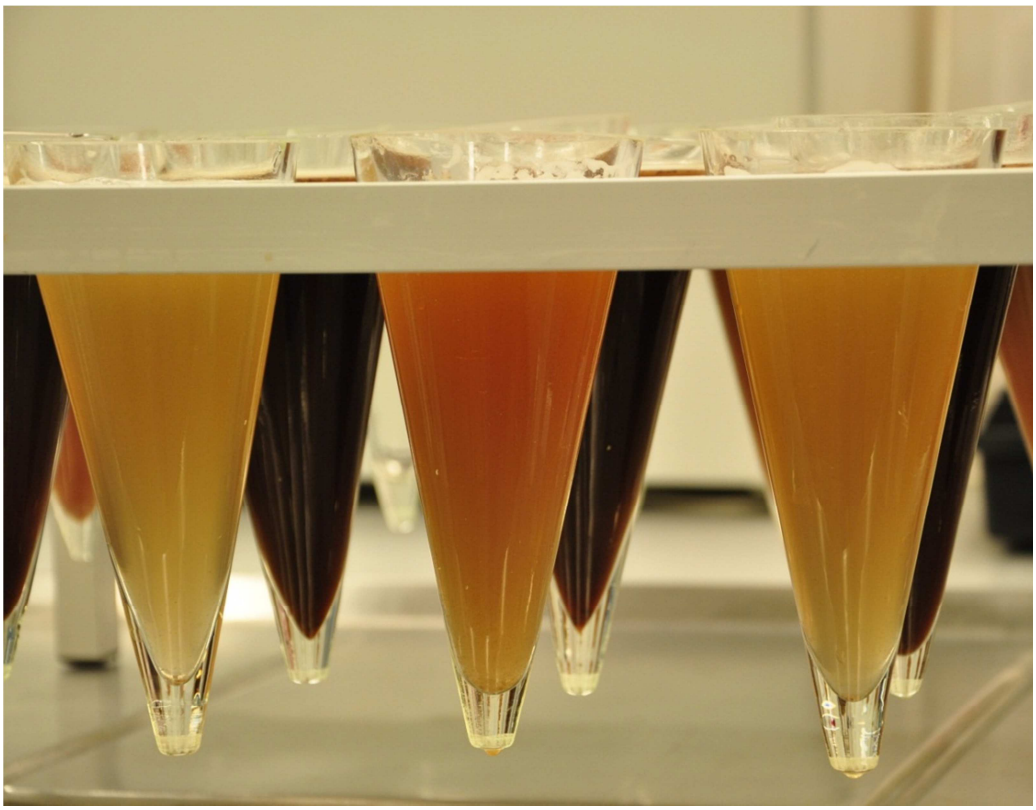

Fig 4. Digested lungs in sedimentation cones to recover *Ascaris suum* larvae

3.7.3 Parasite recovery from the small intestine

The small intestine was separated from its mesentery (Fig 5) and the contents were squeezed into a bucket. The intestine was then opened along its length and the mucus was scrapped off gently from the mucosal surface by placing between two pencils tied together with a rubber band. The intestine was then rinsed in saline which was combined with the intestinal content.

Macroscopic worms were collected and washed with physiological saline and preserved in 70% ethanol. Later, sexing was done and worms were measured using a ruler. Parasites having curved posterior end were male and the female parasite were long, thick and slender compared to the male parasite.

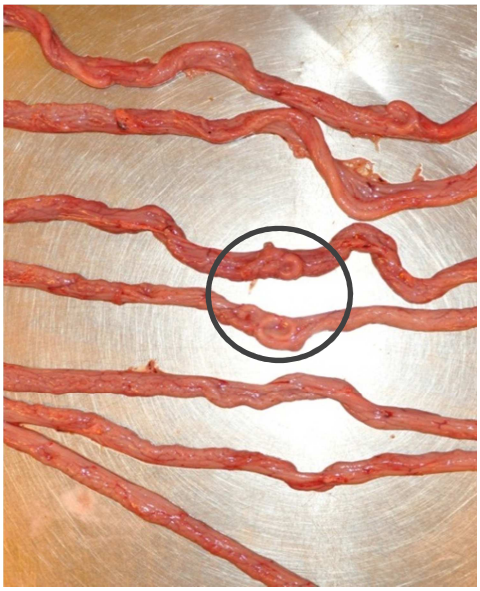

Fig 5. *Ascaris suum* (inside the circle) in the small intestine of necropsied pig

All the intestinal contents (except for finishers where 50% subsample were examined) were collected in the bucket. The contents were homogenized using ladle and mixed with an equal volume of 2% agar. The 2% agar was prepared by dissolving it in boiling tap water and subsequently cooling to 52°C in a water bath. Content that was mixed with agar gel was embedded on cloths (Johnson and Johnson® medical cloth) (Fig 6) and was incubated for 3 hours in plastic jar containing saline at 37°C (Fig 7) (Slotved et al., 1997). The larvae that had migrated out of the gels and settled at the bottom of the containers were recovered by sieving the fluid through a 20 µm sieve and preserved in 70% ethanol. Later, all the larvae were counted using a stereo microscope.

Parasites recovered the small intestine were categorized according to the length, the very young *A. suum* (≤1 cm), the young (male: >1 - <12 cm and female: >1 - <15 cm) and the adult male (≥12 cm) and female (female: ≥15 cm) parasites were categorized as described by Roepstorff and Murrell (1997).

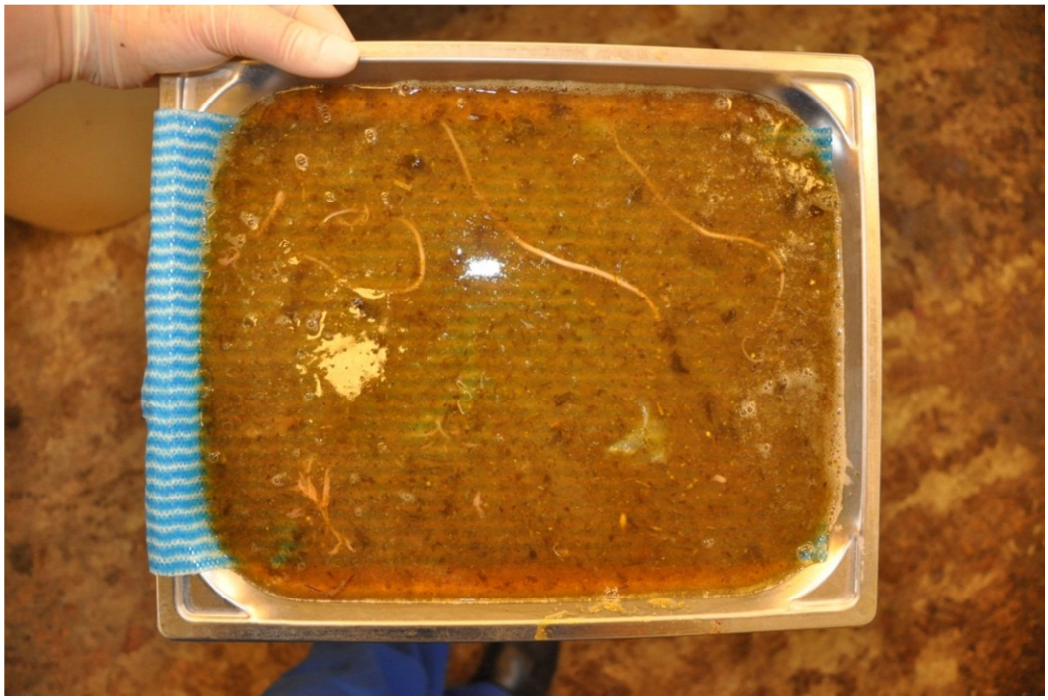

Fig 6. *Ascaris suum* embedded in agar gel

Fig 7. Agar gel embedded in clothes and hung in plastic containers to recover young and very young *Ascaris suum* from the small intestine

3.7.4 Liver spots

Superficial white spots on the liver were enumerated by a single person to avoid between-individual variation in counting the size and the appearance. The spots were classified into two groups: Grade A (clear white mesh-like diffuse or single pearl like round lymphonodular fresh spots) and Grade B (partially healed greyish spots which were also either mesh-like diffuse or as a well-defined single lesion.

3.8 Statistical analysis

Data analysis was performed using SAS® (SAS 9.3, SAS institute Inc, Cary, North Carolina). The level of significance was set at $\alpha = 0.05$. The number of white spots in liver, *A. suum* in the lungs and the small intestine, faecal egg count and the number of eggs present in soil and pens were count data which were assumed to follow a poisson distribution. Therefore, the data were first modelled using a PROC GENMOD procedure fitting the poisson distribution. However, over-dispersion was noticed for each data. Therefore, one was added to each value to minimize overdispersion seen as a result of excessive zero counts, and negative binomial distribution was fitted to overcome the problem of over-dispersion. When overall differences were observed individual comparison between the variables (age group, farms and pens) were done using least square means. The correlation between the adult female *A. suum* and epg were calculated using PROC CORR procedure.

4 Results

4.1 Climatic conditions

The overall mean temperature of summer (June, July and August) 2013 in Denmark was 16.1°C (2.4°C to 33.3°C) which was 0.9°C higher than the normal (calculated on the period 1961 to 1990) temperature (15.2°C). The total precipitation was 136 mm (125 to 170 mm) which was 28% below normal (188 mm). Similarly, in the autumn (September, October and November), the average temperature recorded was 9.9°C (-7.8°C to 26°C) which was 1.1° C above the normal temperature (8.8°C). The total precipitation was 263 mm (173 to 347 mm) which was 15% above normal (228 mm). In the winter (December, January and February) it was 3.7°C (-10.5°C to 13.1°C) which was 3.2°C than the normal temperature (0.5°C) (Danish Metereological Institute).

4.2 Pasture infectivity

The soil from the farrowing pasture of farm A had 21% moisture and it was covered by the grass except around the huts and feeding areas. Vegetation around these areas (huts and feeding areas) was scarce as mainly the sows had spent most of their time over there whereas piglets roamed around the entire farrowing pasture. Examination of soil samples from the farrowing pasture revealed that the mean number of infective, developing and dead *A. suum* eggs per gram dry soil were <1 infective, 1 and <1, respectively.

The moisture content of soil from the farrowing pasture on farm B was 24%. Similar to farm A, the soil was covered by grass except around huts and feeding areas. The farrowing pasture had <1 infective, 2 developing and <1 dead *A. suum* eggs per gram dry soil.

4.3 Pen infectivity

Ascaris suum egg contamination on the entire WG and the GF pens of both farms is presented on Table 1. The large majority of eggs were at the early stage of development while only a few eggs were dead and very few were infective (Table 1; Fig 8). On overall, total number of eggs was higher in the WG pens than the GF pens ($\chi^2 = 5.64$, df = 1, $p < 0.0175$).

On farm B, all areas of five WG pens were contaminated with *A. suum* eggs. Similar to farm A, most of the eggs were developing (early stage) (Table 1; Fig 8). In contrast to farm A, the number of eggs found in the WG pen was not significantly higher than the eggs found in the GF pen ($\chi^2 = 0.04$, df = 1, $p < 0.8471$).

