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Gastrointestinal parasites in pigs

Prevalence, risk factors and control

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Cover: Swedish pig looking over the edge of a pen
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Abstract

Gastrointestinal parasites are common in pigs worldwide, and in all production types. Clinical disease is rare and mainly associated with heavy nematode infections, or piglets infected with the coccidia *Cystoisospora suis*. Subclinical infections are more common and may result in reduced growth and poor feed utilisation. This can in turn affect pig health as well as the sustainability and productivity of the farm.

The aim of this thesis was to update the knowledge of gastrointestinal parasites in Swedish pig herds, as this was last done in the 1980s. Since then, major changes in the national pig production have occurred with e.g., higher demands on animal welfare and improved biosecurity.

Management routines related to parasite control were investigated using a questionnaire. Strategic hygiene and biosecurity practices were commonly practiced for growing pigs but less so for adult animals. Moreover, antiparasitic drugs were frequently used by routine. The occurrence of gastrointestinal parasites was assessed in three different studies. *Oesophagostomum* spp. were the most common parasites and found mainly in sows. The prevalence of *Ascaris suum* in growing pigs was reduced compared to previous studies. *C. suis* was common in piglets on a herd basis and *Cryptosporidium* spp. were found on all sampled farms. Finally, the efficacy of the available anthelmintic drugs was investigated, and for the first time in Sweden a reduced efficacy of ivermectin on *Oesophagostomum* spp. was identified.

In conclusion, several changes in both the prevalence and control of gastrointestinal parasites were identified in this thesis. This new knowledge can in turn contribute to healthier pigs and a more sustainable and profitable pig production.

Keywords: *Ascaris suum*, *Cryptosporidium, Cystoisospora suis*, *Eimeria*, helminth, *Oesophagostomum,* protozoa, *Trichuris suis*

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Mag-tarmparasiter hos gris

Sammanfattning

Mag-tarmparasiter är vanligt förekommande hos grisar världen över. Klinisk sjukdom är dock sällsynt och främst associerad med kraftiga maskinfektioner eller med smågrisar infekterade med koccidien *Cystoisospora suis.* Vanligare är subkliniska infektioner vilka kan leda till minskad foderomvandlingsförmåga och hämmad tillväxt. Detta kan i sin tur påverka grisens hälsa och gårdens produktivitet.

Syftet med den här avhandlingen var att uppdatera och öka kunskapen om mag-tarmparasiter i svenska grisbesättningar, vilket inte har undersökts sedan 1980-talet. Sedan dess har stora förändringar i den nationella grisproduktionen skett, med exempelvis högre krav på djurskydd och förbättrad biosäkerhet.

Rutiner relaterade till parasitkontroll undersöktes med en enkätstudie och strategiska hygien- och smittskyddsrutiner var vanliga för växande grisar, men mindre vanliga för vuxna djur. Avmaskningsmedel användes ofta rutinmässigt. Förekomsten av mag-tarmparasiter undersöktes i tre olika studier. *Oesophagostomum* spp. var vanliga och hittades huvudsakligen hos suggor. Förekomsten av *Ascaris suum* hos växande grisar hade minskat jämfört med tidigare studier. Besättningsprevalensen av *C. suis* var hög och *Cryptosporidium s*pp. hittades på alla provtagna gårdar. Slutligen undersöktes effekten av registrerade avmaskningsmedel, och för första gången i Sverige sågs en reducerad effekt av ivermektin på *Oesophagostomum* spp.

Sammantaget identifierades flera förändringar i både förekomst och kontroll av mag-tarmparasiter i den här avhandlingen. Den nya kunskapen kan i sin tur bidra till friskare grisar och en mer hållbar och lönsam grisproduktion.

Nyckelord: *Ascaris suum*, *Cryptosporidium, Cystoisospora suis, Eimeria*, helminter, *Oesophagostomum*, protozoer, *Trichuris suis*

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Dedication

To Stellan. For being the most wonderful little person there is, I will always give you all the love that I possibly can. And now also a book about pig parasites.

If having a soul means being able to feel love and loyalty and gratitude, then animals are better off than a lot of humans.

James Herriot

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Pettersson E*, Sjölund M, Wallgren T, Osterman Lind E, Höglund J, Wallgren P (2021). Management practices related to the control of gastrointestinal parasites on Swedish farms. *Porcine Health Management.* 7,12

II Pettersson E*, Hestad S, Möttus I, Skiöldebrand E, Wallgren P (2019). Rotavirus and *Cystoisospora suis* in piglets during the suckling and early post weaning period, in systems with solid floors and age segregated rearing. *Porcine Health Management*. 5, 7

III Pettersson E*, Sjölund M, Dórea F, Osterman Lind E, Grandi G, Jacobson M, Höglund J, Wallgren P (2021). Gastrointestinal parasites in Swedish pigs: prevalence and associated risk factors for infection in herds where animal welfare standards are improved. (submitted)

IV Pettersson E*, Ahola H, Frössling J, Wallgren P, Troell K (2020). Detection and molecular characterisation of *Cryptosporidium* spp. in Swedish pigs. *Acta Veterinaria Scandinavica.* 62 (1), 40

V Pettersson E*, Halvarsson P, Sjölund M, Grandi G, Wallgren P, Höglund J (2021). First report on reduced efficacy of ivermectin on *Oesophagostomum* spp. on Swedish pig farms. (submitted)

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The contribution of Emelie Pettersson to the papers included in this thesis was as follows:

I Took major part in the planning of the questionnaire and designing the study, performed the data analysis and interpreted the results together with the other co-authors and had the main responsibility in writing the manuscript.

II Interpreted the results together with the co-authors and had the main responsibility in writing the manuscript.

III Took part in the planning of the study, performed all the data collection and faecal analysis, performed the data analysis together with one of the co-authors, had the main responsibility in writing the manuscript.

IV Performed the data collection and some of the faecal analysis, performed the data analysis together with one of the co-authors and had the main responsibility in writing the manuscript.

V Performed all the data collection and faecal analysis, performed the data analysis together with one of the co-authors, had the main responsibility in writing the manuscript.

Abbreviations

AsHb	Ascaris suum haemoglobin
BZ	Benzimidazole
CI	Confidence interval
DPI	Days post infection
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EPG	Eggs per gram
EU	European Union
FBZ	Fenbendazole
FEC	Faecal egg count
FECR	Faecal egg count reduction
FECRT	Faecal egg count reduction test
FITC	Fluorescein isothiocyanate
GLMM	Generalised mixed models
IVM	Ivermectin
ITS-1	Internal transcriber spacer 1its 1
ITS-2	Internal transcriber spacer 2
LM	Linear models
ML	Macrocyclic lactone
OIE	World Organisation for Animal Health
OPG	Oocysts per gram
OR	Odds ratio
PCR	Polymerase Chain Reaction
PPP	Prepatent period
rDNA	Ribosomal deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid

SBA	Swedish Board of Agriculture
SPF	Specific pathogen free
Spp	Species

1. Introduction

Pigs (*Sus scrofa domesticus*) have been domesticated and lived in the proximity of humans for around 9000 years (Giuffra et al., 2000). Humans have also for thousands of years been aware of, and been affected by the parasites of pigs (Hoeppli, 1956). There is even an ongoing discussion if in fact humans and pigs share a common parasite, and if *Ascaris lumbricoides* (human roundworm) and *Ascaris suum* (pig roundworm) are one species, although this is yet not fully established (Leles et al., 2012, Betson et al., 2013, Søe et al., 2016, Easton et al., 2020).

Pigs are kept in different management systems, from small backyard farms with only a few pigs, to large farms with thousands of animals. Regardless of the production type, gastrointestinal parasites tend to be common. In modern pig production, the most commonly found parasites are the helminths *Ascaris suum*, *Oesophagostomum* spp. and *Trichuris suis* as well as protozoa such as coccidia (Roepstorff et al., 1998, Eijck and Borgsteede, 2005, Kochanowski et al., 2017, Raue et al., 2017). Although gastrointestinal parasites rarely cause severe clinical disease in infected pigs, their impact on pig health and welfare, as well as on the sustainability and productivity of the farms can be substantial (Kipper et al., 2011, Vlaminck et al., 2015, Martínez-Pérez et al., 2017).

To reduce the potential negative effects of parasites in a pig herd, adequate control measures are essential. Control is achieved through a combination of strategic management routines and the use of antiparasitic drugs (Roepstorff and Jorsal, 1990, Roepstorff, 1997). However, due to a worldwide escalating problem with resistance to antiparasitic drugs, responsible and prudent use is necessary (Wolstenholme et al., 2004, Sangster et al., 2018).

The occurrence of gastrointestinal parasites has not been thoroughly investigated in Sweden for more than 30 years. During this period, the global pig production has undergone several big changes and the Swedish pig production is no exception. The number of farms has declined but the herds have gradually turned larger with more intensified production systems. A subsequent positive result of this has been improved hygiene and biosecurity practices (Maes et al., 2020, Alarcón et al., 2021). Increased biosecurity measures may reduce the risk of pathogens, and larger herds have indeed been found to have a lower occurrence of gastrointestinal parasites compared to smaller herds (Roepstorff and Jorsal, 1989, Roepstorff and Jorsal, 1990, Kochanowski et al., 2017).

Consequently, one could expect the parasite status to now be improved in Sweden, due to the more intensified production and the implementation of improved biosecurity measures. However, other changes have transpired during the same period that may have had the opposite effect. In the late 1980s a new animal welfare law was introduced, and Sweden now has some of the strictest regulations on pig welfare in the world. Pigs are to always be loose-housed, including sows throughout the entire reproductive cycle and during suckling. Manipulative rooting material such as straw must be provided to all pigs, and at least 70% of the floors need to be solid, i.e. fully slatted floors are not allowed (SJV, 2018). These are all factors that may enhance survival and transmission of gastrointestinal parasites (Roepstorff and Jorsal, 1990, Joachim et al., 2001, Sanchez-Vazquez et al., 2010, Martínez-Pérez et al., 2017, Kochanowski et al., 2017).

To achieve the best possible control of gastrointestinal parasites in pigs, thorough knowledge of the parasites, and the host response towards, them is essential. As important is updated information on the occurrence of parasites and the measures available to control them. As many decades have passed since gastrointestinal parasites were paid proper attention to in Sweden, the aim of this thesis was to update the knowledge on the occurrence of gastrointestinal parasites as well as on the measures undertaken to control them. This new knowledge will in turn contribute to healthier pigs and a more sustainable and profitable pig production.

2. Gastrointestinal parasites of pigs

2.1 Helminths

2.1.1 General information

Helminths are parasitic worms that are divided into two major phyla, Nematoda (roundworms) and Platyhelminthes (flatworms), that in turn contain the classes Trematoda (flukes) and Cestoda (tapeworms) (Taylor et al., 2016). In Swedish pig production the most significant helminths all belong to the phylum Nematoda.

2.1.2 *Ascaris suum*

Ascaris suum is the largest of the nematodes found in pigs and adult worms measure between 25-40 cm in length. Pigs are infected through ingestion of embryonated eggs from the environment (Murrell, 1986). Following ingestion, infective third stage larvae hatch from the eggs when in the caecum and the proximal colon and penetrate the intestinal wall. The larvae then enter the bloodstream and after one to three days they reach the liver (Murrell et al., 1997, Dold and Holland, 2011). Larval migration through the liver causes tissue damage and evokes an immune response, resulting in cell infiltrates and interlobular depositions of fibrous tissue. Macroscopically this is seen as white spots on the liver, also known as “milk spots” (Ronéus, 1966). There is no association between the number of adult worms in the intestine and the degree of liver white spots and despite the tissue damage, there is no impact on liver function (Eriksen et al., 1991). Instead the changes are mainly of economic importance, as affected organs are condemned at slaughter, resulting in a price deduction for the farmer (Wallgren, 2012).

White spots may appear as early as three days post infection (DPI) but have been seen in experimental studies to be most frequent at seven DPI (Roepstorff et al., 1997). Around six to eight DPI, larvae continue to migrate through the lungs, where extensive damage to the tissue may arise including haemorrhage, emphysema, and oedema. Clinical signs in pigs may include a cough and dyspnoea and although they may be subtle and overlooked, *A. suum* infections could also be a cause of acute respiratory disease outbreaks (Haimi-Hakala et al., 2017, Lassen et al., 2019). Migrating larvae and the subsequent damage may also predispose to infections with other pathogens such as *Mycoplasma hyopneumoniae* (Eriksen et al., 1991). Following the lung migration, larvae penetrate through the alveoli, move up the respiratory tract and eventually reach the pharynx where they are swallowed and return to the gastrointestinal tract around 10 DPI (Roepstorff et al., 1997). More than 95% of the larvae will at this stage be expelled from the gastrointestinal tract and hence be eliminated from the host (Urban, 1986). The remaining larvae however continue to develop and around 24 DPI the final moult takes place in the small intestine. Once adult worms are present, sexual reproduction can occur and eggs are produced (Dold and Holland, 2011). A female worm may produce more than one million eggs per day (Kelley and Smith, 1956), but this will decrease if the total burden of adult worms in the small intestine increases (Sinniah and Subramaniam, 2009). The prepatent period (PPP), i.e. the time it takes from infection to when the adult parasites start producing eggs, is between six to eight weeks (Dold and Holland, 2011). Adult worms may live in the proximal part of the small intestine for up to one year and degrade carbohydrates to assist their energy metabolism. Most adult worms are however expelled from the gastrointestinal tract around 23 DPI (Wang et al., 2013).

Once the eggs are in the environment, development and embryonation takes 10-14 days if conditions are ideal, but often it takes longer for them to become infective to new hosts (Nilsson, 1982, Murrell, 1986). Embryonation is dependent on for example humidity, temperature, and adequate oxygen tension. Temperatures below 15°C or a humidity under 78.5% are for example inhibiting for further egg development. Also pig urine is ovistatic and has ovicidal effects. In contrast, excess water or moisture will shorten the embryonation time (Nilsson, 1982). Non-embryonated eggs of *A. suum* are very hardy in the environment and may survive around 15 years if the conditions are right (Dold and Holland, 2011).

Continuous exposure to *A. suum* results in strong protective immunity in the gut and larval migration has been shown to reduce over time in pigs that are repeatedly infected (Urban, 1986). This is due to the build-up of what is referred to as the pre-hepatic barrier, that prevents larvae from completing the migratory route (Nejsum et al., 2009b). However, since immunity builds up gradually, growing pigs may still be repeatedly infected (Masure et al., 2013) but it has been suggested that the adult worms that become established in the small intestine are likely originating from the initial infection (Nejsum et al., 2009b). Therefore, the *A. suum* burdens gained early in the pre-fattening period may determine the infection intensity for the rest of the rearing period (Nilsson, 1982). Infections with *A. suum* are not equally dispersed in the host population and instead tend to aggregate in a few individuals. In pigs it has been shown that approximately 20% of the host population carry 80% of the worm population (Nejsum et al., 2009a).

In growing pigs, infections may result in a reduced growth rate and reduced feed conversion ability, which in turn will affect farm productivity (Nilsson, 1982, Hale et al., 1985, Vlaminck et al., 2015).

2.1.3 *Oesophagostomum* spp.

Oesophagostomum spp. are large intestinal nematodes. Four species mainly infect pigs, *O. brevicaudum*, *O. georgianum*, *O. quadrispinulatum* and *O. dentatum,* with the two latter being the most common (Murrell, 1986, Roepstorff and Nansen, 1994, Joachim et al., 1999). Transmission is direct through the ingestion of infective third stage larvae from the environment. When ingested, the larvae penetrate the large intestinal mucosa and once in the submucosa a further moult into a fourth stage larvae occurs, forming reactive nodules in the tissue. *Oesophagostomum* spp. are therefore also referred to as nodular worms. After one to three weeks, the larvae emerge from the nodules to enter the large intestinal lumen where a final moult occurs. The now adult worms, measuring between 8-21 mm in length, then become sexually mature and can reproduce. Hypobiosis is also possible, with fourth stage larvae remaining in the nodules for several months. Sexual reproduction leads to female worms excreting numerous eggs into the environment with the faeces. The PPP varies and may be dependent on for example the type of carbohydrates, or the amount of fiber in the diet (Petkevicius et al., 1997, Petkevicius et al., 2001). A mean PPP of 20.2±1.4 days has been shown following experimental infections, indifferent of the

species (Talvik et al., 1997). Once in the environment, the eggs hatch within one to two days, first stage larvae emerge, and within approximately one week the larvae moult twice and develop into infective third stage larvae (Murrell, 1986). Although the L3 are sensitive to cold temperatures and dry and hot conditions, they can survive for almost one year in the outside environment (Murrell, 1986).

Unlike *A. suum*, infections with *Oesophagostomum* spp. do not induce a strong immunity and hence adult pigs are considered an important source of infection at the herd level (Nilsson, 1982).

In growing pigs, *Oesophagostomum* spp. have not been shown to affect weight gain or feed conversion ability and infections are mainly subclinical (Hale et al., 1981). In adults however, heavy burdens may result in weight loss and also cause reproductive disturbances such as a reduced number of piglets born as well as low weaning weights (Pattison et al., 1979).

2.1.4 *Trichuris suis*

Trichuris suis, or the pig whipworm, is found in the caecum and colon. Pigs become infected through the ingestion of embryonated eggs containing infective first stage larvae. Once ingested, the larvae hatch and penetrate the intestinal mucosa where a further moult occurs in the submucosa. Following this, the posterior end of the larvae emerges into the intestinal lumen and only the thinner anterior end remains buried in the mucosa. Following another three moults, an adult worm is developed, and sexual reproduction can occur. The PPP is approximately six weeks (Murrell, 1986). The tissue damage that occurs during the larval phase may result in inflammation and a subsequent diarrhoea, that may become haemorrhagic. Further clinical signs may include dehydration, anaemia, anorexia, and reduced growth rates. In severe cases the pigs may die (Batte et al., 1977, Murrell, 1986). The parasite distribution within the herd resembles that of *A. suum,* with a few individuals hosting the majority of the worm population (Nejsum et al., 2009a). Just as with *A. suum*, infections with *T. suis* induce a strong immune response and younger pigs are hence the most susceptible to infections (Murrell, 1986).

2.1.5 *Hyostrongylus rubidus*

Hyostrongylus rubidus, or the red stomach worm, is a nematode found in the stomach. Pigs are infected through ingesting third stage larvae from the environment in similar way as was described for *Oesophagostomum* spp (see

above). After ingestion, larvae invade the gastric glands in the stomach and complete another two moults to become adult worms. This may result in gastritis and gastric ulcers may occur with heavy infections. Adult worms in the stomach feed partly on blood which may result in anaemia and weight loss of the host (Murrell, 1986) and heavy burdens may also result in reduced weight gain and poor performance of growing pigs (Stewart et al., 1985).

2.1.6 *Strongyloides ransomi*

Strongyloides ransomi, or the threadworm, is found in the small intestine of pigs (Murrell, 1986). Infections are mainly a problem in young piglets where clinical signs include e.g., diarrhoea, poor feed conversion and reduced weight gain (Hale and Marti, 1984, Thamsborg et al., 2016). Pigs are infected by third stage larvae that may enter the host either through ingestion or through penetration of the skin or the oral mucosa. Another efficient transmission route is the lactogenic route, where sows shed infective larvae via the colostrum and hence piglets become infected during their first day of life (Stewart et al., 1976, Murrell, 1986). Pre-natal infections, i.e. through the placenta, also occur but are uncommon (Thamsborg et al., 2016). Once inside the host, larvae migrate through the tissues, including the lungs, and end up in the oral cavity where they are swallowed down to the small intestine. Further development results in parthenogenetic (capable of non-sexual reproduction) adult, egg producing females. Unlike the other above-mentioned nematodes*, S. ransomi* is a facultative parasite with a free-living life cycle (Murrell, 1986, Thamsborg et al., 2016).

2.2 Protozoa

2.2.1 General information

Protozoan parasites are unicellular eukaryotes that include several phyla. In pigs three genera of the phylum Apicomplexa are commonly found, including the coccidians *Cystoisospora suis* and *Eimeria* spp. as well as *Cryptosporidium* spp. In the phylum Ciliophora, *Balantidium coli* can be found in pigs (Taylor et al., 2016).

2.2.2 *Cystoisospora suis*

Cystoisospora suis (previously known as *Isospora suis*) is mainly found in young piglets, aged eight to ten days. Although *C. suis* also can infect older animals, clinical disease in pigs older than three weeks is uncommon (Joachim and Schwarz, 2014). Piglets become infected when ingesting sporulated (infective) oocysts from the environment. The sporulated oocyst contain two sporocysts, each with four sporozoites. Following ingestion, these sporozoites are released and invade the small intestinal epithelial cells, where further development and both asexual and sexual reproduction occurs. (Joachim and Schwarz, 2014, Worliczek et al., 2007). The subsequent damage to the small intestinal epithelium results in inflammation and a loss of the resorptive ability of the gut. Clinical signs include diarrhoea that often is yellow and pasty and is associated with dehydration and reduced growth rates (Mundt et al., 2006, Joachim and Schwarz, 2014). Morbidity is generally high but mortality low, although concurrent infections may worsen clinical signs (Vitovec et al., 1991, Mengel et al., 2012).

Oocysts from infected animals are excreted with the faeces after a PPP of 4-7 days (Joachim and Schwarz, 2014). The oocyst excretion is often cyclic with a peak of a high oocyst shedding followed by a drop, and then another peak (Joachim et al., 2014). This together with the often uneven oocyst shedding by individuals in an infected litter, means that several animals should be sampled and more than one sampling occasion is recommended to avoid false negative results (Sotiraki et al., 2007, Worliczek et al., 2009, Joachim et al., 2018). It should also be noted that diarrhoea and the excretion of oocysts do not always occur simultaneously, hence sampling should also include animals without clinical signs (Koudela and Kučerová, 1999, Mundt et al., 2006). Once in the environment, sporulation of the oocysts is rapid and may occur within 24 hours under optimal conditions (Larsen, 1996, Joachim and Schwarz, 2014).

2.2.3 *Eimeria* spp.

There are eight species of *Eimeria* known to infect pigs, *E. debliecki, E. suis, E. scabra, E. perminuta, E. spinosa, E. polita, E. porci* and *E. neodeblieci*. Infections occurs, just as for *C. suis*, through the ingestion of sporulated oocysts from the environment. In contrast to *C. suis*, each sporulated oocyst contain four sporocysts with sporozoites, a feature that apart from size and shape can assist in differentiating the genera microscopically (Taylor et al.,

2016). Clinical disease caused by *Eimeria* spp. is rare but may occur if there are other predisposing conditions such as stress. The PPP is between 4-10 days depending on the species. Sporulation times in the environment are also slower compared to *C. suis,* between 5-13 days depending on the species (Joachim and Schwarz, 2014).

3.2.4 *Cryptosporidium* spp.

The genus *Cryptosporidium* can infect all vertebrates, but there are many different species and genotypes that usually are adapted to only one or a few host species (Fayer, 2008, Ryan et al., 2014). Pigs are mainly infected with the pig adapted *C. suis* and *C. scrofarum,* although other species, for example the zoonotically important species *C. parvum* have also been found (Zintl et al., 2007, Kváč et al., 2009, Němejc et al., 2013, Kváč et al., 2013, Petersen et al., 2015). Transmission of infective oocysts is faeco-oral and unlike the oocysts of *C. suis* and *Eimeria* spp., those of *Cryptosporidium* spp. are infective directly when excreted from the host and hence do not require any time in the environment to sporulate (Fayer, 2008). The infective dose is low, with as little as 10 oocysts required to infect a new host. This combined with the ability of oocysts to survive for long in the environment, increases the risk of transmission (Guselle et al., 2003). Once ingested, the oocysts release sporozoites that invade and damage epithelial cells in mainly the small intestine. In pigs, infections tend to be subclinical but clinical signs such as diarrhoea, weight loss or reduced growth rates may occur (Guselle et al., 2003, Maddox-Hyttel et al., 2006). Clinical signs may also be exacerbated by other concurrent infections with for example rotavirus or *C. suis* (Enemark et al., 2003).

2.3 Consequences of parasitic infections in pigs

2.3.1 Pig health and welfare

Clinical disease may occur due gastrointestinal parasite infections, as has been discussed for the individual parasites above. More often however, infections in pigs tend to be subclinical, i.e. we do not actually observe any obvious signs of disease. An absence of clinical signs does however not exclude that infections impact the host. Intestinal helminths may reduce the feed intake and the feed conversion ability, leading to reduced weight gain

in pigs (Kipper et al., 2011). As discussed above, infections with gastrointestinal parasites may exacerbate, or predispose to infections with other pathogens. For example lung clearance of bacteria may be reduced in pigs concurrently infected with *A. suum* (Curtis et al., 1987). Parasitic infections may also lead to secondary effects by for example reducing the effects of vaccinations, something that has been shown in pigs vaccinated against *Mycoplasma hyopneumoniae* and concurrently infected with *A. suum* (Steenhard et al., 2009).

2.3.2 Productivity and sustainability of the farm

Gastrointestinal parasites may influence both productivity and sustainability of the farm by the effects they pose on their hosts. The direct effects of parasitism, such as clinical disease or the reduction in daily weight gain may incur costs for veterinary care and drugs, as well as a decrease in the farm productivity. For every additional day it takes a pig to reach market weight the costs increase for the farmer due to increased labour and use of resources, such as feed (Wallgren, 2012). This in turn will affect the sustainability of the farm. In Sweden, feed production is the main contributor to the carbon footprint in pig production, contributing 54% of the total carbon dioxide emissions in the production cycle (RISE, 2020). When parasitic infections decrease the ability of efficient feed conversion, the consequence is increased feed usage and hence a larger carbon footprint.

Organ condemnations at slaughter may also impact the profitability of the farms due to price deductions. A liver condemned at slaughter results in a deduction of approximately 2 euros at abattoirs in Sweden (Wallgren, 2012).

Neonatal coccidiosis may also have large consequences on farm productivity as reduced weight gain and a subsequent increase in feed usage may result in economic losses of up to 100 euros per sow and year (Kreiner et al., 2011).

The use of antiparasitic drugs may be beneficial in reducing the negative impact induced by parasitic infections, and by doing so productivity and the financial gain for the farmer may increase (Kreiner et al., 2011, Kipper et al., 2011). However, unnecessary use of antiparasitic drugs do not only carry a risk for the development of antiparasitic resistance (see below) but also induce increased labour and carry a financial cost, as well as a risk of environmental contamination. Residual anthelmintic drugs that are excreted in the faeces may disrupt the fauna and for example IVM has been shown to

be toxic to dung-breeding arthropods and both BZs and IVM may have toxic effects, and cause toxic disruption in aquatic ecosystems (Arends and Vercruysse, 2002, Wagil et al., 2015, Bundschuh et al., 2016).

2.3.3 Human health

Gastrointestinal parasites of pigs may have both a direct as well as an indirect impact on human health. Some parasites are zoonotic or have zoonotic potential such as *C. parvum,* an important cause of gastrointestinal disease in humans (Kváč et al., 2009, Zintl et al., 2007). The pig adapted species of *Cryptosporidium*, *C. suis* and *C. scrofarum,* are also able to infect humans, although this appears to be rare (Xiao et al., 2002, Kvác et al., 2009). *Ascaris suum* can cause zoonotic infections and more so in countries where infections with the closely related human roundworm *A. lumbricoides* are rare (Nejsum et al., 2005). Close contact with pigs or with pig faeces, including manure used as fertiliser, are risk factors for infection (Nejsum et al., 2005, Nejsum et al., 2012). Humans can be experimentally infected with *T. suis*, but little is known as to what extent this is harmful (Nejsum et al., 2012). Actually, infections with *T. suis* may even have beneficial effects as this parasite has been shown to reduce the severity of clinical signs in humans with for example inflammatory bowel disease (Harnett and Harnett, 2010). Excretory/secretory products of the same helminth have also been shown to reduce various inflammatory responses and may hence be protective in other medical conditions (Laan et al., 2017).

Parasites may also influence human health indirectly by their effect on growth and productivity, as was discussed above, since this in turn may reduce human food availability.