Table 1. Moisture content (%) of samples taken from different areas of the weaner-grower (WG) and the grower-finisher (GF) pens of farm A and farm B and the percentage of developing, dead and infective *Ascaris suum* eggs on these areas

Farm	Pen	Number of pens	Number of samples per area	Area	Moisture %	Developing eggs %	Dead eggs %	Infective eggs %
A	WG	1	5	LiA	34	68.2	31.4	0.30
		1	5	RA	58	86.1	13.8	0.05
		1	5	SF	78	94.3	5.6	0.03
		1	5	LA	80	90.2	9.7	0.01
		1	5	WA	78	87.9	11.9	0.10
		1	5	FA	29	81.6	18.3	0.08
	GF	1	5	LiA	42	69.8	29.8	0.29
		1	5	RA	46	92.6	7.3	0.06
		1	5	SF	69	90.5	9.3	0.11
		1	5	LA	81	90.0	9.7	0.21
		1	5	WA	64	89.7	10.1	0.11
		1	5	FA	24	77.3	22.6	0
B	WF	5	3	LiA	67	85.8	14.1	0.07
		5	3	RA	75	89.7	10.2	0.01
		5	3	SFLA	82	93.0	6.8	0.02
		5	4	WAFA	42	80.1	17.9	0.08
	GF	5	3	LiA	65	87.5	12.2	0.15
		5	3	RA	69	84.5	15.4	0.05
		5	3	SFLA	77	89.8	10.1	0.01
		5	4	WAFA	62	85.1	14.7	0.05

LiA= litter area; RA=running area; SF=slatted floor; LA=latrine area; WA=watering area; FA=feeding area; SFLA: slatted floor/latrine area, WAFA=watering/feeding area

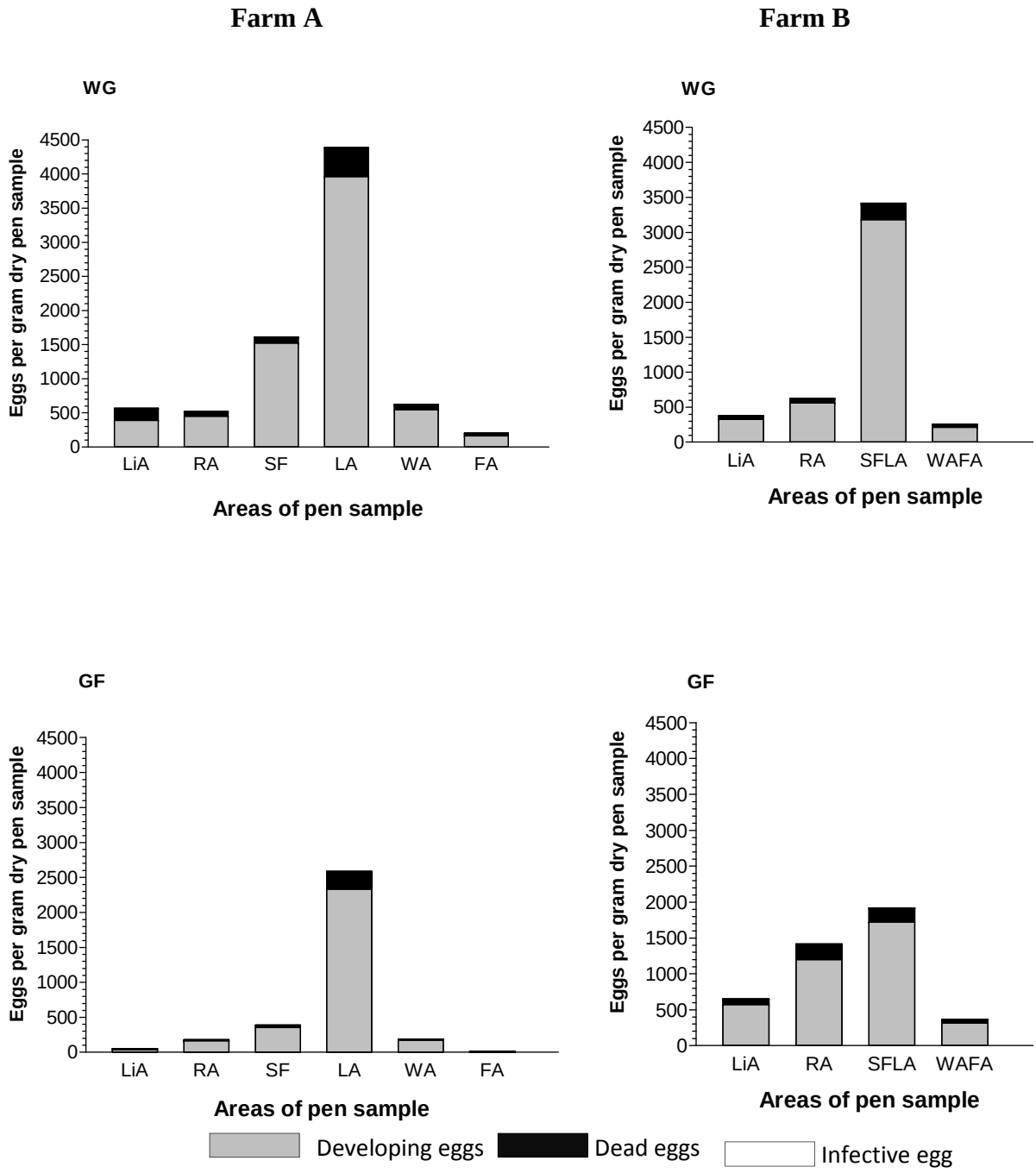

Fig 8. Mean number of *Ascaris suum* eggs per gram dry material from litter area (LiA), running area (RA), slatted floor (SF), latrine area (LA), watering area (WA), feeding area (FA), slatted floor/latrine area (SFLA), watering/feeding area (WAFA) from the weaner-grower (WG) pens and the grower-finisher (GF) pens on farm A and B. Infective eggs were present in all areas in the pens but they were very few (<1 to 2) to be visible on the graph.

4.4 Body weight of pigs

The mean body weight of the weaners (n=15) from farm A was 17.6 kg and from farm B (n=15) was 11.0 kg. Growers from farm A (n=15) and farm B (n=15) weighed 27.0 kg and 31.2 kg, respectively. The mean body weight of finishers from farm A (n=15) was 97.0 kg and farm B (n=15) was 105.6 kg.

4.5 Liver white spots

Out of 45 pigs from farm A, 84% had Grade A and 78% had Grade B spots on their livers (Fig 9), respectively. There was a significant difference on total number of white spots between the three age groups (χ^2 = 45.99, df = 2, $p < 0.0001$) (Fig 10). The weaners had significantly higher number of liver spots than the growers ($p < 0.0020$) and the finishers ($p < 0.0001$). Finishers of farm A had significantly less number of white spots than the growers ($p < 0.0001$).

Out of 45 pigs, 82% had Grade A spots and 78% had Grade B. There was a significant difference on the total number of white spots between three age groups ($\chi 2 = 35.05$, df = 2, $p < 0.0001$) (Fig 10). The total number of liver spots was higher in the weaners than the growers ($p < 0.0001$) and finishers ($p < 0.0001$). The finishers of farm B had high number of white spots on the liver than the growers ($p = 0.0023$).

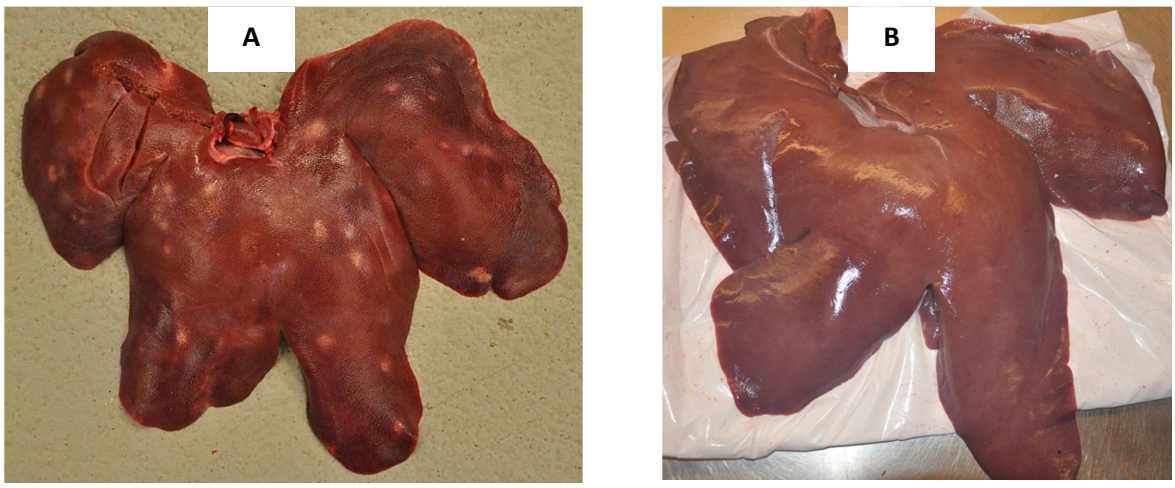

Fig 9. Livers of the weaner (A) with white spots and the finisher (B) without white spots

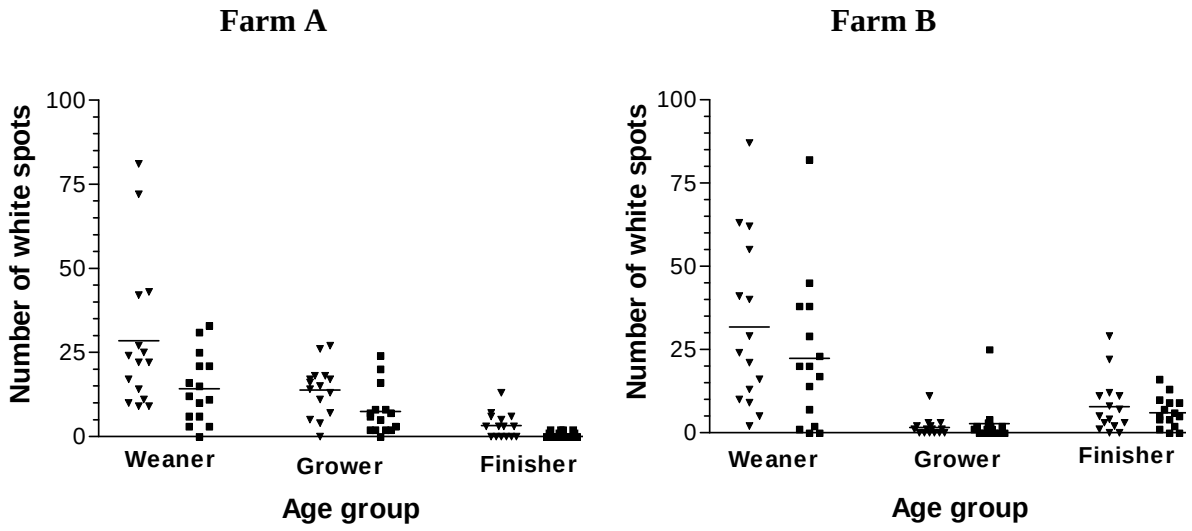

Fig 10. Number of Grade A (▼) and Grade B (■) superficial liver white spots in the weaner, the grower and the finisher pigs

from farm A and farm B.

***** Horizontal bars indicate mean values

4.6 *Ascaris suum* burden in the lungs

From farm A, on overall comparison, there was a significant difference ($\chi^2 = 31.24$, df = 2, $p < 0.0001$) in the number of *A. suum* larvae found in the lungs between the three age groups of pigs, the trend being decreasing with age (Fig 12). Similarly, the prevalence seemed to decreasing over time with the highest prevalence in the weaners followed by the growers and zero prevalence in the finishers (Table 2). There was a significant difference on the number of larvae present in the weaners and the finishers ($p < 0.0001$) and the growers and the finishers ($p < 0.0001$) but no significant difference was seen in the weaners and the growers ($p = 0.3250$).

On farm B, there was a significant difference ($\chi^2 = 7.96$, df = 2, $p = 0.0187$) in the number of *A. suum* larvae found in the lungs among three groups of pigs. Number of *A. suum* larvae in

the lungs thus appeared highest in the weaners followed by the growers, whereas no lung larvae were seen in the finishers (Fig 12). Although, the trend was decreasing over time, a significant difference in the number of lung larvae was observed only in the finishers and the growers ($p = 0.0048$) and no significant difference was seen between the weaners and the growers ($p > 0.05$) and the weaners and the finishers ($p > 0.05$).

4.7 *Ascaris suum* burden in the small intestine

Of 45 pigs, 91% from farm A harboured worms in the small intestine (Table 2). There was a significant difference among three age groups in the number of very young worms (≤1 cm) present in the small intestine ($\chi^2 = 34.29$, df = 2, $p < 0.0001$). The weaners had significantly more number of very young worms than the finishers ($p < 0.0001$) but no such significant difference was observed between the weaners and the growers ($p = 0.3250$). Similarly, there was a significant difference in the number of young worms present in the small intestine among pigs of three age groups ($\chi^2 = 13.56$, df = 2, $p = 0.0001$). The young worm count was significantly higher in the weaners than the growers ($p = 0.0003$) and the finishers ($p = 0.0373$). Significant difference was observed in the adult worm (Fig 11) count between the three age groups as well ($\chi^2 = 19.59$, df = 2, $p < 0.0001$). The finishers had significantly more number of adult worms than the growers ($p < 0.0001$) but marginal significance was observed between the finishers and the weaners ($p = 0.0648$).

From farm B, 71% pigs had worms in the small intestine. There was a significant difference in the number of the very young worms present between the three age groups ($\chi^2 = 34.29$, df = 2, $p < .0001$). The very young worm count was highest in the weaners and gradually decreased in the growers and the finishers (Fig 12). However, significant difference was observed only between the finishers and the weaners ($p = 0.026$) but not between the weaners and the growers ($p = 0.2498$). Like farm A, the young worm counts were significantly different between the three age group ($\chi^2 = 20.14$, df = 2, $p < .0001$). Significantly higher young worm count was observed in the weaners than the growers ($p < 0.0001$) and the finishers ($p = 0.0002$). Similarly, there was a significant difference in the adult worm count between three age group ($\chi^2 = 19.94$, df = 2, $p < 0.0001$). Adult worms were significantly higher in the finishers than in the weaners ($p = 0.0044$) and the growers ($p < 0.0001$).

Fig 11. Adult *Ascaris suum* in 70% ethanol recovered from the small intestine of the finishers of farm A

From the data of farm A of all age group, it has been shown that the number of larvae in the lungs was negatively associated with the number of adult worm present in the small intestine (χ^2 = 5.54, df = 1, P = 0.0186). The number of the young worms had no association with the number of adult worms in the small intestine (χ^2 = 1.54, df = 1, p = 0.2145).

Similarly from farm B, it has been shown that there was no association between the number of larvae in the lungs and the number of adult worms present in the small intestine (χ^2 = 1.63, df = 1, P = 0.2019). Likewise, the number of young worms had no association with the number adult worms in the small intestine (χ^2 = 0.09, df = 1, p = 0.7705).

There was no significant difference on over all worm burden (χ^2 = 1.32, df = 1, P = 0.25), liver white spots (χ^2 = 0.07, df = 1, P = 0.7929) and epg (χ^2 = 0.40, df = 1, P = 0.5283) between the two farms and the course of infection was similar except that the weaners from farm A had harboured adult worms but no adult worms were found on the weaners of farm B. Of 45 pigs from farm A, 49% had harboured *Oesophagostommum* spp. in the large intestine whereas all the pigs from farm B had been infested with *Oesophagostommum* spp.

In total 9% pigs from farm A and 24% from farm B had harboured *Trichuris suis* in the large intestine.

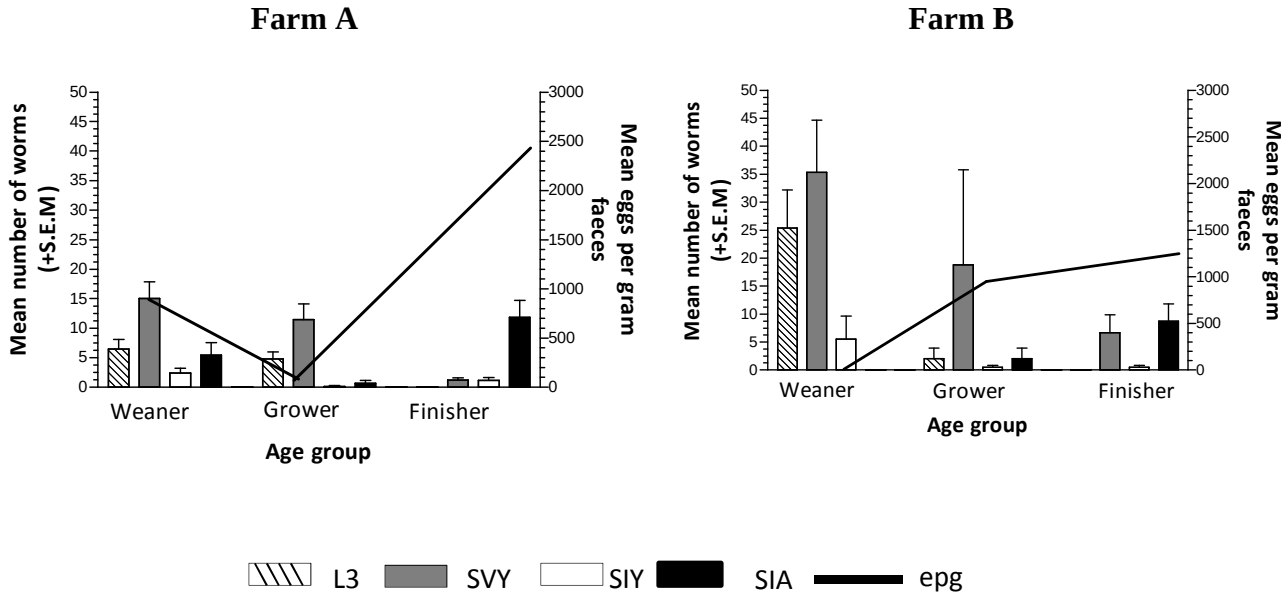

Fig 12. Mean number (+S.E.M) of *Ascaris suum* in the lungs (L3), the very young (≤1 cm) worms (SVY), the young (male: >1- <12 cm and female: >1 - <15 cm) worms (SIY) and the adult (male: ≥12 cm and female: ≥15 cm) worms (SIA) in the small intestine and eggs per gram faeces (epg) in the slaughtered weaners (n=15), growers (n=15) and finishers (n=15) from farm A and farm B.

Table 2. Mean number (min-max) and prevalence (%) of *Ascaris suum* in the lungs (L3), very young (≤1 cm), young (male: >1- <12 cm and female: >1 - <15 cm) and adult (male: ≥12 cm and female: ≥15 cm) *A. suum* in the small intestine and eggs per gram faeces (epg) of weaner (W), grower (G) and finisher (F) pigs from farm A and farm B

Farm	Age	Lungs			Small intestine						Total worm burden	Prevalence %	epg
		N	L3	Prevalence %	≤1 cm	Prevalence %	> 1 cm	Prevalence %	Adult	Prevalence %			
A	W	15	6 (0-23)	87	15 (0-40)	93	2 (0-10)	60	5 (0-24)	33	29 (5-54)	100	970 (0-6000)
	G	15	5 (0-14)	73	11 (0-31)	87	<1 (0-2)	7	1 (0-7)	20	17 (0-43)	93	93 (0-1360)
	F	15	0	0	1 (0-4)	47	1 (0-7)	40	12 (0-29)	87	14 (0-31)	93	2427 (0-9760)
B	W	15	25 (4-106)	100	35 (0-130)	93	5 (0-60)	33	0	0	66 (5-200)	100	0
	G	15	2 (3-28)	13	19 (0-256)	47	1 (0-4)	27	2 (2-29)	13	23 (0-284)	60	988 (0-14820)
	F	15	0	0	7 (0-36)	40	<1 (0-5)	13	9 (0-34)	47	16 (0-70)	60	1239 (0-3800)

4.8 *Ascaris suum* length

The weaners on farm A had worm of all sizes, the maximum of which was 22.5 cm (Fig 13). The growers had two worm populations having length <1-2 cm and 15-25 cm. Likewise, in the finishers, the length varied from ≤ 1 to 30 cm but no worms between 2 and 5 cm were found.

On farm B, the length of worms in the weaners did not exceed 9 cm and most of them measured ≤1 cm (Fig 13). Like farm A, two worm populations were found in the growers of farm B having length 1-4 cm and 15-20 cm. Similarly, in the finishers, three worm populations were found where one population was ≤1-1 cm, other was 12-30 cm and only two worms of length 5 and 9 cm were found.

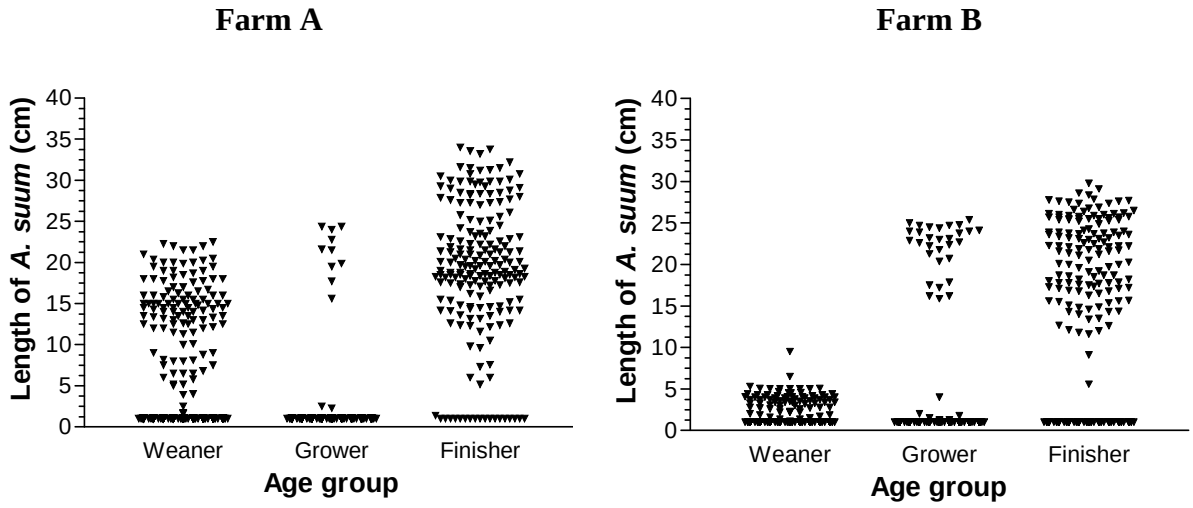

Fig 13. Length of individual *Ascaris suum* recovered from the weaners (n=15), growers (n=15) and finishers (n=15).