2.4 Diagnosis of gastrointestinal parasites in pigs

2.4.1 Macroscopic visualisation in pigs

The most common helminths of pigs (e.g., *A. suum*, *Oesophagostomum* spp. and *T. suis*) are as adults large and can easily be seen with the naked eye. Finding adult worms expelled in the faeces, at necropsy or at slaughter confirms a diagnosis (Figure 1). However, not finding adult worms does not exclude infection since pigs may harbour only the immature stages and larvae cannot be seen macroscopically (Vlaminck et al., 2014). Protozoa

such as coccidia or *Cryptosporidium* spp. cannot be seen macroscopically either, but can be detected histologically in the intestinal wall at necropsy (Larsen, 1996, Fayer, 2008).

Secondary changes due to parasitic infections, such as the lesions caused by the migrating larvae of *A. suum* may be noted during necropsy or at slaughter (Figure 1). These changes include the white spots on the liver, as well as pneumonic lesions in the lungs. White spot lesions may appear three to seven days post infection and are hence an indication of a recent infection. The scars do disappear however, and in an experimental study a marked reduction of the white spots was noted 21-56 days after infection (Roepstorff et al., 1997). Therefore, using the degree of white spots on the liver as an indicator of *A. suum* infection clearly has limitations as both early and late infections can be missed (Roepstorff et al., 1997, Nejsum et al., 2009b). Also, if pigs are continuously infected, immunity builds up at the level of the intestine, and very few or no scars will form (Vlaminck et al., 2012). Other infections with e.g., *Toxocara* spp., brucellosis, tuberculosis or other bacterial infections may also induce lesions that resemble white spots (Vlaminck et al., 2015). Ocular inspection of livers is hence a subjective diagnostic method, especially at the abattoir where little time is spent doing this, making it an insensitive method for diagnosing *A. suum* infections (Vlaminck et al., 2014). Also, during the inspection at the abattoir a minimum of 10 lesions should be recorded on the liver surface in order for the lesions to be registered and the organ condemned (Livsmedelsverket, 2020). This again shows that using the degree of lesions registered at the abattoir is a relatively insensitive method for *A. suum* diagnosis.

Migrating larvae of *A. suum* may also cause pneumonic lung lesions that can be used as a possible indicator of *A. suum* infection (Lassen et al., 2019) but this method must be regarded as a highly insensitive diagnostic tool.

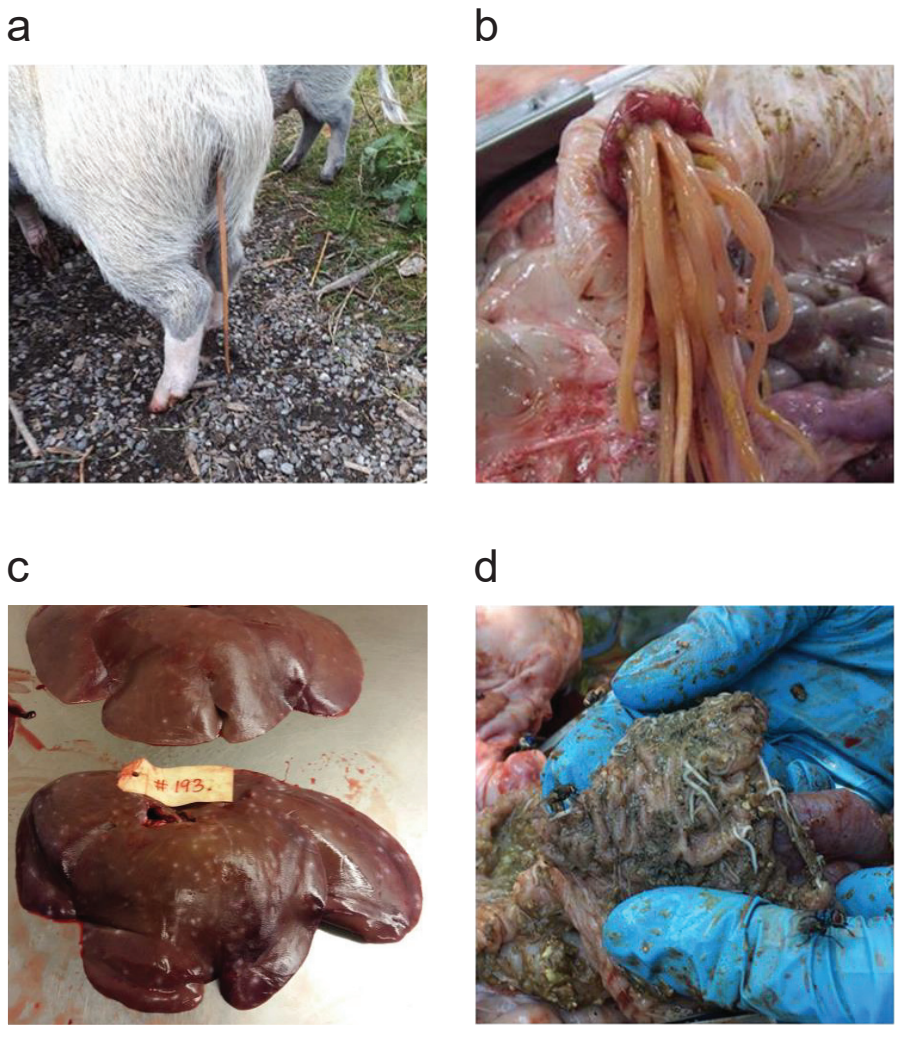

Figure 1. Macroscopic findings of gastrointestinal parasite infections. a) Adult *Ascaris suum* protruding under the tail of a miniature pig (photo Katarina Hultberg), b) Multiple adult *A. suum* in the intestine at necropsy (photo Malin Cerne), c) Pig liver with multiple white spot lesions caused by *A. suum* found at necropsy (photo Emelie Pettersson), d) Adult *Trichuris suis* in the intestine, found at necropsy (photo Elin Gertzell).

2.4.2 Faecal examination

There are several methods available to analyse faecal samples for gastrointestinal parasites. Some methods are qualitative, meaning that the parasite species are identified by for example egg or oocyst morphology (Figure 2) but not the level of infection. With quantitative methods, the parasite species as well as the level of infection, measured in eggs or oocysts per gram of faeces (EPG/OPG) can be determined. What method to use depends on the clinical question, the type of samples as well as available resources (e.g., equipment). Coproscopical examinations are relatively easy, non-invasive, and inexpensive to perform (Vlaminck, 2013). There are however some limitations. First, pigs are coprophagic and may ingest non-infectious eggs of for example *A. suum* or *T. suis*, resulting in false positive results (Boes et al., 1997, Boes et al., 1998). False negative results are also possible if pigs for example only harbour single sex adult worms and no egg excretion occurs (Roepstorff et al., 1997). Diagnostics may also be carried out at a time when no adult worms are present or when no eggs are excreted (Boes et al., 1997, Vlaminck et al., 2012). It should however be noted that a false positive sample still is relevant if investigating the farm prevalence as it clearly indicates that the parasite is present in the herd (Vlaminck et al., 2014).

Given the circumstances discussed above and the knowledge that some parasites (e.g., *A. suum* and *T. suis*) have an aggregated distribution, several animals of the same age category in a herd should be sampled if performing faecal analysis. The same principle is relevant if sampling is done for *C. suis* or *Cryptosporidium* spp. where not all pigs in a litter or a group shed oocysts at the same time. Hence pooling of samples, or multiple sampling occasions to account for biphasic or intermittent shedding of oocysts, should be considered (Enemark et al., 2002, Joachim et al., 2018).

Below are some brief descriptions of common methods used to examine faecal samples.

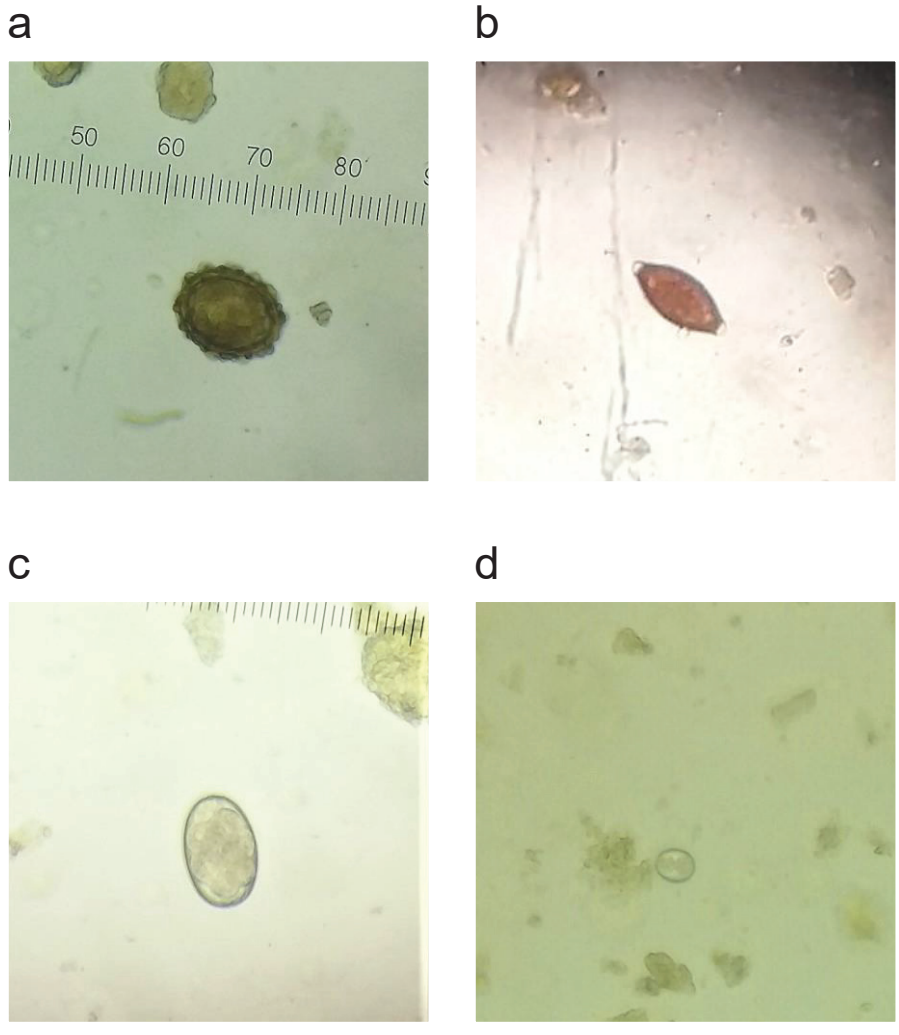

Figure 2. Faecal samples examined under the microscope (100x magnification). a) *Ascaris suum* egg, b) *Trichuris suis* egg c) strongyle-type egg d) *Cystoisospora suis* oocyst (Photos Emelie Pettersson).

2.4.2.1 Faecal smears

A faecal smear is considered a qualitative or a semiquantitative diagnostic method and requires very little equipment. To prepare a faecal smear, the faecal sample is mixed with an equal amount of water and a thin layer is spread on a slide before examining by microscope (Taylor et al., 2016). Several different stains may be used when examining the smears, for example carbol-fuchsin can be used to detect oocysts from coccidia or *Cryptosporidium* spp. (Fayer, 2008, Joachim et al., 2018). Using autofluorescence microscopy to examine faecal smears in the detection of *C. suis* oocysts is the most reliable and sensitive method currently available (Joachim et al., 2018).

2.4.2.2 Faecal floatation

Faecal flotation methods utilise the density of parasite eggs or oocysts that are suspended in a fluid with a higher specific gravity (>1.10-1.20). By allowing a sample to sit for a period in a tube, or using centrifugation, faecal debris will sink to the bottom and the eggs or oocysts will float to the top to then be further identified by microscopy. For a qualitative examination of a sample a direct flotation can be used. If a quantitative assessment is desired, the modified McMaster technique can be used, and one method is as follows: 3 g of faeces is placed into a glass bottle. Care should be taken to ensure that faeces from several parts of the sample is included. A volume of 42 ml of tepid tap water is added and the bottle is shaken vigorously. The sample should then be filtered through a fine meshed sieve into a bowl. The suspension is then be transferred into a Clayton-Lane tube and centrifuged. Following this the supernatant should be removed using suction, and the remaining pellet in the tube vortexed. When the sample is to be analysed the tube is filled with a flotation fluid e.g., a saturated glucose-salt fluid, and the sample should be mixed. Finally, a McMaster chamber is filled and examined for nematode eggs and/or coccidian oocysts in the microscope. With this method the number of detected eggs or oocysts are multiplied by 50 to get the number of EPG/OPG (Coles et al., 1992, Taylor et al., 2016). When examining faecal samples from young piglets or if samples are steatorrhoeic, faecal smears or faecal sedimentation should be considered instead (Taylor et al., 2016, Joachim et al., 2018).

2.4.2.3 Faecal sedimentation

When using faecal sedimentation, the high specific gravity of eggs or oocysts is used to allow them to settle at the bottom of a tube. This is done by mixing the faecal sample with a solution with a lower specific gravity compared to the eggs or oocysts, and by adding a solvent such as ether. This allows for separation of debris from the sample and a sediment is formed that then can be further examined using microscopy (Taylor et al., 2016, Soares et al., 2020).

2.4.2.4 Larval identification

Strongyle-type eggs (*Oesophagostomum* spp. and *Hyostrongylus rubidus*) cannot readily be identified to genus based on the egg morphology. Instead, these eggs need to be developed to third stage larvae by culturing the faecal sample under aerated conditions, in a warm and moist environment. Once the larvae are hatched they can be recovered and identified to genus based on morphology (Taylor et al., 2016).

2.4.3 Immunoassays

Different immunoassays can be used in for example the analysis of *Cryptosporidium,* as it shows an advantage over the use of light microscopy in being faster and more reliable (Jex et al., 2008). By e.g. staining faecal smears with fluorescein isothiocyanate (FITC) anti-*Cryptosporidium* antibodies that recognises surface epitopes on the oocysts, the oocysts can be viewed using fluorescence microscopy (Fayer, 2008, Jex et al., 2008).