* Worms of length ≤1 cm was not measured so it was set to 1.0 cm.

4.9 Faecal egg counts

There was a significant difference (χ^2 = 11.56, df = 2, p = 0.0031) in the epg between the three age groups from farm A (Fig 14). On pairwise comparison, no significant difference was seen between the finishers and the weaners (p = 0.2544). The faecal egg counts were higher in the finishers, followed by the weaners and the growers, reflecting the overall worm burdens.

For farm B, there was also a significant difference (χ^2 = 11.56, df = 2, p = 0.0031) in faecal egg counts between the weaners, the growers and the finishers (Fig 14). The faecal egg counts were also higher in the finishers followed by the growers while no eggs (or adult worms) were found in the weaners.

A positive correlation was observed between epg and the number of adult *A. suum* females (r^2 = 0.54, p < 0.0001) that were recovered from the necropsied pigs from both the farms (Fig 15). A false positive egg count was observed in 13% growers and 13% finishers of farm A and 33% finishers of farm B.

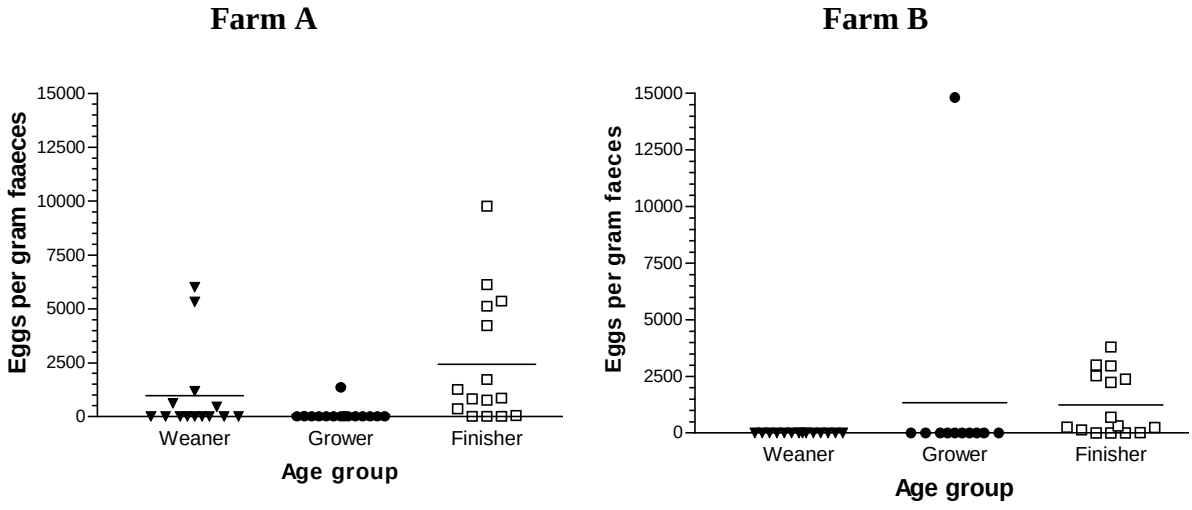

Fig 14. Eggs per gram faeces of *Ascaris suum* in slaughtered weaners (▼; n=15), growers (•; n=15) and finishers (□; n=15) on two farms (A and B)

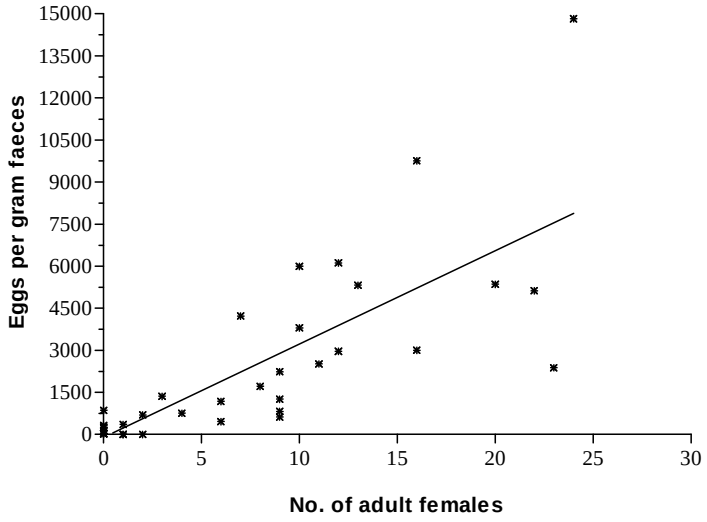

Fig 15. Correlation between the number of *Ascaris suum* eggs per gram faeces (epg) and the number of adult *Ascaris suum* females (pooled data from farm A and farm B)

5 Discussion

A number of experimental studies have studied the population biology and the transmission dynamics of *A. suum* without fully representing the natural scenario on farm. To my best knowledge, this may be the first observational study that was conducted to describe the infection dynamics of *A. suum* on organic pig farms over time through necropsies, thus confirming the experimental data of other studies. The results indicated that organic pigs are continuously exposed to the infective eggs of *A. suum* until they are slaughtered. Piglets take up *A. suum* infection at an early age from contaminated farrowing paddocks and continue to be exposed in the indoor pens. Young pigs seemed to be more susceptible to the pre-intestinal stages as they harboured higher numbers of migrating larvae (liver white spots and the lung larvae) than the older. Over time, most of the pigs expelled the worm and got reinfected with infective eggs present in the environment. After reinfection, some worms might have been established in the small intestine and become adult while the other newly hatched larvae might have been prevented from the migration due to the development of host acquired immunity. Thus, the established adult worm population had started to produce eggs and contaminated the environment. Furthermore, the results of the present study also showed that the infective eggs could be found in all areas of the pens, subsequently becoming source of infection to the following batch of pigs if not properly cleaned.

5.1 Contamination in farrowing paddocks

On Danish organic farms, farrowing sows and piglets are kept on pastures throughout the year and the minimum age of the piglets at weaning is 7 weeks. The weaners are then either moved to weaning pasture or to indoor pens (Anonymous, 2010). During the study period, the weaners from both farms were moved to the indoor pens. The results of the soil samples revealed that the farrowing paddocks of the farms were contaminated with the infective *A. suum* eggs suggesting young pigs could have acquired infection from the farrowing pasture at an early age. The preceding summer was hot and dry, therefore, it was assumed that the egg mortality might have been higher than usual. During the summer, *A. suum* eggs either developed faster (Roepstorff and Murrell, 1997) than other seasons or the eggs might have died due to higher temperature (compared to spring, autumn and winter) and dehydration (Larsen and Roepstorff, 1999), ensuring low numbers of eggs in the autumn compared to if the summer had been wetter. Under Danish conditions, Larsen and Roepstorff (1999) have

underlined that the eggs deposited in the autumn, winter and spring develop in the following summer only. The infective *A. suum* eggs found in the current study could have been excreted by the pigs in the late summer 2013 and some might have developed faster and became infective in the late autumn due to warm weather. However, some eggs could have been excreted during the previous year too. The later eggs (eggs excreted in 2012) might have been protected being buried in soil or covered by vegetation as sun-exposed soil compared to unexposed have higher mortality (Beaver, 1952). As *A. suum* eggs lose water very slowly unless the temperature increases (Wharton, 1979), it could be speculated that the eggs from 2012 were protected from long term dehydration.

Furthermore, on the farms, rotational and strip grazing was practiced. The presence of *A. suum* eggs in the pasture indicated that pasture rotations for shorter period (upto 3 years) might have little effect on the transmission of *A. suum.*

5.2 Contamination in the WG and the GF pens with *A. suum* eggs

The majority of the eggs were found in the LA followed by the SF which were adjacent to the LA and the least number of eggs were present in the FA on farm A. Similar findings were observed in all the WG and the GF pens of farm B. High number of eggs was found in the LA and the SF which could reflect the defaecation pattern of the pigs as well. The eggs present in all areas of the pens suggest that pigs could have disseminated the faeces containing eggs from the LA or the SF (where they normally defecated) to other areas. However, eggs were found in the area where pigs were not able to reach (in the roof above the LiA on farm A, data not shown). Therefore, dissemination of the eggs could have occurred through contaminated equipment or from the person handling the farm too.

The infective eggs were present in all areas of the WG and the GF pens of both farms although the number was much lower compared to developing and dead eggs. Therefore, it could be speculated that there is a risk of continuous possible transmission of *A. suum* to the following batch (upcoming pigs) in the indoor pens if the pens are not properly cleaned after removal of the pigs.

5.3 *Ascaris suum* in weaners, growers and finishers

Following a single inoculation, Roepstorff et al. (1997) found that the larvae had reached lungs at day 3 post inoculations (p.i.), the majority of white spots in livers were seen at day 10 p.i., and that the migrating larvae had returned to the small intestine at day 10 p.i. These newly migrated larvae in the small intestine at day 11 p.i. measured 0.18-0.20 cm in length (Douvres et al., 1969). Assuming the early moderate natural infection to be similar to that of single inoculation, in the current study, 60% of the weaners from farm A and 33% from farm B had worms of more than 1 cm length which suggests that the pigs might have become infected at least 10 days before necropsy. A Danish study indicated that the pigs born and raised on contaminated paddocks had become infected approximately at 1½ weeks of age (Mejer and Roepstorff, 2006). The scenario could be similar for this study too, as 33% weaners from farm A had harboured adult worms and excreted eggs in the faeces at weaning (7-9 weeks old). This means that these piglets might have become infected 6-7 weeks before the necropsy or within the first weeks of life because normally adult female *A. suum* begins to excrete eggs within six to eight weeks p.i. (Roepstorff et al., 1997). In other words, piglets could have ingested infective eggs in the first week of life when they were confined to the contaminated farrowing huts or shortly after getting access to the pasture as such.

Kelley and Nayak (1965) underlined that piglets can get partial protection until the age of 3-5 weeks by maternal antibodies which they receive via colostrum. However, negative effect or no effect had also been found in piglets that had received colostrum from experimentally long-term exposed sows (Boes et al., 1998). In consistent to findings of Boes et al. (1998), on an experimental study where two groups of sows were exposed with moderate to high *A. suum* infection and the other group without exposure, Mejer (unpublished) observed no effect of *A. suum* infection in piglets born from these sows irrespective of the sows immune status. Although sows were not included in the present study, it is likely that they had also been exposed to *A. suum* and transferred antibodies to the new-born piglets via colostrum. The presence of worms in the weaners suggests that maternal antibodies could have little effect on *A. suum* transmission.

The worm burden in the lungs and the small intestine, and the number of white spots on the liver of growers from both farms were lower than for the weaners. Masure et al. (2013) have described that a self-cure mechanism by primary infection in the host elicits increased gut

motility that causes the expulsion of the newly migrated larvae/very young worms from the intestine by weep (increased luminal secretion) and sweep (increased gut movement) mechanism (Anthony et al., 2007). However, the adult worm populations are able to counteract this mechanism (Masure et al., 2013). Perhaps, the weaners of both farms must have expelled some larvae/very young worms in the farrowing paddock by weep and sweep mechanism but the very young, young and adult worms that were in the weaners should have reflected in the growers, too. The substantial decrease in the worm burden in the growers suggests that other complex factors might have played role in the expulsion process. One possible explanation could be that post weaning diarrhoea which occurs commonly in the weaners when they are moved from farrowing paddock to the WG pens (Helena Mejer, personal communication). The onset of diarrhoea might have benefited the pigs due to the expulsion of the worms from the small intestine by some unknown mechanism. The very young worms that were harvested from growers of both farms could have been acquired after the pigs recovered from the post weaning diarrhoea. A study by Boes et al. (1998) has suggested that pigs might be reinfected after the expulsion of adult worms from the intestine. Furthermore, after anthelmintic treatment, it has been found that pigs get reinfected to *A. suum* if the environmental contamination is present (Pattison et al., 1980; Roepstorff, 1997). Although, anthelmintic treatment is kept at a minimum on these farms, the growers might have expelled the worms either by a non-immune mediated transitory self-cure mechanism or might have been eliminated as a consequence of post-weaning diarrhoea. The growers must have been reinfected by newly migrated larvae which were reflected by the presence of adult worm population in the finishers.

In contrast to farm A, a few growers from farm B had lung larvae (farm A: 73%, farm B: 13%), very young (farm A: 87%, farm B: 47%) and young worms (farm A: 87%, farm B: 47%) in the small intestine. In addition, the number of white spots on the liver of growers was less compared to the weaners of farm B. It could be speculated that the growers from farm B could have started to become immune as there were infective eggs present in the environment.

In the finishers, the adult worm population was predominant on both farms. On long term exposure to infective eggs, pigs might develop almost complete acquired immunity by 19 weeks (Urban et al., 1988) which is reflected by the gradual development of an immune

mediated pre-hepatic barrier, which might almost completely inhibits newly hatched larvae from migrating while not affecting the established adult worms (Eriksen et al., 1992a; Masure et al., 2013; Urban et al., 1988). The same phenomenon could have occurred in the current study where the acquired immunity might have acted on newly migrated larvae reducing its migration to the lungs and the intestine while not affecting adult worms that had successfully established. This adult population then could have posed a threat of continued contamination of the GF pens. Thus, potentially ensuring infective eggs, that could infect later batches of pigs, if the pens are not properly cleaned.

Furthermore, two populations of the adult and the very young worms (≤1 cm) were present in the finishers of farm B. Consistent with the current findings, other studies had also found two worm populations in pigs that were exposed continuously on pasture and on repeated experimental exposure (Mejer and Roepstorff, 2006; Nejsum et al., 2009b). The authors from those studies have argued that the pre-hepatic barrier did not completely prevent reinfection but after completing migration the newly recruited larvae got expelled from the intestine after sometime. A similar phenomenon could have occurred in the current study as well since there was no young worm population in any of the pigs. Therefore, the very young worms could have been the newly migrated worms that were not expelled. Moreover, it could be possible that the adult population present in the small intestine of the finishers might have played role in the expulsion of the larvae that had completed migration by concomitant immunity as suggested by Roepstorff (2003).

Apart from *A. suum*, *Oesophagostommum* spp. was recovered from the large intestine of all the slaughtered pigs of farm B and 49% from farm A. The anterior part of the large intestine is the predilection site of *Oesophagostommum* spp. (Christensen et al., 1995) and also the site of penetration of newly hatched *A. suum* larvae (Murrell et al., 1997). Though these two parasite species shared the same site, a weak antagonistic interaction between these two worms had been observed by Helwigh et al. (1999) where *O. dentatum* was shorter and had lower faecal egg counts if *A. suum* was present but no effect was seen on *A. suum*. Therefore, in the current study, the presence of *Oesophagostommum* spp. could have had less effect on *A. suum* population.

On a study by Roeptorff et al. (1997), a significant positive correlation was observed between the number of adult worms and epg. In consistent to his finding, a positive correlation between adult female *A. suum* and epg was observed on the current study. This suggests that the detection of eggs in the faeces reflect the presence of the adult worms in the small intestine. However, there might be some possibility of getting false positive (due to coprophagic behaviour) or false negative results (unisex or immature infection). Furthermore, in the current study, false positive result was found in a few growers and finishers and but not in the weaners which could be due to the availability of large surface area per pig on an outdoor pasture compared to the indoor pens.

6 Conclusion

From the results of the present study it can be concluded that the transmission of *A. suum* in organic pigs occurs at a very early age on the farrowing pasture contaminated with infective *A. suum* eggs. All the pigs thus acquire infection by the time they are weaned. The infective eggs can be found in the indoor pens due to infrequent cleaning of pens and use of straw bedding. Therefore, the transmission could occur in the indoor pens as well. In the indoor pens after weaning, most of the pigs expel worms and get reinfected with infective eggs from the environment. The overall intensities and prevalence decreases as the pigs grow older presumably to some level of immunity. It has been substantiated that there is a complex interaction between the host, parasite and environment in *A. suum* infection where establishment of parasites in the host depends upon level of exposure to infective eggs, interaction of parasite within the host and most likely the immune response of the host.

7 Perspectives

The present study has broadened the knowledge on the dynamics of infection of *A. suum* on farm which may be used for further studies to control the parasite by using alternative approaches. In addition, many ideas and possibilities have arisen for future studies.

The present study on two farms was performed from late autumn 2013 to February 2014. However, the availability of infective eggs in the environment might vary according to the season, year, etc due to variation in microclimatic conditions of the environment. Eventually, there might be difference in the dynamics of *A. suum* infection according to year, season and batch of the pigs. Similarly, the dynamics might also vary according to the farm management system as infection pattern might be different on farms having facilities of weaning pasture and on farms that directly move the weaners into the indoor pens. Further research on *A. suum* could be done on transmission pattern on farms that provide weaning pasture and farms that only house the weaners in indoor pens.

The current study relied on the findings on worm burden, faecal egg count and number of white spots present on the liver. Testing the pig sera for antibodies specific against *A. suum* excretory or secretary antigens (Roepstorrf *et al.*, 1997, Eriksen *et al*., 1992a) along with the procedures that were followed in the current study could be done to find out whether the

worm free pigs had been previously exposed to the *A. suum.* This may also give an overview of the susceptibility/resistance against *A. suum.*

The viability of the eggs was confirmed using morphological examination by visual inspection of eggs under microscope. Although, examining eggs by visual inspection is easy to perform and faster than other methods such as incubation of eggs for six weeks to test ability to embryonate, it was difficult to distinguish between the live and dead eggs if the eggs had not undergone vacuolization, cytolysis or any shrinkage. Therefore, it is necessary to develop new methods to distinguish between viable and non-viable eggs.

On farm level, it is difficult to control *A. suum* transmission in outdoor pasture but if control measures are applied in the indoor pens, then the transmission could be minimized. For an example, if anthelmintic treatment (after diagnosing) is combined with proper cleaning and drying of pens when the pigs are moved from farrowing pasture to the indoor pens, then it could reduce transmission. For cleaning, natural products such as slaked lime which has shown negative effect on the survival of *A. suum* eggs (Ibsen, 1999) could be used.

References

Anderson, T.J., 1995. *Ascaris* infections in humans from North America: molecular evidence for cross-infection. Parasitology 110, 215-219.

Anonymous 2010. Guidelines for organic husbandry and agriculture. In Vejledningomokologiskjordbrugsproduktion. (Ministry of Food, Agriculture and Fisheries Danish Plant Inspectorate), p. 175.

Anthony, R.M., Rutitzky, L.I., Urban, J.F., Jr., Stadecker, M.J., Gause, W.C., 2007. Protective immune mechanisms in helminth infection. Nature reviews. Immunology 7, 975-987.

Barrett, J., 1976. Studies on the induction of permeability in *Ascaris lumbricoides* eggs. Parasitology 73, 109-121.

Beaver, P.C., 1952. Observations on the epidemiology of ascariasis in a region of high hookworm endemicity. The Journal of parasitology, 445-453.

Boes, J., Medley, G.F., Eriksen, L., Roepstorff, A., Nansen, P., 1998. Distribution of *Ascaris suum* in experimentally and naturally infected pigs and comparison with *Ascaris lumbricoides* infections in humans. Parasitology 117 589-596.

Boes, J., Nansen, P., Stephenson, L.S., 1997. False-positive *Ascaris suum* egg counts in pigs. International journal for parasitology 27, 833-838.

Borgsteede, F.H., Jongbloed, A.W., 2001. [Organic pig farming: what about parasitic infections?]. Tijdschrift voor diergeneeskunde 126, 39-42.

Carstensen, L., Vaarst, M., Roepstorff, A., 2002. Helminth infections in Danish organic swine herds. Veterinary parasitology 106, 253-264.

Christensen, C., Barnes, E., Nansen, P., Roepstorff, A., Slotved, H.-C., 1995. Experimental *Oesophagostomum dentatum* infection in the pig: Worm populations resulting from single infections with three doses of larvae. International journal for parasitology 25, 1491-1498.

Douvres, F.W., Tromba, F.G., Malakatis, G.M., 1969. Morphogenesis and migration of *Ascaris suum* larvae developing to fourth stage in swine. The Journal of parasitology, 689-712.

Eriksen, L., Andersen, S., Nielsen, K., Pedersen, A., Nielsen, J., 1980. Experimental *Ascaris suum* infection in pigs. Serological response, eosinophilia in peripheral blood,

occurrence of white spots in the liver and worm recovery from the intestine. Nordisk veterinaermedicin 32, 233-242.

Eriksen, L., Lind, P., Nansen, P., Roepstorff, A., Urban, J., 1992a. Resistance to *Ascaris suum* in parasite naive and naturally exposed growers, finishers and sows. Veterinary parasitology 41, 137-149.

Eriksen, L., Nansen, P., Roepstorff, A., Lind, P., Nilsson, O., 1992b. Response to repeated inoculations with *Ascaris suum* eggs in pigs during the fattening period. I. Studies on worm population kinetics. Parasitology research 78, 241-246.

Fairbrother, J.M., Nadeau, E., Gyles, C.L., 2005. *Escherichia coli* in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Animal health research reviews / Conference of Research Workers in Animal Diseases 6, 17-39.

Forsum, E., Nesheim, M., Crompton, D., 1981. Nutritional aspects of *Ascaris* infection in young protein-deficient pigs. Parasitology 83, 497-512.

Früh, B., Bochicchio, D., Dippel, S., Edwards, S., Gunnarsson, S., Leeb, C., Lindgren, K., Mejer, H., Prunier, A., 2011. Organic Pig Production in Europe, Health Management in Common Organic Pig Farming. In Technical guide from the COREPIG project (Research Institute of Organic Agriculture (FiBL)) pp. 4.

Galvin, T.J., 1968. Development of human and pig ascaris in the pig and rabbit. The Journal of parasitology 54, 1085-1091.

Gantzer, C., Gaspard, P., Galvez, L., Huyard, A., Dumouthier, N., Schwartzbrod, J., 2001. Monitoring of bacterial and parasitological contamination during various treatment of sludge. Water research 35, 3763-3770.

Geng, J., 2002. Secretion of a Novel, Developmentally Regulated Chitinase (Family 19 Glycosyl Hydrolase) Into the Perivitelline Fluid of the Parasitic Nematode, *Ascaris Suum*. Molecular and Biochemical Parasitology 124, 11-21.