Furthermore, serological methods are also available for the indirect detection of some of the gastrointestinal parasites of pigs. For example, over the past decade two Enzyme-linked immunosorbent assays (ELISA) have been developed for *A. suum* diagnosis, using *A. suum* haemoglobin (AsHb) or a water-soluble complete homogenate of L3 larvae that have migrated through the lungs (Vlaminck et al., 2012, Vandekerckhove et al., 2017).

2.4.4 Molecular diagnostics

Molecular tools using DNA-based methods are highly sensitive and have become more extensively used in the field of veterinary parasitology over the past few decades. Their use is not only in the field of diagnostics but also for investigating genes involved in drug resistance or in the area of drug

development, however mainly for nematodes in ruminants (Prichard and Tait, 2001, Kotze et al., 2020).

In terms of diagnostics, molecular tools can be used as a complement to e.g. microscopy (Prichard and Tait, 2001). For instance, PCR can be used to differentiate *Eimeria* spp. which can be challenging using microscopy alone (Ruttkowski et al., 2001).

Molecular tools are also valuable when other diagnostic methods are insufficient. For example, species of *Oesophagostomum* cannot be differentiated at the egg or larval stage. Thus, PCR based protocols and sequencing are instead useful tools when no adult worms are available (Várady et al., 1996, Lin et al., 2008). Likewise, molecular tools are required for species identification of *Cryptosporidium* as this cannot be done by microscopy alone (Jex et al., 2008).

2.5 Control and prevention

2.5.1 Overall aims of control and prevention

All the major gastrointestinal parasites in pigs are faeco-orally transmitted and relying on antiparasitic drugs alone for control has been shown to be ineffective (Nilsson, 1982). Antiparasitic drugs should instead be used in combination with strategic management routines (Nilsson, 1982, Roepstorff and Jorsal, 1990, Roepstorff, 1997, Martínez-Pérez et al., 2017) and the overall emphasis needs to be on a) reducing the risk of eggs or oocysts being excreted from infected hosts, b) minimising parasite survival and development in the environment and c) reducing the risk of parasite transmissions to new hosts (Figure 3).

It has also been suggested that in well managed pig herds the routine use of antiparasitic drugs could be made redundant (Roepstorff, 1997). Instead, pigs could be analysed for the presence of gastrointestinal parasites and treatments only used when deemed necessary and to selected groups of animals with a pre-determined level of infection. This would resemble the routines that have been implemented with targeted treatments for e.g., ruminants or horses (Charlier et al., 2014, Nielsen et al., 2014, Tydén et al., 2019).

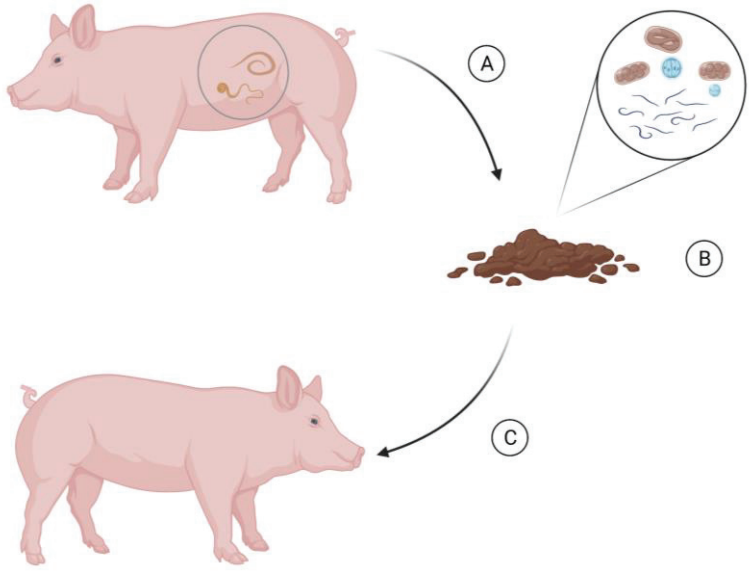

Figure 3. The three areas to focus on in order to reduce gastrointestinal parasites in a pig herd: A) reduce the risk of eggs and/or oocysts being excreted from infected hosts, B) minimise parasite survival and embryonation in the environment and C) reduce the risk of parasite transmissions to new hosts. Illustration by Emelie Pettersson (Created with BioRender.com)

2.5.2 Biosecurity

Adequate biosecurity measures, aimed at reducing parasite introductions are essential. This can first of all be in the form of external biosecurity, where parasites are prevented from being introduced to the farm in the first place. For example farms that purchase growing pigs, in contrast to keeping their own breeding stock, have a higher risk of parasite introductions (Vlaminck, 2013). For farms that do purchase pigs, the number of sources used should be limited and only piglet suppliers with high hygiene standards should be used, in order to reduce the risk of parasite introductions (Joachim et al., 2001). Treating new pigs with antiparasitic drugs on arrival should also be considered a preventative measure (Vlaminck, 2013).

Parasite infections can easily also be transmitted within a pig herd, emphasising the need for good internal biosecurity, i.e., preventing parasite transmission within the farm itself. Parasite eggs and oocysts are easily spread on fomites such as farm equipment or footwear (Langkjaer and Roepstorff, 2008, Vlaminck, 2013) and ensuring good internal biosecurity is hence essential to reduce the risk of parasite transmission between different sections and different age categories of pigs.

Age-segregated rearing, a strategy where pigs are reared batch-wise and do not mix with pigs of other ages, has in studies been shown to be effective in reducing within-farm transmissions (Roepstorff and Jorsal, 1990, Joachim et al., 2001, Kochanowski et al., 2017, Martínez-Pérez et al., 2017). Batch production also allows time between batches for adequate cleaning and drying of the pens (Nilsson, 1982).

Sufficient rodent control is another aspect of ensuring good external and internal biosecurity as rodents may carry pathogens into the farm as well as within the farm (Backhans and Fellström, 2012). Rodents have for example been shown to carry the pig specific species of *Cryptosporidium* (Zhao et al., 2018).

2.5.3 Hygiene

In modern indoor pig production, the main source of infection is likely residual eggs or oocysts in the environment, rather than for example sows infecting her piglets (Nilsson, 1982, Nilsson et al., 1984, Martinsson and Nilsson, 1986, Sotiraki et al., 2007). The buildup of bedding material and manure can provide a microclimate that is suitable for parasite survival, highlighting the importance of proper cleaning routines and hygiene (Persson and Lindqvist, 1975, Roepstorff and Jorsal, 1990). Although eggs and oocysts generally can survive well in the environment, certain factors, such as temperature, humidity, pH and oxygen levels, all influence egg embryonation as well as larval development and survival. For example, if temperatures fall below 15ºC, eggs of *A. suum* may survive but will not embryonate and will hence not be infective to other pigs (Nilsson, 1982, Katakam et al., 2014, Jankowska-Mąkosa and Knecht, 2015). Both helminth eggs and coccidian oocysts are sensitive to desiccation, and lack of sufficient humidity will prevent embryonation (Roepstorff and Nansen, 1994, Roepstorff, 1997, Langkjaer and Roepstorff, 2008). On the other hand, excess moisture, caused by for example water spillage in the pens, may

favour larval development and increase risk of parasite transmission (Nilsson, 1982). This emphasises the need for strategic localisation of for example drinkers, as well as allowing pens to thoroughly dry after cleaning and disinfecting.

Many of the commonly used disinfectant are not effective against nematode eggs and coccidian oocysts and care should be taken to use a product also aimed at parasites, such as a cresol-based product (Straberg and Daugschies, 2007, Oh et al., 2016).

Several housing factors may limit the ability to thoroughly clean and dry pens, such as utilising worn out facilities, having solid floors or providing bedding material (Roepstorff and Jorsal, 1990, Dangolla et al., 1996, Joachim et al., 2001, Sanchez-Vazquez et al., 2010, Martínez-Pérez et al., 2017). Housing pigs in deep litter straw beds may however have a protective effect against gastrointestinal parasites as the microenvironment, with an unfavorable pH and low oxygen levels, are unsuitable for parasite development (Katakam et al., 2014, Jankowska-Mąkosa and Knecht, 2015).

Outdoor access on pasture is another risk factor for parasite transmission and infection (Roepstorff and Nansen, 1994, Eijck and Borgsteede, 2005, Lai et al., 2011). The contamination of pastures may be caused by previous grazing with infected pigs or through the spread of for example contaminated manure (Lindgren et al., 2020). Eggs of e.g., *A. suum* and *T. suis* survive well in the soil (Roepstorff and Nansen, 1994, Lindgren et al., 2020). The eggs and non-infective larval stages of *Oesophagostomum* spp. are more sensitive to environmental factors such as low humidity, but the infective L3 stage may survive well in outdoor environments (Roepstorff and Nansen, 1994). Pig parasites that require an intermediate host such as the earthworm (e.g., the pig lungworm *Metastrongylus* spp.) are also more commonly found in outdoor pig herds due to fact that they have access to the actual intermediate host (Murrell, 1986).

2.5.4 Antiparasitic drugs

Antiparasitic drugs are used to control clinical disease and to reduce the negative effects that may occur due to subclinical infections. By treating infected pigs, the contamination of the environment with eggs and/or oocysts is also reduced. In Sweden two substances are registered for the treatment of helminth infections (anthelmintics) in pigs, the macrocyclic lactone (ML) ivermectin (IVM) and the benzimidazole (BZ) fenbendazole (FBZ)

(Läkemedelsverket, 2021). The MLs bind to and activate glutamate-gated chloride ion channels on nerve and muscle cells that are present in invertebrates, such as nematodes and arthropods. This results in an increased permeability to chloride ions, hyperpolarisation and finally paralysis and death of the parasite. The MLs are effective against ectoparasites and both the larval and adult stages of nematodes in pigs. Ivermectin is available in formulations for both oral and subcutaneous administration (Arends and Vercruysse, 2002, Taylor et al., 2016). The BZs act by binding to the parasite β-tubulin protein which are essential constituents of microtubules. This results in disruption of cell structure and energy metabolism, ultimately killing the parasite (Lacey, 1990, Taylor et al., 2016). Benzimidazoles are approved for use against the larval and adult stages of the common nematodes of pigs. However, *T. suis* requires a higher dose of BZs compared to other nematodes, possibly due to a reduced drug uptake and an increased ability to detoxify the drug (Hansen et al., 2014, Hansen et al., 2017). Fenbendazole is only available in formulations for oral administration (Läkemedelsverket, 2021).

The anticoccidial triazinetrione derivative, toltrazuril is available for metaphylactic control of neonatal coccidiosis caused by *C. suis* and acts by disrupting parasite mitochondrial respiration (Noack et al., 2019, Läkemedelsverket, 2021). Toltrazuril is given in day 3-5 of life, either as an oral formulation or as an injection, and in the European Union (EU) it is commonly used by routine during the piglet's first week of life (Shrestha et al., 2017). Toltrazuril reduces the clinical signs of neonatal coccidiosis as well as the shedding of oocyst. This in turn reduces the risk of *C. suis* transmission to new hosts (Joachim and Mundt, 2011).

There are no available therapeutic or prophylactic drug treatments for infections with *Cryptosporidium* spp. (Ryan et al., 2014, Björkman et al., 2018).

2.5.5 Resistance to antiparasitic drugs

Drug resistance is defined as a heritable trait, present when there is a larger frequency of individuals in a population that tolerates a dose of a compound, compared to a normal population of the same species (Sangster et al., 2018). Resistance to antiparasitics is an emerging problem with enormous consequences in livestock around the world (Wolstenholme et al., 2004, Sangster et al., 2018). Resistance to, or a reduced efficacy of all the major

classes of anthelmintics including MLs, BZs and pyrantel/levamisole have been reported in *Oesophagostomum* spp. of pigs (Roepstorff et al., 1987, Bjørn et al., 1990, Gerwert et al., 2002, Macrelli et al., 2019). There are however no reports of resistance in *A. suum* or *T. suis* (Hansen et al., 2014). Resistance to the anticoccidial drug toltrazuril has also recently been identified (Shrestha et al., 2017).

2.5.6 Assessing the efficacy of antiparasitic drugs

It is of great value to accurately detect and assess the efficacy of antiparasitic substances at an early stage. On a farm level, the recommended test for assessing anthelmintic drug efficacy in the field is the Faecal Egg Count Reduction Test (FECRT) (Kaplan and Vidyashankar, 2012, Levecke et al., 2018, Sangster et al., 2018). The FECRT is done by analysing faecal samples before and 10-14 days after treatment with an anthelmintic drug. The McMaster method, or equivalent techniques, are used to identify and enumerate the eggs whereafter the reduction in FEC post treatment is calculated. In pigs it is recommended to sample a minimum of 10 animals and a faecal egg reduction (FECR) of less than 90% is suggestive of resistance (Coles et al., 2006).

Routine faecal testing to assess the efficacy of toltrazuril has also been suggested as a way of ensuring durable control of neonatal coccidiosis (Shrestha et al., 2017).

There are several other ways to assess treatment effect or to investigate possible resistance, such as post-mortem examination of the gastrointestinal tract and other appropriate organs (e.g., the liver and lungs if looking for larvae of *A. suum*) after antiparasitic treatment, or using *in vitro* tests (Coles et al., 2006, Hennessy et al., 2006). Although fewer *in vitro* tests are established for pig parasites than in other host species (Coles et al., 2006), they are available and a larval migration assay to assess anthelmintic efficacy on *A. suum* was recently developed as one example (Zhao et al., 2017).

2.5.7 Alternative control strategies including vaccines

Given that there are only a few drugs registered for the treatment of gastrointestinal parasites in pigs, combined with a growing concern of drug resistance, alternative methods of control are required (Thamsborg et al., 2010, Williams et al., 2021). Strategic hygiene and biosecurity practices have been discussed above and it has been suggested that routine use of

anthelmintic drugs could be replaced with selective treatments in well managed pig herds (Roepstorff, 1997) as has already been discussed. A growing field of research is also investigating the effect of nutrition, diet, and gut microbiota on gastrointestinal helminths (Williams et al., 2021).

Vaccines are successfully used as a way to prevent and control many infectious diseases and they are widely used in the field of pig medicine (Zimmerman et al., 2012). However, there are only a few vaccines aimed for livestock parasites available in the world (Joachim, 2016). Attempts have been made to develop a vaccine against *A. suum* using for example purified AsHb, a protein found in the pseudocoelomic fluid of adult worms, but has not been successful (Vlaminck et al., 2011). The option of vaccinating sows to protect the piglets against *C. suis* is however something that has been suggested as a possible future target in the area of vaccine development against pig parasites (Joachim, 2016).