Hale, O.M., Stewart, T.B., Marti, O.G., 1985. Influence of an experimental infection of *Ascaris suum* on performance of pigs. Journal of animal science 60, 220-225.

Haugegaard, J., 2010. Prevalence of nematodes in Danish industrialized sow farms with loose housed sows in dynamic groups. Veterinary parasitology 168, 156-159.

Helwigh, A.B., Christensen, C.M., Roepstorff, A., Nansen, P., 1999. Concurrent *Ascaris suum* and *Oesophagostomum dentatum* infections in pigs. Veterinary parasitology 82, 221-234.

Hinck, L.W., Ivey, M.H., 1976. Proteinase activity in *Ascaris suum* eggs, hatching fluid, and excretions-secretions. The Journal of parasitology 62, 771-774.

Ibsen, A.E.W., 1999. Occurence of *Ascaris suum* eggs in the danish swine breeding. Special veterinary report. Center for Experimental Parasitology, Royal Veterinary and Agricultural School, Copenhagen, Denmark, pp. 18.

IFOAM, 2000. The World Grows Organic In: Proceedings in 13th International IFOAM Scientific Conference, Basel, Switzerland, 28 to 31 August, 2000.

Katakam, K.K., 2014. Ecology and viability of eggs of the pig round worm (*Ascaris suum*) on-farm and laboratory studies. PhD Thesis. University of Copenhagen, Denmark, pp. 108-132.

Katakam, K.K., Roepstorff, A., Popovic, O., Kyvsgaard, N.C., Thamsborg, S.M., Dalsgaard, A., 2013. Viability of *Ascaris suum* eggs in stored raw and separated liquid slurry. Parasitology 140, 378-384.

Kelley, G.W., Nayak, D.P., 1965. Passive immunity to *Ascaris suum* transferred in colostrum from sows to their offspring. American journal of veterinary research 26, 948-950.

Kraglund, H.-O.R., 1999. Survival, development and dispersal of the free-living stages of *Ascaris suum*, *Oesophagostomum dentatum* and *Trichuris suis* at the pasture. PhD Thesis. Department of Ecology, Zoology Section, The Royal Veterinary and Agricultural University, Copenhagen, Denmark, pp.105-119.

Kraglund, H.O., Roepstorff, A., Gronvold, J., 2001. The impact of season and vegetation on the survival and development of *Oesophagostomum dentatum* larvae in pasture plots. Parasitology 123, 415-423.

Kransnonos, L.N., 1978. [Long-term survival of ascarid eggs (*Ascaris lumbricoides L.*, 1758) in the soil of Samarkand]. Meditsinskaia Parazitologiia i Parazitarnye Bolezni 47, 103-105.

Larsen, M.N., Roepstorff, A., 1999. Seasonal variation in development and survival of *Ascaris suum* and *Trichuris suis* eggs on pastures. Parasitology 119, 209-220.

Lunney, J.K., Urban, J.F., Jr., Johnson, L.A., 1986. Protective immunity to *Ascaris suum*: analysis of swine peripheral blood cell subsets using monoclonal antibodies and flow cytometry. Veterinary parasitology 20, 117-131.

Masure, D., Wang, T., Vlaminck, J., Claerhoudt, S., Chiers, K., Van den Broeck, W., Saunders, J., Vercruysse, J., Geldhof, P., 2013. The intestinal expulsion of the roundworm *Ascaris suum* is associated with eosinophils, intra-epithelial T cells and decreased intestinal transit time. PLoS neglected tropical diseases 7(12), e2588. doi:10.1371/journal.pntd.0002588

Mejer, H., Roepstorff, A., 2006. *Ascaris suum* infections in pigs born and raised on contaminated paddocks. Parasitology 133, 305-312.

Miquel, N., Roepstorff, A., Bailey, M., Eriksen, L., 2005. Host immune reactions and worm kinetics during the expulsion of *Ascaris suum* in pigs. Parasite immunology 27, 79-88.

Murrell, K.D., Eriksen, L., Nansen, P., Slotved, H., Rasmussen, T., 1997. *Ascaris suum*: A revision of its early migratory path and implications for human ascariasis. The Journal of parasitology 83, 255-260.

Nejsum, P., Parker, E.D., Jr., Frydenberg, J., Roepstorff, A., Boes, J., Haque, R., Astrup, I., Prag, J., Skov Sorensen, U.B., 2005. Ascariasis is a zoonosis in denmark. Journal of clinical microbiology 43, 1142-1148.

Nejsum, P., Roepstorff, A., Jorgensen, C.B., Fredholm, M., Goring, H.H., Anderson, T.J., Thamsborg, S.M., 2009a. High heritability for *Ascaris* and *Trichuris* infection levels in pigs. Heredity 102, 357-364.

Nejsum, P., Thamsborg, S.M., Petersen, H.H., Kringel, H., Fredholm, M., Roepstorff, A., 2009b. Population dynamics of *Ascaris suum* in trickle-infected pigs. The Journal of parasitology 95, 1048-1053.

Oksanen, A., Eriksen, L., Roepstorff, A., Ilsoe, B., Nansen, P., Lind, P., 1990. Embryonation and infectivity of *Ascaris suum* eggs. A comparison of eggs collected from worm uteri with eggs isolated from pig faeces. Acta veterinaria Scandinavica 31, 393-398.

Olsen, L.S., Kelley, G.W., Sen, H., 1958. Longevity and egg-production of *Ascaris suum*. Transactions of the American Microscopical Society 77, 380-383.

Passey, R.F., Fairbairn, D., 1955. The respiration of *Ascaris lumbricoides* eggs. Canadian journal of biochemistry and physiology 33, 1033-1046.

Pattison, H.D., Thomas, R.J., Smith, W.C., 1980. A survey of gastrointestinal parasitism in pigs. The Veterinary record 107, 415-418.

Permin, A., Yelifari, L., Bloch, P., Steenhard, N., Hansen, N.P., Nansen, P., 1999. Parasites in cross-bred pigs in the Upper East region of Ghana. Veterinary parasitology 87, 63-71.

Rhodes, M.B., McCullough, R.A., Mebus, C.A., Klucas, C.A., Ferguson, D.L., Twiehaus, M.J., 1977. *Ascaris suum*: hatching of embryonated eggs in swine. Experimental parasitology 42, 356-362.

Roepstorff, A., 1997. Helminth surveillance as a prerequisite for anthelmintic treatment in intensive sow herds. Veterinary parasitology 73, 139-151.

Roepstorff, A., 1998. Natural *Ascaris suum* infections in swine diagnosed by coprological and serological (ELISA) methods. Parasitology research 84, 537-543.

Roepstorff, A., 2003. *Ascaris suum* in pigs, Population Biology and Epidemiology. Doctorate Thesis. Royal Veterinary and Agricultural University, Copenhagen, Denmark, pp 38-40.

Roepstorff, A., Eriksen, L., Slotved, H.C., Nansen, P., 1997. Experimental *Ascaris suum* infection in the pig: worm population kinetics following single inoculations with three doses of infective eggs. Parasitology 115, 443-452.

Roepstorff, A., Jørgensen, R.J., Nansen, P. 1992. Parasitter hos økologiske svin. In Rapport over projekt finansieret af Jordbrugsdirektoratet under Landbrugsministeriet, København, Danske Slagterier, p. 2.

Roepstorff, A., Murrell, K., 1997. Transmission dynamics of helminth parasites of pigs on continuous pasture: *Ascaris suum* and *Trichuris suis*. International journal for parasitology 27, 563-572.

Roepstorff, A., Murrell, K., Boes, J., Petkevičius, S., 2001. Ecological influences on transmission rates of *Ascaris suum* to pigs on pastures. Veterinary parasitology 101, 143-153.

Roepstorff, A., Nansen, P., 1994. Epidemiology and control of helminth infections in pigs under intensive and non-intensive production systems. Veterinary Parasitolology 54, 69-85.

Roepstorff, A., Nansen, P. 1998. Epidemiology, diagnosis and control of helminth parasites of swine. A FAO handbook. FAO, Rome, pp. 51, 112.

Roepstorff, A., Nilsson, O. 1991. En fællesnordisk prævalensundersøgelse. In Epidemiologien ved parasitære infektioner hos svin: Parasitære infektioner hos svin, vedrørende parasitære infektioner hos svin, undersøgelser over epidemiologi, diagnostik, vært/parasitrelationer og bionomi under forskellige produktionssystemer, København, Den kgl. Veterinær- og Landbohøjskole, pp. 32-33, 35.

Roepstorff, A., Nilsson, O., Oksanen, A., Gjerde, B., Richter, S.H., Örtenberg, E., Christensson, D., Martinsson, K.B., Bartlett, P.C., Nansen, P., Eriksen, L., Helle, O., Nikander, S., Larsen, K., 1998. Intestinal parasites in swine in the Nordic countries: prevalence and geographical distribution. Veterinary parasitology 76, 305-319.

Ronéus, O., 1966. Studies on the aetiology and pathogenesis of white spots in the liver of pigs. Journal Acta Veterinaria Scandinavica 16, 1-112.

Rose, J.H., Small, A.J., 1981. The relationship between pasture herbage and the development and survival of the free-living stages of *Oesophagostomum dentatum*. Journal of helminthology 55, 109-113.

Seamster, A.P., 1950. Developmental studies concerning the eggs of *Ascaris lumbricoides var. suum*. American Midland Naturalist 43, 450-470.

Slotved, H.C., Barnes, E.H., Eriksen, L., Roepstorff, A., Nansen, P., Bjorn, H., 1997. Use of an agar-gel technique for large scale application to recover *Ascaris suum* larvae from intestinal contents of pigs. Acta veterinaria Scandinavica 38, 207-212.

Steenhard, N.R., Jungersen, G., Kokotovic, B., Beshah, E., Dawson, H.D., Urban, J.F., Jr., Roepstorff, A., Thamsborg, S.M., 2009. *Ascaris suum* infection negatively affects the response to a *Mycoplasma hyopneumoniae* vaccination and subsequent challenge infection in pigs. Vaccine 27, 5161-5169.

Thamsborg, S.M., Nejsum, P., Mejer, H., 2013b. Impact of *Ascaris suum* in livestock, In: HOLLAND, C. (Ed.) ASCARIS: THE NEGLECTED PARASITE. Elsevier Inc. , Dublin, pp. 363-381.

Thomsen, L.E., Mejer, H., Wendt, S., Roepstorff, A., Hindsbo, O., 2001. The influence of stocking rate on transmission of helminth parasites in pigs on permanent pasture during two consecutive summers. Veterinary parasitology 99, 129-146.

Urban, J.F., Jr., Alizadeh, H., Romanowski, R.D., 1988. *Ascaris suum*: development of intestinal immunity to infective second-stage larvae in swine. Experimental parasitology 66, 66-77.

Weng, Y., Hu, Y., Li, Y., Li, B., Lin, R., Xie, D., Gasser, R., Zhu, X., 2005. Survey of intestinal parasites in pigs from intensive farms in Guangdong Province, People's Republic of China. Veterinary parasitology 127, 333-336.

Wharton, D., 1980. Nematode egg-shells. Parasitology 81, 447-463.