3. Pig production in Sweden

3.1 The national pig population

There are approximately 1150 pig producers registered with the Swedish Board of Agriculture (SBA) in Sweden and of these approximately 60% keep breeding sows. The majority (85%) of the total number of sows in the country are however kept on only 26% of the farms indicating a continuing trend of farms becoming larger and larger. Out of the registered farms, 80% produce fatteners and around 2.6 million pigs are slaughtered each year. The majority of the pig farming in Sweden occurs in the southern and central parts of the country (SJV, 2020). Around 2% of the registered farms are Specific Pathogen Free (SPF) farms and 2% are registered as organic farms (Lannhard Öberg, 2019). Generally breeding sows are a Yorkshire and Landrace cross and they are inseminated with Hampshire or Duroc semen, resulting in three-breed crosses that are reared to market weight.

3.2 Pig breeding systems

Sows in Sweden produce on average 2.2 litters of piglets per year. In each litter an average of 14.6 piglets are born and out of those 12 are weaned (WinPig, 2019). The piglets remain with the sow until weaning, which is not done by routine before 28 days of age at an individual level (SJV, 2018). From the time of weaning until around 12 weeks of age, and a weight of approximately 30 kg, the pigs are referred to as growers. From that age until reaching a market weigh of around 120 kg body weight and a mean age of 170 days, pigs are referred to as fatteners or fattening pigs. Farms may also keep gilts (unmated sows) as replacement animals i.e., to replace older sows

in the herd. A majority of all matings are done via artificial insemination, although boars are used for teasing. Sows that are not lactating are referred to as dry sows. Pigs of different age categories can be seen in Figure 4.

Age-segregated, batch-wise rearing is commonly practiced on Swedish pig farms (Backhans et al., 2015). It is accomplished by simultaneous weaning of groups of sows that are then inseminated after four to seven days post weaning, when coming into heat. During the dry period sows are group-housed, often on deep litter straw beds. A few days prior to farrowing, the pre-partum sows are moved to the farrowing units where they remain until their piglets are weaned. All piglets in a batch are then reared to market weight without mixing with pigs of other age groups (Einarsson et al., 2014). In contrast, in continuous production systems, age-segregation is not practiced, and growing pigs of different ages are mixed. Age-segregated rearing became widely used in Sweden following the ban of using antibiotics as growth promoters in 1986 (Wallgren, 2009).

Pigs can be produced in different breeding systems. Sow herds may raise pigs from birth to slaughter on so called farrow-to-finish farms or they may sell the pigs to specialised fattening farms when the growers weigh around 30 kg. Specialised fattening farms do not house any sows but instead purchase pigs from piglet producing farms and the fattening farms may buy pigs from several different farms.

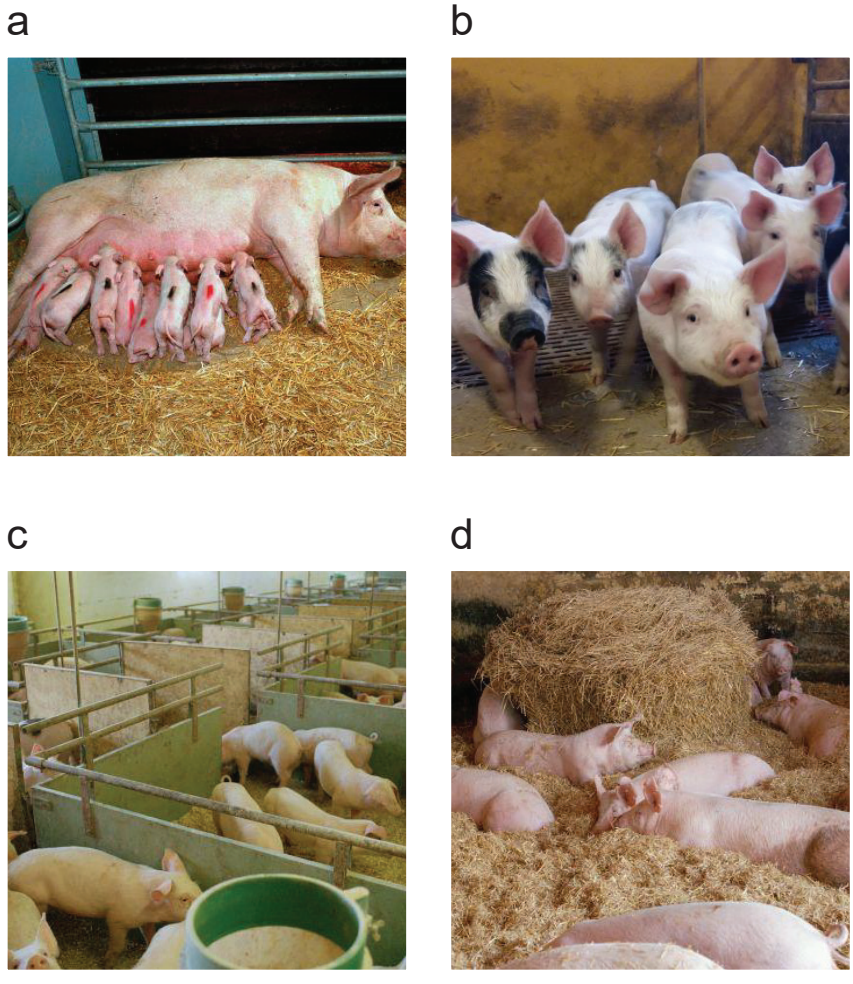

Figure 4. The different age categories of pigs in their different housing environments. a) suckling piglets in the farrowing pen, b) growers in a pen with a partly slatted floor, c) fatteners in fattening pens and d) dry sows in a deep litter straw bed (Photos a, c and d SVA, photo b Emelie Pettersson).

3.3 Pig health and welfare

Swedish pigs are declared free from Aujeszky's disease (Robertsson J, 1996), porcine respiratory and reproduction syndrome (Carlsson et al., 2009), atrophic rhinitis (Wierup and Wallgren, 2000) as well as from the diseases on the former list A of the World Organisation of Animal Health (OIE). The use of growth promoters has been banned since 1986, and the routine use of metaphylactic antibiotics is not carried out. Farms that are SPF are also declared free of *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae, Brachyspira hyodysenteriae,* swine influenza and sarcoptic mange (Wallgren and Vallgårda, 1993).

Sweden has some of the strictest animal welfare laws in the EU, and according to national legislation pigs are to always be loose-housed, including sows with piglets. Fully slatted floors are not allowed and at least 70% of the floor must be solid. Manipulative bedding material such as straw or saw dust must be provided in all production systems. The routine docking of tails is not allowed (SJV, 2018). On organic pig farms, outdoor access must be provided all year round (SJV, 2019).

4. Hypotheses and aims of the thesis

In Sweden, the area of pig parasitology has not been given much attention in the past 30 years, a time coinciding with extensive husbandry and management changes made in the national pig production. The hypothesis of this thesis was that the parasite prevalence, as well as the general approach towards treatment and control, had changed since this was last thoroughly investigated in the 1980s.

The overall aim of this thesis was therefore to establish updated information on the prevalence, risk factors and control of pig parasites in Swedish pig herds. Such updated information will have a practical significance and serve as a guide when discussing treatment and control of parasites in the Swedish pig herds of today and ought to be of wider interest as production systems with increased requirements on welfare are discussed. The overall intent of this gained information was to contribute to improved pig health and more sustainable and profitable pig farms.

The specific aims of this theses were to:

- To investigate the occurrence of gastrointestinal helminths and protozoa in conventional Swedish pig herds
- To document the current management practices related to parasite treatment and control on Swedish pig farms
- To assess the efficacy of the anthelmintic drugs used on Swedish pig farms

5. Comments on Materials and Methods

The following chapter is a summary of the material and methods used in Study I-V. Detailed descriptions are found in the respective papers.

5.1 Study population

The study population consisted of the pig herds registered with the SBA. For Study II, the registry of SBA was used to select the herds. However, for the remaining studies, that registry was no longer available. Therefore, the three major pig health organisations in Sweden: a) Gård och Djurhälsan, b) Lundens djurhälsa and c) Distriktsveterinärerna assisted in contacting farms for Study I, III and IV. In Study I, participating farms were able to indicate if they were interested in partaking in a prevalence study (Study III). Farms from Study III were hence selected from participation in Study I. Farms with a high FEC in Study III were further included in Study V, where anthelmintic efficiency was investigated (Figure 5).

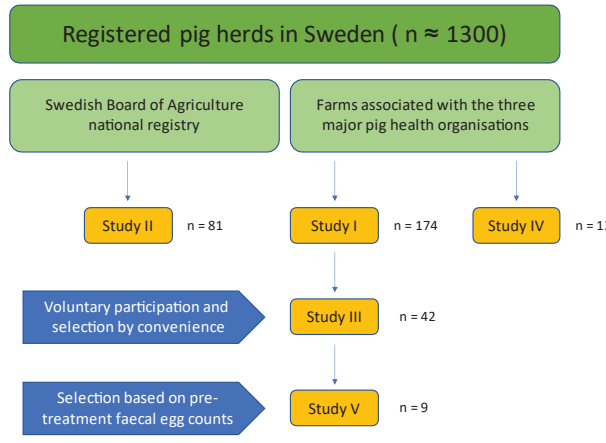

Figure 5. The selection process of the different study populations used for Study I-V.

5.2 Herd factors and management routines (I)

Information on herd factors and management routines, associated with control and prevention of parasitic infections, were assessed using an online questionnaire with 30 questions (Appendix 1). The questionnaire was designed and distributed by Questback Essentials (Questback Sweden Ltd, Stockholm, Sweden). The information from this questionnaire was used in Study I, III, IV and V. In Study II, the management routines were assessed using a different questionnaire that was filled in at the time of sampling (data not shown).

5.3 Sample collections (II-V)

In Study II, 81 farms were included, and 10 litters were repeatedly sampled on each farm when the piglets were aged two, four and six weeks. Ten fresh faecal sub-samples were collected from the floor of each pen and pooled into one sample per pen. Repeated and pooled samplings were done as *C. suis* oocysts often are shed in a biphasic pattern and not all individuals in the same litter may be shedding oocysts simultaneously (Joachim et al., 2018). In Study III, 42 farms were included, and a total of 1615 faecal samples were collected from the age categories a) post-weaning piglets aged 5-6 weeks (n=337); b) growers (n=345); c) fatteners (n=308); d) dry sows (n=277) and

e) pre-partum sows (n=348). For age categories a-d, several faecal sub-samples were collected from the pen floors and pooled into one sample per pen. For age category e, individual animals were sampled, either directly from the rectum or from the floor promptly after defecation.

In Study IV, 13 farms and 222 pooled samples collected from the age categories a) piglets aged 0-5 weeks (n=48); b) growers (n=57); c) fatteners (n=67) and d) adults (n=50) were included.

In Study V, 104 samples collected from individual pre-partum sows, on nine different farms from Study III, were included and re-sampled 14 days after anthelmintic treatment. Five of the nine farms used FBZ for anthelmintic treatment and four farms used IVM. Out of the four farms that used IVM, one farm had previously used FBZ and very recently changed into using IVM. The anthelmintics were administered to the sows just prior to farrowing on all nine farms.

5.4 Parasite detection (II-V)

5.4.1 Study II

In Study II, centrifugal sedimentation according to Telemann was used to detect *C. suis* oocysts. In brief, 1 g of the faecal sample was suspended and shaken in 5 ml 5% acetic acid. Once the suspension had settled for 1 min it was filtered through a sieve into a tube. An equal amount of ether was added, the mixture shaken and then centrifuged. The sediment was then examined using light microscopy.

5.4.2 Study III

In Study III, centrifugal flotation and a modified McMaster technique were used to identify and quantify nematode eggs and coccidian oocysts, with a lower detection limit of 50 EPG/OPG. In short, 3 g of faeces were placed into a glass bottle and care was taken to ensure faeces from several parts of the sample was included. Forty-two milliliters of lukewarm tap water were added, and the bottle shaken vigorously. The sample was then filtered through a 150µm sieve into a plastic bowl. The suspension was transferred into a Clayton-Lane tube and centrifuged. The supernatant was removed using suction and the remaining pellet was vortexed. The tube was then filled with a saturated glucose-salt floatation fluid with a specific gravity of

1.300g/ml, the sample mixed with a plastic pipette and a McMaster chamber was filled and examined for nematode eggs and coccidian oocysts using light microscopy.

When strongyle-type eggs were identified, the samples were cultured to hatch third stage larvae for genus identification. In short, approximately two thirds of a 180 ml plastic container was filled with faecal material and covered with a perforated lid. Samples were then incubated at 28°C for 10 days and larvae recovered in accordance with Roberts and O'Sullivan (Roberts and O'Sullivan, 1950). Approximately 50 L3 per sample were identified based on morphological criteria as previously described (Thienpont et al., 1979).

5.4.3 Study IV

In Study IV, centrifugal flotation and epifluorescence microscopy were used to detect *Cryptosporidium* oocysts. In short, 1 g of faeces was suspended in 7 ml of Phosphated Buffered Saline (PBS) with Tween 20 (PBS-Tween) and care was taken to ensure faeces from several parts of the sample was included. The suspension was filtered through sieve and underlaid with a saline-glucose flotation fluid with a specific gravity of 1.07 g/ml to a total volume of 12 ml and centrifuged. The supernatant was transferred to a clean tube, washed using MilliQ water and centrifuged again. A sample volume of one milliliter was finally obtained. For oocyst detection, 10µl of each cleaned sample were placed on a Teflon printed 3-well slide (Immuno-Cell Int, Belgium) and air dried, fixated and stained with diluted fluorescein isothiocyanate (FITC)-labelled monoclonal anti-*Cryptosporidium* antibodies (Waterborne Inc., New Orleans, LA, USA) according to the manufacturer's instructions. The wells were examined and quantified using epifluorescence microscopy. The lower theoretical detection limit of this method was 100 OPG.

Faecal samples positive on microscopy were further investigated by molecular diagnostics to determine species. DNA was extracted from individual faecal samples using a commercial kit (DNeasy PowerLyzer PowerSoil Kit) according to instructions of the manufacturer, and PCR and sequencing of the 18S rRNA gene was done for species determination. Additional sequencing of the 28S rRNA gene was done for seven of the samples.

5.4.4 Study V

In Study V, samples were examined using centrifugal flotation, a modified McMaster technique and larval cultures as was described above (section 5.4.2 Study III). To determine the species of *Oesophagostomum* present in the sample, DNA was extracted from the faecal samples collected both before and after anthelmintic treatment, using the Nucleospin DNA Tissue kit (Macherey Nagel, Germany) according to instructions of the manufacturer. DNA was amplified using PCR with species specific primers, targeting the second partial internal transcribed spacer (ITS-2) of the nuclear ribosomal DNA (rDNA) of *O. dentatum* and the partial internal transcribed spacer (ITS-1), complete 5.8S and partial ITS-2 rDNA of *O. quadrispinulatum*, modified from (Lin et al., 2008). Gene sequencing was done on samples that produced a PCR product for either *O. dentatum* and/or *O. quadrispinulatum*.

5.5 Assessing anthelmintic efficacy (V)

In Study V, anthelmintic efficacy was assessed using FECRT. Faecal samples were analysed before and 14 days after anthelmintic treatments administered by the farmers. Pigs were treated with the drug that was normally used on the farm, either FBZ at the recommended dose of 5 mg/kg given in the feed, or IVM at 0.3 mg/kg body weight, administered as a subcutaneous injection. The farmers were informed to dose the sows for a minimum of 330 kg of body weight to avoid the risk of underdosing. The FECR 14 days post-treatment was calculated using the "eggCounts 2.3" package in R (v 1.1.456) (R Core Team, 2018). A FECR of 95% or less, and a lower confidence interval (CI) of 90% was considered a reduced efficacy. This is a modification of the recommended 90% reduction cut off that has previously been recommended to use when assessing anthelmintic efficacy in pigs (Coles et al., 2006).

5.6 Data analysis (I-V)

In Study I, data were analysed by examining descriptive statistics as well as applying Chi-square tests when analysing categorical variables and the expected values were ≥5. When comparing management parameters in relation to herd size, the criteria for statistical analysis using chi- square tests

were often not fulfilled and only descriptive data were reported. Data were analysed using Microsoft Excel and SAS® software version 9.4 (SAS Inst. Inc., Cary, NC, USA),

In Study II, data were analysed using descriptive statistics as well as paired student t tests and Spearman rank correlation tests to compare the prevalence between age groups, and to assess the impact of certain management factors. Data were analysed using Microsoft Excel and SAS® software version 9.4 (SAS Inst. Inc., Cary, NC, USA),

In Study III, the associations between parasite prevalence and the different management factors identified in the questionnaire were investigated using logistical regression. For this analysis, each age category was considered one observation and considered positive if at least one sample was positive. Each parasite species was investigated individually for possible risk factors or protective factors. The statistical analyses were performed in the statistical programming environment R, version 4.0.3 (R Core Team, 2020).

In Study IV, descriptive statistics and Fisher's exact test were used to investigate the differences in occurrence of *Cryptosporidium* spp. between the age categories of pigs. Data management and statistical analysis were performed using Stata (StataCorp. 2017. Stata Statistical Software: Release 15.1. College Station, TX: StataCorp LLC.).

For Study V, a linear model (LM) was used to compare the FEC of *A. suum* and *Oesophagostomum* spp. between the different farms. A generalised linear mixed model (GLMM) was used to investigate if the choice of anthelmintic influenced the pre-treatment FEC of *Oesophagostomum* spp. These statistical analyses were performed in the statistical programming environment R, version 4.0.2 (R Core Team, 2020). The faecal egg count reduction (FECR) 14 days after treatment was calculated using the "eggCounts 2.3" package, also in R (v 1.1.456) (R Core Team, 2018).

For all statistical analysis, the results were considered significant if $p < 0.05$.

Diagrams were made using Prisma GraphPad Version 8.4.2 (GraphPad Software, La Jolla California USA).

6. Main Results

The following chapter is a summary of the main results from Study I-V. Detailed results are presented in the respective papers.

6.1 Herd factors and management routines (I)

Herd factors and management routines were investigated in 174 farms using a questionnaire study. Out of the responding farms, 78% kept sows, either as farrow-to-finish farms (46%) as piglet producers (31%) or as central units in a sow pool (1%). Out of the 174 farms, 68% produced fatteners either as farrow-to-finish farms (46%) or as specialised fattening farms (22%). Eight percent of the participating farms were organic or had outdoor production and two percent were SPF farms. Fourteen percent of the participating farms had less than 100 sows, 46% kept 100-400 sows and 18% kept more than 400 sows. Overall, the participating farms represented the structure of Swedish pig production reasonably well (see Chapter 3). Outdoor access was provided on all the registered organic farms and on 3% of the conventional farms, all which had less than 100 sows.

The most common pen type for all age cage categories of growing pigs were conventional pens with partly slatted floors (maximum 30% as per national law). Deep litter straw beds on solid floors were the most used pen type for dry sows and this type of bedding was also used for fatteners on 6% of the farms. Straw was the most common type of bedding material used for all age categories followed by wood shavings and peat. Liquid feed was used alone or combined with dry feed for the growers on 59% of the farms and for the fatteners and the dry sows on 82% and 67% of the farms, respectively. Water was generally supplied using nipple drinkers and the water source was placed over the slatted part of the floor in the farrowing pens on 52% of the

farms. The corresponding figures for the grower pens, fattening pens and dry sow pens were 79%, 78% and 21% respectively.

6.2 Biosecurity and hygiene practices (I)

Strict batch-wise production was practiced for the farrowing units on 88% of the farms and for the grower units on 80% of the farms. This was significantly ($p<0.05$) more common compared to strict batch-wise production in the fattening units which was done on 75% of the farms (Figure 6). Although it could not be verified statistically for all age categories, strict batch-wise production seemed to be more common on medium and large sized farms compared to small sized farms. Cleaning and disinfection between each new batch was more commonly practiced in the farrowing, grower and fattening units compared to dry sow units. Likewise, it was more common to have a downtime period of more than four days in sections for growing pigs as compared to those for dry sows (Figure 6). Again, strict hygiene practices were more common in the farrowing, grower, and fattening units on large and medium sized farms, although this difference could not always be shown to be statistically significant. For the dry sow units, cleaning and disinfecting between each batch was however numerically more commonly practiced in the smaller herds compared to the medium and large herds.

Farms used a great variety of disinfectants (results not shown) but very few used a product that was effective against coccidian oocysts and nematode eggs such as cresol-based products for example.

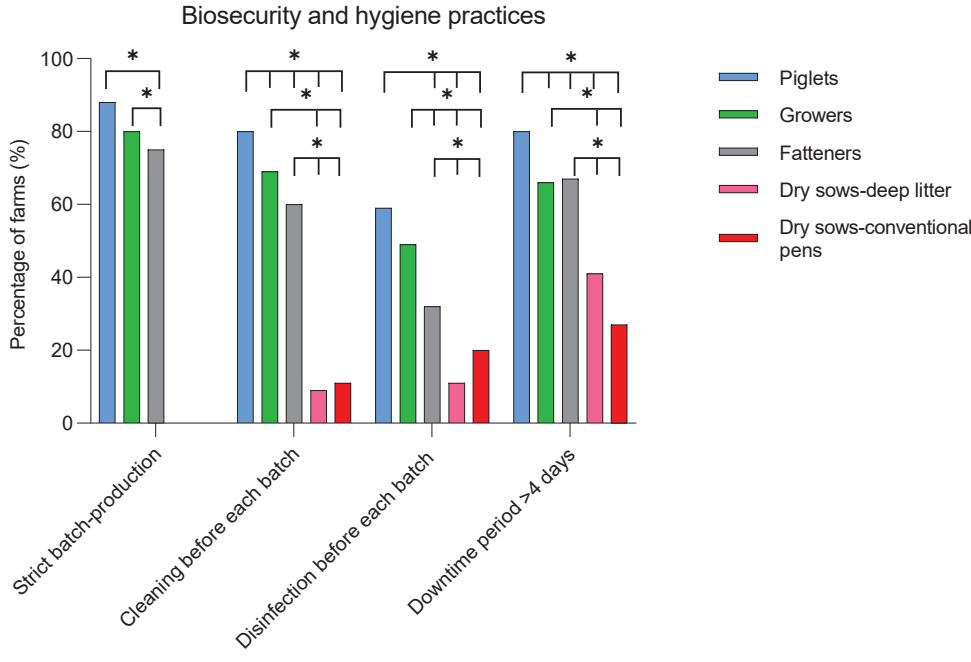

Figure 6. Biosecurity and hygiene practices as reported by 174 farms in Study I. An asterisk indicates that there was a significant ($p<0.05$) difference between the separate age categories.

6.3 Faecal analysis for parasites (I)

Regular faecal analysis for parasites was done for piglets on 3% of the farms, for growers on 4%, for fatteners on 2% and for sows on 4% of the farms.

6.4 Use of antiparasitic drugs (I)

Antiparasitic drugs were used on 69% (120/174) of the total number of participating farms and the most common practice was to treat pre-partum sows with anthelmintics, either FBZ or IVM. On 13% of the farms, sows were treated at several time points. Replacement animals were treated on 50% of the farms with either FBZ or IVM. Growers and fatteners were treated with anthelmintics on 13% and 3% of the farm respectively and FBZ was the only drug used for these age categories. Piglets were not treated with anthelmintics on any farm but 9% reported to use toltrazuril for the

management of neonatal coccidiosis. Ivermectin was used to control sarcoptic mange twice or more per year on 21% of the farms (Figure 7) but it was not specified what animals were treated. Some farms did not use any antiparasitic drugs at all (n=54). Out of these, 37 were specialised fattening farms (69%), 10 were conventional farrow-to-finish farms (19%) and 7 were registered organic farms (12%).

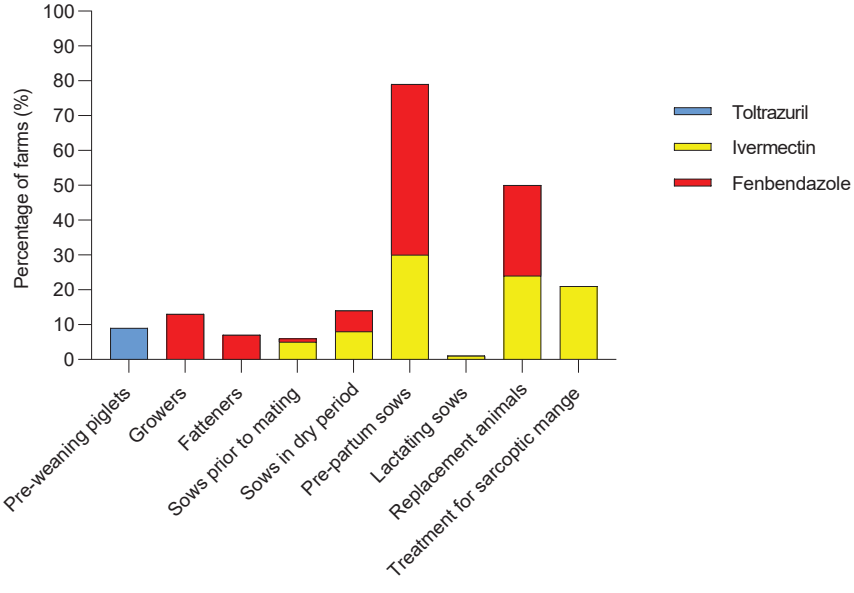

Figure 7. The use of antiparasitic drugs shown by age and stage of production as reported in Study I. A total of 69% (120/174) of the farms used antiparasitic drugs and these were on some farms administered to several age categories and at several stages of production. The denominator for each calculated percentage was the number of farms with the respective age category present.

6.5 Parasite prevalence (II-V)

Parasitic prevalence was investigated in Studies II, III and IV and the results are shown per parasite in Figure 8. Additional comments about each specific parasite, including faecal egg, or oocyst counts are discussed in sections 6.5.1-6.5.6.

6.5.1 *Cystoisospora suis*

The prevalence of *Cystoisospora suis* was examined in suckling and recently weaned piglets in Study II, and in post-weaning piglets and older pigs in Study III. In Study II the herd prevalence as well as the sample prevalence was the highest in the two-week-old piglets and reduced at the two subsequent samplings. However, if cumulative data were calculated, the number of infected herds increased with age from 58% positive herds at two weeks of age, to 75% positive herds at four weeks, and 84% at six weeks of age (data not shown in Figure 8). In Study III, *C. suis* was detected on a total of 60% of the sampled farms and in 5% of the samples and the highest prevalence was in post-weaning piglets. In Study III the oocyst range was 50-20,300 OPG with a mean of 620 OPG. Faecal oocyst counts were not available from Study II.

6.5.2 *Cryptosporidium* spp.

Cryptosporidium spp. were examined in Study IV and was found to be most prevalent in growers and fatteners. In pre-weaning piglets and growers both *C. suis* and *C. scrofarum* were detected. In fatteners only *C. scrofarum* was found and in adult sows both *C. suis* and *C. parvum* were detected. The overall faecal oocyst count ranged from 100-30,600 OPG with a mean of 2,789 OPG.

6.5.3 *Eimeria* spp.

Overall, *Eimeria* spp. were detected on 64% of the farms and in 9% of the individual faecal samples in Study III. The highest prevalence was found in the adult sows. The overall faecal oocyst count ranged from 50-218,300 OPG with a mean of 5,202 OPG.
No species determination was done.

6.5.4 *Ascaris suum*

Eggs from *A. suum* were overall detected on a total of 43% of the farms and in 5% of the samples in Study III. The highest prevalence was found in pre-partum sows. The overall FEC ranged from 50-8,250 EPG with a mean of 963 EPG.

6.5.5 *Trichuris suis*

Overall, 10% of the farms and <1% of the total amount of samples were positive for *T. suis* in Study III and only samples from adult sows were positive. Overall faecal egg counts ranged from 50-250 EPG with a mean of 96 EPG.