Wharton, D.A., 1979. *Ascaris* sp.: Water loss during desiccation of embryonating eggs. Experimental parasitology 48, 398-406.

Williams-Blangero, S., Criscione, C.D., VandeBerg, J.L., Correa-Oliveira, R., Williams, K.D., Subedi, J., Kent, J.W., Jr., Williams, J., Kumar, S., Blangero, J., 2012. Host genetics and population structure effects on parasitic disease. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 367, 887-894.

Appendices

Appendix 1

Table 1a. Raw data on number of *Ascaris suum* in the lungs (L3), the very young (≤1 cm) worms (SVY), the young (male: >1- <12 cm and female: >1 - <15 cm) worms (SIY) and the adult (male: ≥12 cm and female: ≥15 cm) worms (SIA) in the small intestine, white spots on liver (Grade A: clear white lymphonodular or diffuse and Grade B: greyish white lymphonodular or diffuse) and eggs per gram (epg) faeces of the necropsied **weaners** of farm (A & B)

Farm	Pig	Lungs	Small intestine				Liver		Mc Master
		L3	≤ 1 cm	>1cm	Female	Male	Grade A	Grade B	epg
A	1	2	6	10	13	8	24	3	5320
	2	4	12	1	0	0	14	6	0
	3	0	25	2	12	12	9	21	6000
	4	5	40	2	0	0	11	16	0
	5	9	32	1	9	3	42	21	620
	6	8	7	6	6	7	10	10	1180
	7	0	10	0	0	0	25	25	0
	8	17	13	0	0	0	43	15	0
	9	4	20	0	0	0	81	33	NA
	10	7	6	2	0	0	17	6	0
	11	2	18	7	0	0	27	31	0
	12	5	6	5	9	2	22	3	460
	13	23	14	0	0	0	72	0	0
	14	5	0	0	0	0	9	11	0
	15	6	17	0	0	0	22	12	0
B	1	30	1	1	0	0	24	20	0
	2	5	53	0	0	0	9	2	0
	3	6	8	0	0	0	21	7	0
	4	7	20	0	0	0	87	17	0
	5	38	59	0	0	0	5	0	0
	6	23	14	13	0	0	13	1	0
	7	23	16	0	0	0	40	38	0
	8	5	0	0	0	0	16	20	0
	9	35	36	0	0	0	62	23	0
	10	38	25	0	0	0	2	0	0
	11	4	33	0	0	0	10	29	0
	12	106	28	0	0	0	63	82	0
	13	7	13	4	0	0	41	45	NA
	14	43	94	3	0	0	55	38	0
	15	10	130	60	0	0	29	14	0

Table 1b. Raw data on number of *Ascaris suum* in the lungs (L3), the very young (≤1 cm) worms (SVY), the young (male: >1- <12 cm and female: >1 - <15 cm) worms (SIY) and the adult (male: ≥12 cm and female: ≥15 cm) worms (SIA) in the small intestine, white spots on liver (Grade A: clear white lymphonodular or diffuse and Grade B: greyish white lymphonodular or diffuse) and eggs per gram (epg) faeces of the necropsied **growers** of farm (A & B)

Farm	Pig	Lungs	Small intestine				Liver		Mc Master
		L3	≤ 1 cm	>1cm	Female	Male	Grade A	Grade B	epg
A	16	5	22	0	0	0	18	2	0
	17	3	5	0	0	0	14	3	0
	18	0	18	0	0	0	0	2	0
	19	3	3	0	0	0	7	2	0
	20	11	8	0	1	0	11	0	0
	21	3	6	0	2	0	15	8	0
	22	12	31	0	0	0	27	24	0
	23	0	0	0	0	0	5	2	0
	24	0	1	0	0	0	16	7	0
	25	3	0	0	0	0	17	7	0
	26	0	1	2	0	0	13	6	0
	27	14	14	0	3	4	17	8	1360
	28	4	25	0	0	0	18	16	20
	29	4	16	0	0	0	26	20	0
	30	10	22	0	0	0	4	5	20
B	16	0	0	0	0	0	1	3	0
	17	0	0	0	0	0	1	0	0
	18	28	256	0	0	0	11	25	0
	19	0	0	0	0	0	0	0	0
	20	0	1	0	1	0	3	0	0
	21	3	0	1	0	0	1	0	0
	22	0	0	0	0	0	0	2	NA
	23	0	1	0	0	0	1	0	0
	24	0	5	0	0	0	2	2	NA
	25	0	0	0	0	0	0	1	0
	26	0	13	4	0	0	0	0	0
	27	0	0	0	0	0	3	1	NA
	28	0	0	2	0	0	2	4	NA
	29	0	4	1	24	5	0	1	14820
	30	0	2	0	0	0	0	2	0

Table 1c. Raw data on number of *Ascaris suum* in the lungs (L3), the very young (≤1 cm) worms (SVY), the young (male: >1- <12 cm and female: >1 - <15 cm) worms (SIY) and the adult (male: ≥12 cm and female: ≥15 cm) worms (SIA) in the small intestine, white spots on liver (Grade A: clear white lymphonodular or diffuse and Grade B: greyish white lymphonodular or diffuse) and eggs per gram (epg) faeces of the necropsied **finishers** of farm (A & B)

Farm	pig	Lungs	Small Intestine				Liver		Mc Master
		L3	≤ 1 cm	>1cm	Female	Male	Grade A	Grade B	epg
A	31	0	0	0	1	0	3	0	860
	32	0	0	0	9	16	13	2	1260
	33	0	0	0	0	2	6	2	0
	34	0	2	1	4	2	7	2	760
	35	0	0	7	9	11	3	0	820
	36	0	2	1	22	5	5	0	5120
	37	0	2	0	1	1	6	2	360
	38	0	0	0	0	0	0	1	40
	39	0	0	3	16	11	0	0	9760
	40	0	0	4	8	2	3	0	1720
	41	0	0	1	0	0	0	1	0
	42	0	2	0	7	3	0	0	4220
	43	0	4	0	12	5	3	0	6120
	44	0	4	0	1	0	0	1	0
	45	0	2	0	20	9	0	0	5360
B	31	0	0	0	0	0	11	1	0
	32	0	0	0	2	1	22	9	700
	33	0	0	0	12	7	7	4	2960
	34	0	0	0	11	6	8	7	2520
	35	0	6	0	0	0	3	10	240
	36	0	0	0	0	0	5	5	320
	37	0	34	5	16	15	2	6	3000
	38	0	36	0	0	0	4	5	140
	39	0	0	0	0	0	1	4	260
	40	0	18	1	9	2	29	9	2240
	41	0	0	0	10	6	0	0	3800
	42	0	2	0	0	0	12	13	0
	43	0	4	0	23	11	3	2	2380
	44	0	0	0	0	0	11	16	20
	45	0	0	0	0	0	0	0	0

Appendix 2

Table 2a. Raw data on moisture percent and number of *Ascaris suum* eggs per gram dry soil of farm A

Sample number	Moisture percent	Developing eggs	Infective eggs	Dead eggs	Total eggs
W1	24	0.79	0.26	0.26	1.31
W2	18	0.49	0.24	0	0.73

Table 2b. Raw data of moisture percent and number of *Ascaris suum* eggs per gram dry soil of farm B

Sample number	Moisture percent	Developing eggs	Infective eggs	Dead eggs	Total eggs
W1	32	1.18	0.59	0.00	1.77
W2	16	3.57	0.00	0.95	4.52

W1: Walking route 1; W2: Walking route 2

Appendix 3

Table 3a. Raw data of moisture percent and number of *Ascaris suum* eggs per gram dry pen sample in weaner-grower (**WG**) pen of farm A

Area	Sample number	Moisture percent	Developing eggs	Infective eggs	Dead Eggs	Total eggs
LiA	1	50	120	7	33	160
	2	0	150	3	86	239
	3	0	139	1	26	166
	4	20	128	0	41	168
	5	33	1023	0	84	1107
RA	1	20	256	1	115	372
	2	62	240	0	40	279
	3	77	882	0	64	946
	4	73	137	0	18	155
	5	59	720	0	121	841
SF	1	84	1783	3	133	1918
	2	76	749	0	67	816
	3	80	1103	0	61	1164
	4	80	1500	0	70	1570
	5	68	2466	0	121	2587
LA	1	78	295	0	92	386
	2	80	5882	0	242	6124
	3	78	7309	2	1000	8311
	4	82	4732	0	388	5120
	5	84	1575	0	405	1980
WA	1	85	543	0	107	650
	2	67	756	0	134	890
	3	80	307	2	17	326
	4	75	581	0	30	610
	5	84	545	1	85	631
FA	1	33	72	0	39	111
	2	20	384	0	78	462
	3	33	170	0	30	200
	4	40	110	1	12	123
	5	17	78	0	24	102

LiA: litter area, RA: running area, SF: slatted floor, LA: latrine area, WA: waterer, FA: feeder

Table 3b. Raw data of moisture percent and number of *Ascaris suum* eggs per gram dry pen sample found in grower-finisher (**GF**) pen of farm A

Area	Sample number	Moisture percent	Developing eggs	Infective eggs	Dead Eggs	Total eggs
LiA	1	50	42	1	23	65
	2	60	47	0	13	60
	3	17	10	0	10	20
	4	67	79	0	28	107
	5	17	7	0	6	13
RA	1	77	486	0	17	503
	2	33	18	0	1	19
	3	42	14	0	1	14
	4	20	83	0	10	93
	5	33	342	0	34	376
SF	1	50	165	0	8	173
	2	70	67	1	8	76
	3	74	486	1	49	536
	4	84	724	0	86	810
	5	67	320	1	29	350
LA	1	78	6468	5	759	7232
	2	78	589	0	98	687
	3	84	1313	6	163	1481
	4	80	3180	15	205	3400
	5	86	114	1	40	156
WA	1	55	164	0	14	179
	2	72	184	0	24	209
	3	74	246	0	38	284
	4	60	62	0	7	69
	5	60	193	1	13	207
FA	1	29	6	0	6	12
	2	33	16	0	7	23
	3	14	14	0	2	16
	4	20	6	0	0	6
	5	25	11	0	0	11

LiA: litter area, RA: running area, SF: slatted floor, LA: latrine area, WA: waterer, FA: feeder

Table 3c. Raw data of moisture percent and number of *Ascaris suum* eggs per gram pen sample found in weaner-grower (**WG**) pens of farm B

Pen	Area	Sample number	Moisture percent	Developing eggs	Infective eggs	Dead Eggs	Total eggs
1	LiA	1	74	888	0	188	1075
		2	72	49	0	4	52
		3	54	22	0	17	40
	RA	1	82	122	0	14	137
		2	68	73	0	65	138
		3	72	111	0	50	161
	LA	1	82	79	0	18	97
		2	82	4152	0	216	4368
		3	80	189	0	24	213
	WA	1	74	78	0	18	96
		2	78	155	0	45	199
		3	20	12	0	2	14
	FA	1	56	83	0	9	91
		2	20	9	0	3	11
		3	40	38	0	13	52
2	LiA	1	78	207	0	44	251
		2	68	131	0	26	158
		3	76	245	0	36	281
	RA	1	68	124	1	26	151
		2	80	77	0	9	86
		3	80	90	0	20	110
	LA	1	84	85	0	20	105
		2	82	252	0	96	348
		3	80	325	0	36	361
	WA	1	60	105	2	40	147
		2	50	128	0	36	164
		3	78	85	0	18	103
	FA	1	58	50	0	7	56
		2	20	15	0	4	18
		3	64	142	0	28	170
3	LiA	1	20	56	0	8	64
		2	78	191	0	68	259
		3	64	121	0	74	195
	RA	1	76	135	0	33	168
		2	80	443	0	40	483
		3	80	680	0	31	711
	LA	1	84	34	0	0	34
		2	82	1357	1	123	1481
		3	82	436	0	54	490
	WA	1	70	197	0	192	389