6.5.6 *Oesophagostomum* spp.

Oesophagostomum spp. were detected on 64% of the farms and in 19% of the individual samples in Study III. The highest prevalence was found in adult sows. The overall FEC ranged from 50-8,550 EPG with a mean of 998 EPG.

6.5.7 Other parasites

The red stomach worm, *Hyostrongylus rubidus* was not detected in any of the samples, nor were eggs of the pig lungworm *Metastrongylus* spp. Single eggs that possibly could have been from *S. ransomi* were detected in five samples from five different herds in Study III, but it could not be excluded they were from free-living nematodes. *Balantidium coli*, a protozoan parasite that generally is considered non-pathogenic in pigs, was detected in 9% of the litters at 2 weeks, in 11% at 4 weeks and in 26% of the litters at 6 weeks of age in Study II.

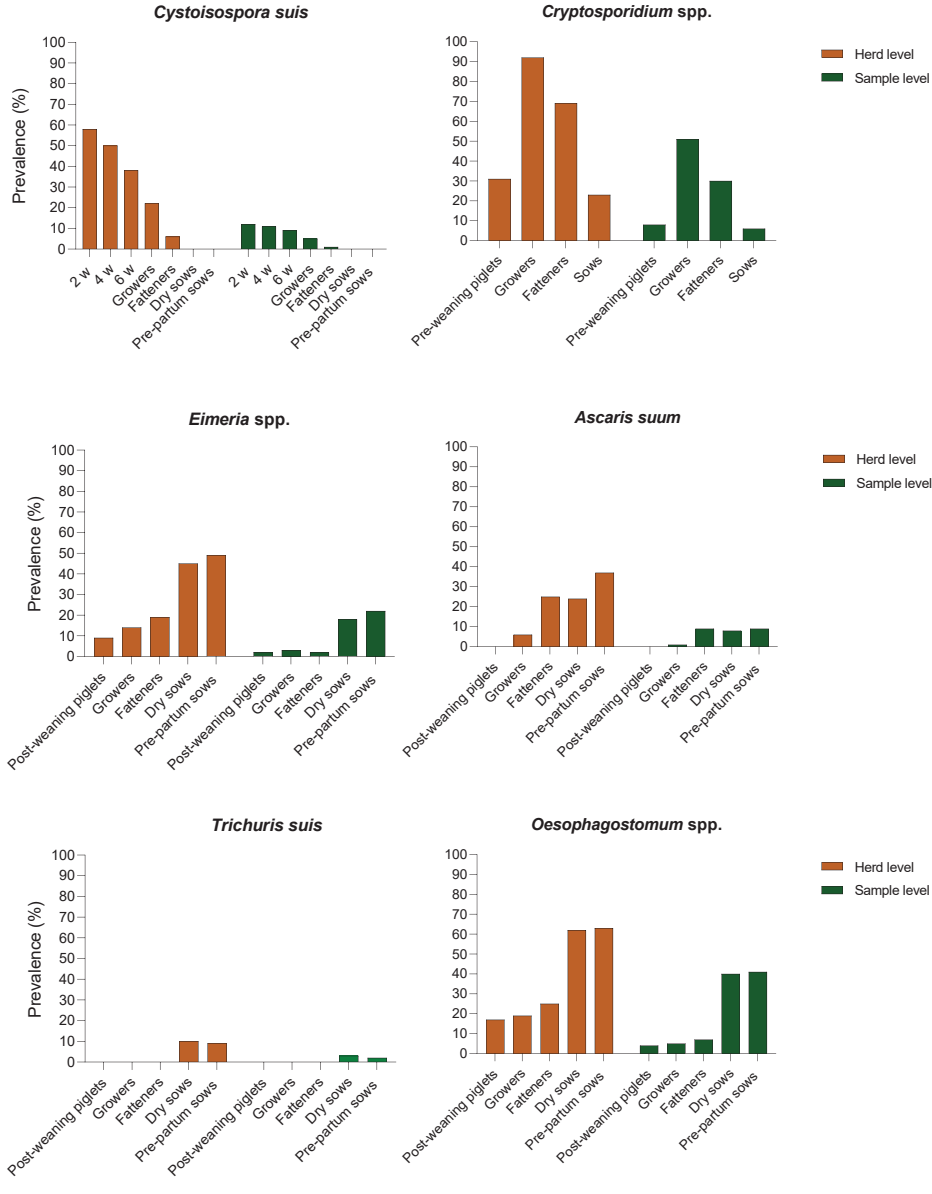

Figure 8. Prevalence of each respective parasite detected in Study II-IV, shown by herd level and sample level.

6.6 Risk factors for parasitic infections (III)

Possible protective factors or risk factors for gastrointestinal parasite infections were investigated using logistic regression in Study III. Each parasite species was investigated separately.

Age was always a significant predictor for parasite occurrence. Due to a small number of observations and a low percentage of positive samples, few specific measured factors identified in the questionnaire were shown to be statistically significant. However, samples from small sized farms were associated with a higher risk of being positive for *A. suum* compared to samples from large sized farms (OR 159, CI 2-10,826). Strict batch-wise production was found to be a protective factor for *T. suis* infections (OR 0.04, CI 0.038-0.039). All models, except the model for *C. suis*, showed a high intra-farm variance. For most models, more than 50% of the observed variability could be explained by farm-level effects, rather than specific differences in the investigated management practices.

6.7 Anthelmintic efficacy (V)

Anthelmintic efficacy of the registered drugs IVM and FBZ was assessed on nine farms using FECRT. Treatment against *A. suum* was effective (FECR >90%) on the five farms that were positive for this parasite, regardless of whether IVM or FBZ had been used for treatment (see Paper V for results).

Oesophagostomum spp. were detected pre-treatment on all nine farms, and on four farms post-treatment (Figure 9). Out of the four farms that were positive post-treatment, three farms (farms no 7-9) had a FECR of <95%. On these three farms, IVM had been used for treatment (Table 1). Out of the six farms where treatment showed good efficacy (FECR 95-100%) against *Oesophagostomum* spp., five had used FBZ (farms no 1-5) and one IVM (farm no 6) (Table 1).

PCR and sequencing showed that both *O. dentatum* and *O. quadrispinulatum* were present on five of the nine farms pre-treatment (farms no 1, 4-6, 8), and on two of the farms post-treatment (farm no 8, 9). Only *O. dentatum* was found on the remaining farms except for farm number 7 where no larvae were recovered post-treatment. All three farms where treatment showed poor efficacy had used IVM as the sole anthelmintic drug for several years, and two farms also used IVM twice or more per year primarily against sarcoptic mange. The one farm that used IVM with good

efficacy as a result had just recently changed from previously using FBZ. The three farms where IVM treatment showed poor efficacy later changed to using FBZ with good effect and a FECR of 100% (data not shown).

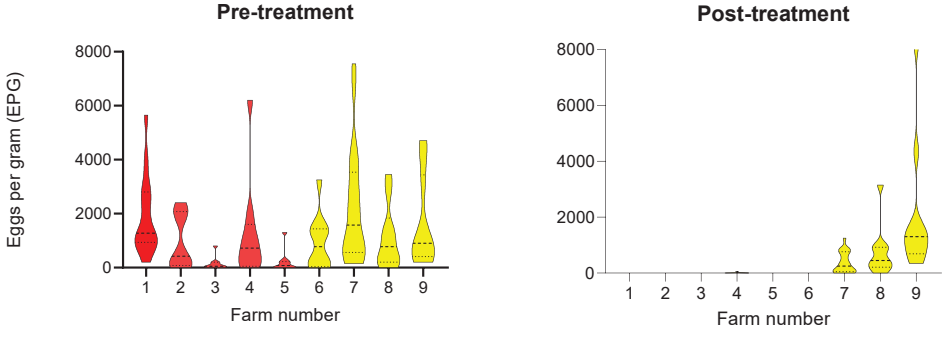

Figure 9. Violin plots showing the faecal egg count (FEC) of *Oesophagostomum* spp. on the nine farms included in Study V. All farms were positive pre-treatment, and four farms were positive post-treatment (farm no 5 had a FEC of 5±16 epg post-treatment and the results are not visible on the violin plot).

Table 1. Faecal Egg Count Reduction (FECR) of strongyle-type eggs with 95% confidence intervals (CI). The samples were collected from sows on nine Swedish pig farms 14 days after treatment with either fenbendazole (FBZ) or ivermectin (IVM)

Farm no	Anthelmintic	Mean FECR	Low 95% CI	High 95% CI
1	FBZ	99.8	99.4	100
2	FBZ	99.5	98.3	100
3	FBZ	96.4	88.6	100
4	FBZ	99.6	98.9	100
5	FBZ	97.5	92.6	100
6	IVM	99.5	98.5	100
7	IVM	83	80	85.9
8	IVM	31.5	16.6	45
9	IVM	1.9	1e-002	5.7

7. Discussion

The overall aim of thesis was to update the knowledge on prevalence, potential risk factors and control of gastrointestinal parasites in Swedish pig herds of today. The last thorough investigation on this topic was performed in the 1980s and since then extensive changes in the national pig production have been made.

New knowledge and insights can assist both veterinarians and pig farmers in the control and treatment of gastrointestinal parasites in the most optimal way. The result will be healthier pigs, more sustainable and profitable farms as well as a reduced risk of antiparasitic drugs losing their efficacy.

It was clear from the studies performed within this thesis that there had been major changes in the control measures of gastrointestinal parasites since the studies that were done 30 years ago, and that these adjustments had also altered the overall occurrence of gastrointestinal parasites.

7.1 Management practices in relation to gastrointestinal parasites

Optimal parasite control is achieved through a combination of biosecurity and hygiene measures, as well as through the strategic use of antiparasitic drugs. The overall aims with control are to reduce the spread of parasites from infected hosts, to prevent parasite survival in the environment and to reduce the risk of parasite transmission to new hosts. The two latter aims can mainly be achieved through hygiene and biosecurity measures. Several management practices used on farms were investigated with the aim to identify possible risk factors as well as protective factors for parasite survival and transmission.

Following the ban of growth promotors in Sweden in 1986, batch-wise production became increasingly more frequent in Sweden (Backhans et al., 2015, Wallgren, 2009) and this was also evident from the results within this thesis. Age-segregated batch-wise production was commonly practiced in the farrowing, grower, and fattening units on a majority of the farms. This is an important management tool that has shown to reduce parasite transmissions within pig herds (Roepstorff and Jorsal, 1990, Joachim et al., 2001, Martínez-Pérez et al., 2017, Kochanowski et al., 2017). Overall, this strategy likely aimed to protect young pigs from pathogen exposure and this practice was indeed less common in the dry sow units, where often more than one farrowing group are housed together, subsequently creating a continuous production system for adults.

It was common for farms to always clean and disinfect pens between batches of growing pigs (piglets, growers, and fatteners). If this is done properly it can reduce the risk of residual faecal material in the pens, something that otherwise could create a favourable environment for parasite survival. Disinfection was done with an array of different disinfectants and many of the commonly used products were not effective against helminth eggs or coccidian oocysts. This highlighted the importance of remembering parasites when deciding what disinfectants to use on a pig farm.

When pens are cleaned and disinfected, it is essential they can thoroughly dry before new pigs are introduced, to prevent a humid environment for parasites to thrive in. It was common for farms to have a downtime period of at least four days between batches in the farrowing, grower, and fattening units, which should be enough time to allow thorough drying of the pens. The overall strict hygiene measures for growing pigs were in line with previous findings of good hygiene and biosecurity on pig farms in Sweden (Postma et al., 2015). There was also an indication that larger farm in general practiced better overall biosecurity and hygiene routines compared to smaller farms which was in line with the hypotheses. However, the strict hygiene practices that were evident for growing pigs were rarely observed in the dry sow units, certainly due to the infrequent practice of batch-wise production among adults as was discussed above.

Limiting dampness is another management practice that can help to reduce gastrointestinal parasites in the environment (Nilsson, 1982). Many of the farms placed the drinking facility (mainly nipple drinkers or automatic waterers) over the slatted part of the floor for growing pigs which reduced

the risk of spilled water creating damp environments that could favour parasite survival and embryonation. This practice was however less common in the dry sow units and could contribute to an increased the risk of parasite survival and embryonation in these units.

It was evident from the studies within this thesis that there had been some changes in the overall occurrence of gastrointestinal parasites within the Swedish pig herds over the past three decades. One of the interesting findings was the reduced prevalence of *A. suum* in growing pigs compared to when this was last investigated in the 1980s. Of the sampled fatteners, only 9% of the pens were positive for *A. suum* compared to almost 35% when this was last investigated (Roepstorff et al., 1998). On a herd level, fatteners on 25% of the farms were positive which again was low compared to older Swedish studies, when the corresponding figure was almost 50% (Nilsson, 1982). Infections with *A. suum* affect feed conversion and growth in a negative way, and it is hence an important parasite of growing pigs. The importance of this parasite in fatteners is also emphasised by the white spot lesions on the liver caused by the migrating larvae. White spot lesions that are recorded at slaughter result in organ condemnation and a price deduction. This further highlights the economic impact that infections with *A. suum* may have. However, it was evident in Study I that farms appeared to have only limited problems with white spot lesions registered at slaughter, as 74% of the herds reported to have less than 5% of the livers condemned for this reason.

A likely explanation for the reduced prevalence of *A. suum* in growing pigs, as well as the low occurrence of white spot registrations at slaughter, could be the common practice of batch-wise production. This practice does first of all improve internal biosecurity by limiting parasite spread between different age categories. Secondly it allows for sufficient cleaning, disinfection and drying of the pens between batches, subsequently reducing the parasite load in the environment. Continuous contamination of the environment has been concluded to be a major source of *A. suum* eggs to new hosts (Nilsson, 1982), emphasising the importance of proper hygiene measures between batches of pigs. Still, batch-wise production could not be verified as a protective factor for *A. suum* infections statistically in this work, most likely because it was practiced in almost all herds.