		2	40	43	2	29	74
		3	67	220	0	29	249
	FA	1	63	292	1	41	334
		2	29	58	0	10	69
		3	50	196	0	46	242
4	LiA	1	84	625	0	158	783
		2	78	279	0	25	305
		3	74	423	0	39	462
	RA	1	52	291	0	43	334
		2	80	2452	0	187	2639
		3	78	249	0	38	287
	LA	1	84	366	0	48	414
		2	82	21654	0	1256	22910
		3	80	8733	7	878	9618
	WA	1	30	47	0	10	57
		2	63	276	0	55	331
		3	71	215	0	34	249
	FA	1	58	114	0	10	124
		2	50	25	0	2	27
		3	40	132	0	21	153
5	LiA	1	68	1133	3	63	1199
		2	74	236	0	29	264
		3	48	249	0	21	271
	RA	1	80	1037	0	105	1142
		2	80	1761	0	202	1963
		3	70	751	0	91	842
	LA	1	82	6289	0	419	6708
		2	82	2992	0	200	3192
		3	80	721	0	141	862
	WA	1	86	1677	0	229	1906
		2	60	31	0	5	36
		3	20	1370	0	333	1703
	FA	1	48	311	1	48	360
		2	20	168	0	37	205
		3	20	23	0	10	33

LiA: litter area, RA: running area, SF: slatted floor, LA: latrine area, WAFA: watering/ feeding area.

Table 3d. Raw data of moisture percent and number of *Ascaris suum* eggs per gram pen sample found in grower-finisher (**WG**) pens of farm B

Pen	Area	Sample number	Moisture percent	Developing eggs	Infective eggs	Dead Eggs	Total eggs
1	LiA	1	82	1350	0	301	1651
		2	78	221	0	25	246
		3	52	42	0	41	83
	RA	1	80	2289	0	277	2566
		2	60	660	0	65	724
		3	74	665	0	119	785
	LA	1	78	1489	2	203	1694
		2	80	4310	0	900	5210
		3	60	779	0	49	828
	WAFA	1	70	215	1	33	248
		2	70	177	0	64	241
		3	40	93	0	26	118
		4	70	538	3	172	713
2	LiA	1	80	2083	0	185	2268
		2	72	106	0	110	216
		3	70	385	0	128	513
	RA	1	72	1807	0	292	2099
		2	70	327	0	300	627
		3	72	2271	1	228	2501
	LA	1	82	3031	0	184	3216
		2	76	798	0	108	907
		3	80	3627	0	160	3787
	WAFA	1	64	188	0	34	222
		2	64	118	0	25	142
		3	66	406	0	60	466
		4	64	229	0	73	303
3	LiA	1	84	1589	0	150	1739
		2	78	97	0	6	104
		3	19	32	0	15	46
	RA	1	80	875	0	101	976
		2	70	347	0	100	447
		3	72	989	0	1125	2114
	LA	1	80	1357	0	209	1566
		2	80	472	0	16	488
		3	80	1180	0	140	1320
	WAFA	1	66	2241	0	88	2329

		2	72	329	0	111	441
		3	72	267	0	69	336
		4	80	435	0	123	558
4	LiA	1	76	1985	15	95	2095
		2	26	24	1	38	62
		3	78	9	0	7	16
	RA	1	70	1108	9	141	1258
		2	24	1321	0	91	1411
		3	70	1249	0	73	1322
	LA	1	66	859	0	51	910
		2	78	1925	0	140	2065
		3	76	2317	0	288	2604
	WAFA	1	58	132	0	25	157
		2	60	242	0	56	298
		3	60	14	0	3	17
		4	64	212	0	35	246
5	LiA	1	58	615	0	70	685
		2	70	30	0	7	37
		3	50	369	0	61	430
	RA	1	68	1348	0	78	1426
		2	66	1188	0	115	1304
		3	82	1506	0	168	1673
	LA	1	70	480	0	47	527
		2	82	1733	0	201	1934
		3	80	1494	0	235	1729
	WAFA	1	60	168	0	27	195
		2	40	32	0	4	36
		3	56	78	0	30	108
		4	50	164	0	31	195

LiA: litter area, RA: running area, SF: slatted floor, LA: latrine area, WAFA: watering/ feeding area.

Appendix 4

Fig 4a. Sketch of the pig pen and sampling areas (**O**) of weaner-grower (WG) and grower-finisher (GF) pens

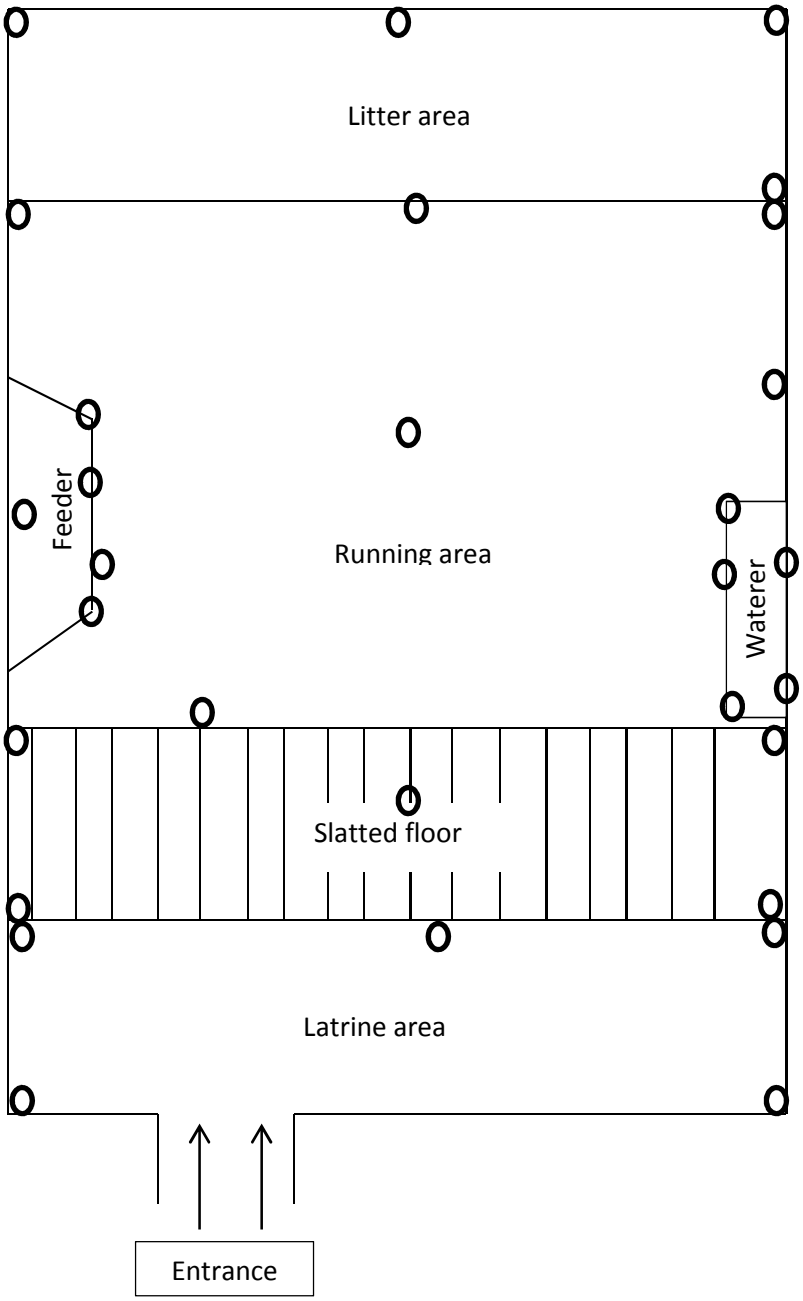

Fig 4b. Sketch of the pig pen and sampling areas (**O**) of weaner-grower (WG) pens of farm B

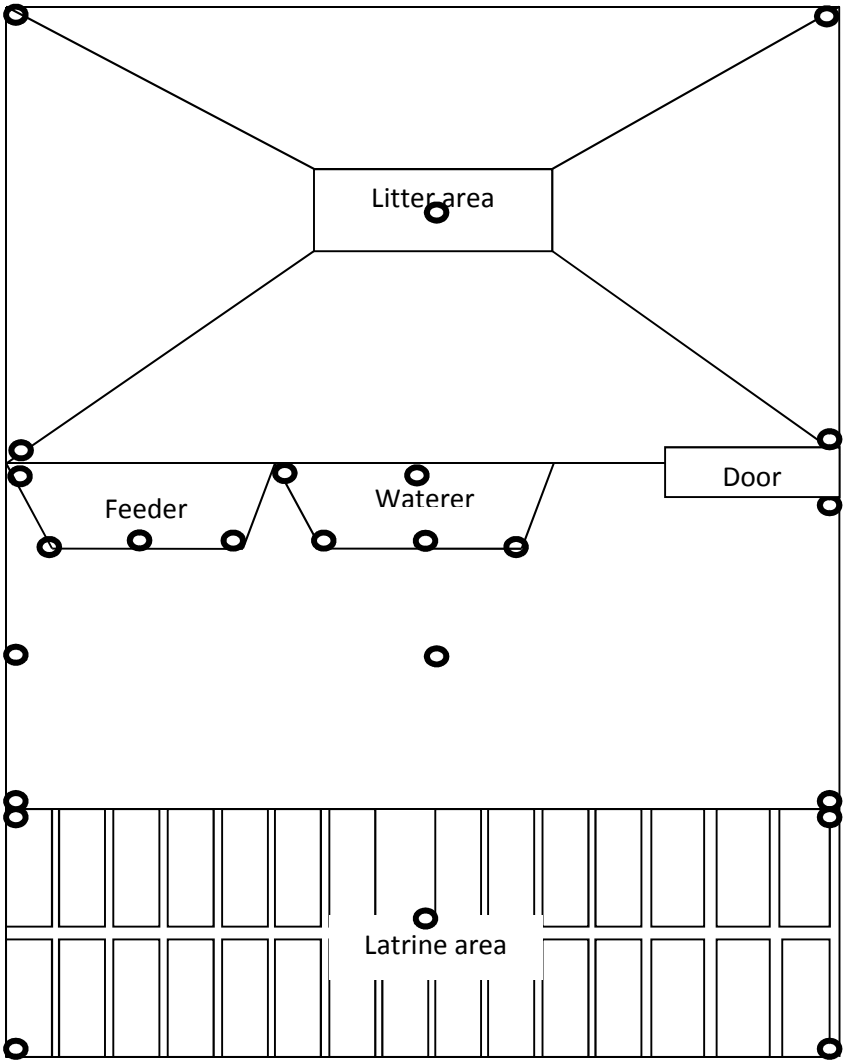

Fig 4c. Sketch of the pig pen and sampling areas (**O**) of grower-finisher (GF) pens of farm B