The age category with the highest prevalence of *A. suum* was the pre-partum sows with 37% of the herds and 9% of the samples being positive. On both a herd level and a sample level, those results were similar to what

was found 30 years ago when 36.4% of the herds (Nilsson, 1982) and 8% of the samples collected from sows were positive (Roepstorff et al., 1998). Generally, *A. suum* tend to have a low clinical and subclinical impact on adult animals and sows are not considered a major direct source of infection to their piglets (Nilsson, 1982). However, infected sows may still have a role in maintaining infections in the herd through continued contamination of the environment.

Overall, *Oesophagostomum* spp. were the most prevalent helminths in the examined pig herds. A total of 64% of the herds and 19% of the samples were positive and the highest prevalence was in the pre-partum sows where 41% of the samples were positive. It is common to find the highest prevalence of this parasite in adult animals and a periparturient rise in egg excretion for *Oesophagostomum* spp. is known to occur (Jacobs, 1970). The current prevalence of *Oesophagostomum* spp. in sows showed a marked increase compared to when this was last examined in the 1980s. At that time, 23% of the samples from lactating sows and 30% from dry sows were positive (Roepstorff et al., 1998). The current prevalence was also high compared to other countries such as Denmark and the Netherlands where only 15% and 22% of the sows respectively were found to be positive for *Oesophagostomum* spp. (Eijck and Borgsteede, 2005, Haugegaard, 2010). The high prevalence can likely be explained by the continuous production systems, with access to bedding material on solid floors, as well as the common lack of regular cleaning, disinfection or a sufficient downtime period reported for this age category of pigs. The subsequent result of insufficient management routines in the dry sow section is that eggs and larvae of *Oesophagostomum* spp. may accumulate in the environment, and new sows that enter these units can become infected.

The prevalence of *Oesophagostomum* spp. was lower (4-7%), in growing pigs which was in line with the results from the previous large national study (Roepstorff et al., 1998). The lower prevalence of *Oesophagostomum* spp. in growing pigs was likely explained by both the biology of the parasite as well as by the more intense hygiene practiced in units of younger pigs.

Trichuris suis was uncommon and only detected on 10% of the farms and in less than 1% of the total amount of samples. Only samples from adult sows were positive and FEC were low (50-250 EPG). This was consistent with both previous national studies (Nilsson, 1982, Roepstorff et al., 1998) as well as other European studies (Joachim et al., 2001, Eijck and Borgsteede, 2005,

Haugegaard, 2010, Raue et al., 2017). Given the low FEC, coprophagia should be considered as a source of potential false positive samples for both *T. suis* as well as for *A. suum* (Boes et al., 1997, Boes et al., 1998), although the presence of eggs still indicate that the parasite is present in the herd.

The prevalence of *C. suis* was investigated in both Study II and Study III. The reason for investigating this parasite separately in Study II was to allow repeated samplings of the same litters which is recommended due to the often biphasic and uneven shedding of *C.suis* oocysts from infected pigs (Joachim et al., 2018). In Study III only post-weaning piglets were included but infections were however quantified which was not done in Study II. In Study II the highest prevalence of *C. suis* was in two-week-old piglets where 58% of the sampled farms and 12% of the total number of litters were positive. This was also the expected time to find a high prevalence of infected piglets in a herd, as clinical signs and oocyst excretion tend to mainly occur in piglets aged eight to ten days of age (Joachim and Schwarz, 2014). However, the cumulative herd prevalence was also calculated and as expected, it was increased at the two subsequent samplings when the same piglets were four and six weeks of age. This was likely explained by the continued shedding of oocysts by already infected piglets, combined with the continuous spread to new hosts, both within the same litter as well as between litters in the same herd.

The main source of *C. suis* infection to new piglets are residual oocysts in the farrowing pens and not direct transmission from the sows (Sotiraki et al., 2007, Langkjaer and Roepstorff, 2008). Keeping in mind that the infective dose can be as low as 100 oocysts (Worliczek et al., 2009), and the mean OPG in Study III was 620, it does not require a lot of residual faecal material to contaminate the farrowing pens. This again emphasises the importance of adequate cleaning and disinfection of the farrowing pens prior to introducing a new litter of piglets.

Overall, the strictest hygiene and biosecurity routines were indeed practiced in the farrowing units. It was evident that there was a focus on protecting the youngest pigs in the herd, which is beneficial regarding the control of *C. suis* as the age of the piglet when acquiring an infection is an important factor for the clinical outcome (Worliczek et al., 2009). Indeed, piglets infected early, especially on the first day of life, appear to have the most pronounced occurrence of diarrhoea as well as reduction of weight gain

compared to piglets infected later in life (Mundt et al., 2003, Worliczek et al., 2009).
Co-infections with for example rotavirus, another common cause of neonatal diarrhoea in piglets, may worsen the clinical disease caused by *C. suis* (Vitovec et al., 1991, Vlasova et al., 2017). In Study II the prevalence of rotavirus was also investigated and found to be prevalent with a cumulative herd prevalence at six weeks of 100% (see Paper II for results).

Eimeria spp. were common and detected on 64% of the farms, mainly in samples from adult sows. Infections with *Eimeria* spp. are generally subclinical, although gastrointestinal disease may occur with heavy infections, especially in younger animals (Joachim and Schwarz, 2014, Karamon et al., 2007).

Cryptosporidium spp. was found on all the 13 sampled farms in Study IV, and in a total of 25% of the pen samples. There are no previous studies that have investigated *Cryptosporidium* spp. in Swedish pigs and the global prevalence is reported to be anywhere between 1-100%, depending on the country and production system (Němejc et al., 2013). The results concluded that *Cryptosporidium* likely was common on Swedish pig farms as it was detected in every sampled herd. These results were also much in line with findings from Denmark where one study found the herd prevalence of *Cryptosporidium* spp. to be 31% in piglets, 100% in weaners and 16% in sows (Maddox-Hyttel et al., 2006).

Infections with *Cryptosporidium* are most common in young animals (Maddox-Hyttel et al., 2006, Ryan et al., 2014) and this is also the case in pigs, where mainly growers and fatteners tend to be infected (Kváč et al., 2013). In Study IV, the highest prevalence of *Cryptosporidium* spp. was in growers aged 6-12 weeks and the lowest prevalence was in the adults. This age distribution follows other studies and was similar to what has been reported from for example Norway (Hamnes et al., 2007), Denmark (Maddox-Hyttel et al., 2006), Canada (Guselle et al., 2003) and Australia (Johnson et al., 2008).

Samples positive for *Cryptosporidium* spp. were further analysed for species determination and the pig specific *C. suis* and *C. scrofarum* were predominantly found. However, *C. parvum,* with a high zoonotic potential was also detected in two samples. Cryptosporidiosis is, apart from being an important disease in animals, also a common cause of gastrointestinal disease in humans, and infections generally occur through contaminated food or

water (Fayer, 2008). As well as causing acute gastroenteritis, infections with *Cryptosporidium* spp. may result in persistent problems such as chronic gastrointestinal disease or joint pain, also in otherwise healthy adults (Lilja et al., 2018). Sweden has seen several large outbreaks of human cryptosporidios over the past few years connected to contaminated water or food (Widerström et al., 2014, Folkhälsomyndigheten, 2021). The common occurrence, as well as the detection of *C. parvum,* highlights the importance of remembering pigs as a potential source of zoonotic cryptosporidiosis, either through direct contact with animals or through faecal contamination e.g., when manure is used as fertiliser.

Another interesting finding in Study IV was that *Cryptosporidium* spp. were identified on a SPF farm. This farm had been established by caesarean sections and no animals had ever been introduced. This combined with the rigorous biosecurity practices on the farm made rodents a suspected potential source of infection as they are known carriers of pig pathogens, including pig specific species of *Cryptosporidium* (Backhans and Fellström, 2012, Zhao et al., 2018). Adequate rodent control should hence be emphasised as an important biosecurity practice on pig farms.

Single eggs that resembled eggs of *Strongyloides ransomi* were detected in five samples from five different farms. However, it could not be excluded these were from free-living nematodes. The red stomach worm, *Hyostrongylus rubidus* could not be detected on any of the sampled farms, which was expected as this parasite mainly is found in outdoor herds and all sampled farms in Study III had indoor production (Nilsson, 1982, Murrell, 1986).

7.2 The use of antiparasitic drugs

One of the key points in the control of gastrointestinal parasites is to reduce the possible parasite contamination of the environment to levels where health and productivity of pigs are minimally affected. Antiparasitic drugs can be used to achieve this, as well as to reduce the negative impact the parasites may have on their actual hosts. In Sweden, only two anthelmintic substances are registered for the treatment of pigs, IVM and FBZ, and toltrazuril is registered for the treatment of neonatal coccidiosis.

The use of anthelmintic drugs was common in the surveyed herds and 69% used either FBZ or IVM by routine. It should be noted that the 31% that did

not use anthelmintics frequently were mainly specialised fattening farms or organic farms where the latter are not allowed to use anthelmintics without prior faecal analysis for parasites.

As regular faecal analysis for parasites very rarely was done in any of the herds, the frequent use of anthelmintic drugs was concluded to be done by routine. In contrast, only approximately 18% of the farms used anthelmintic drugs on a regular basis in the 1980s (Nilsson, 1982) and hence there has been a marked increase in the use of these drugs over the past 30 years.

Regarding toltrazuril, farms must have a confirmed history of *C. suis* to use this anticoccidial drug in Sweden. On the surveyed farms toltrazuril was used in 9% of the herds. The conclusion from the low usage was that *C. suis* most likely was not of large concern, despite being relatively prevalent on a herd level. A low usage of toltrazuril is desired as there are recent reports of toltrazuril showing a reduced efficacy in pig herds in the EU (Shrestha et al., 2017).

7.3 Anthelmintic efficacy

In Study V the efficacy of IVM and FBZ were investigated in sows using FECRT, and for the first time in Sweden, a reduced efficacy of IVM on *Oesophagostomum* spp. was discovered. Sows in three out of the nine sampled herds showed a FECR of <95% after treatment with IVM.

It is important to note that when this reduced efficacy was detected, some common causes of treatment failure, such as sub-optimal drug administration or drug underdosing had been ruled out. All farmers had been instructed to dose the anthelmintic for a minimum weight of 330 kg, which should be adequate for even the heaviest sows. The four farms that used IVM all administered the drug via subcutaneous injections which should ensure that all animals received an adequate dose, which may not be the case when drugs are administered in the feed as was done with FBZ.

A possible explanation for the reduced efficacy may have been related to host-parasite relationship or pharmacokinetics (Várady et al., 1996). The two common species of *Oesophagostomum* are located in different anatomical areas of the large intestine, with *O. quadrispinulatum* being found in the caecum and the proximal part of the colon and *O. dentatum* in the middle and distal parts of the colon. Because the gut transit time is slower in the distal part of the large intestine, digesta tend to accumulate there for longer.

Therefore *O. dentatum* may be exposed to drugs for a longer duration, with a subsequent enhanced drug effect, compared to *O. quadrispinulatum* (Hale et al., 1981, Bjørn et al., 1989, Várady et al., 1996). In addition, earlier studies have also shown that anthelmintic drugs may have different pharmacokinetic effects on the two species of *Oesophagostomum,* where both FBZ and IVM were highly effective against *O. dentatum*, but less effective against *O. quadrispinulatum* (Várady et al., 1996, Praslicka et al., 1997). However, it was not possible to associate the poor efficacy of IVM to the species of *Oesophagostomum* present in the examined herds given that both species were present on farms with both good and poor treatment efficacy prior to treatment, as well as in the post-treatment samples on farms with poor efficacy.

Repeated use of one anthelmintic drug class may predispose to the selection of resistance and has been suggested as a cause of treatment failure of *Oesophagostomum* in pigs (Macrelli et al., 2019). Indeed, the three farms that showed poor treatment efficacy had used IVM as the sole anthelmintic drug for several years and two of the farms also used IVM twice or more per year to control sarcoptic mange. On the contrary, the farm that had used IVM with good efficacy had only recently changed to this drug from previously using FBZ. Following the results of this study, all three farms where IVM showed poor efficacy changed to using FBZ with a good effect as result.

However, the definition of anthelmintic resistance includes both a change in gene frequency of a population, produced by drug selection, as well as being heritable (Shoop, 1993, Prichard et al., 1980). Therefore, based on this study alone, and without any knowledge of previous drug efficacy in the three herds, the conclusion that anthelmintic resistance had been induced by long-term use of only one drug class, could not be made. However, it is striking that poor treatment efficacy was only found in herds that had used IVM for a long time.

The discovery of reduced efficacy of IVM on *Oesophagostomum* spp. was important as it highlighted the merit of performing regular surveillance of anthelmintic efficacy in pigs. As has been discussed, anthelmintic resistance is an emerging global threat, and anthelmintic efficacy must be monitored as part of the actions taken to reduce the risk of further development of resistance (Kotze et al., 2020). This becomes even more important, considering that anthelmintic drugs often are administered in pig herds on a routine basis. The finding was also important when considering that

Oesophagostomum spp. were the most common parasites found in Swedish pig herds. Despite the often subclinical nature of infections in pigs, heavy infections in sows may lead to clinical disease, weight loss as well as low birth rates and reduced growth of her piglets (Pattison et al., 1979). This in turn will affect the welfare of the pigs as well as the productivity of the farm.

The anthelmintic treatments were effective against *A. suum* on the five farms where this parasite was detected, regardless of the anthelmintic used. There is to this point no reports of anthelmintic resistance in *A. suum* and the high efficacy was hence in line with previous studies (Stewart et al., 1996, Borgsteede et al., 2007, Lopes et al., 2014).

7.4 Methodological considerations

There are several factors that need to be taken into consideration when assessing the findings within this thesis. In Study I, the response rate was only 21% and although there were few discrepancies in the answers, and all types of production were represented, it was still only a small proportion of the pig farms in Sweden. One could also suspect that well managed farms are more likely to respond to a questionnaire assessing hygiene, biosecurity, and parasite control. However, it is also more likely that farms with a known parasite problem would consider participating in a prevalence study to have the farm assessed regarding parasite occurrence.

In Study III and V, faecal flotation and a modified McMaster technique were used to analyse the faecal samples. This method first carries the risk of false positive samples due to coprophagia (Boes et al., 1997, Boes et al., 1998). Secondly, false negative samples are also possible due to factors such as the uneven dispersion and shedding of eggs or oocysts of many of the different parasites (Nejsum et al., 2009a, Joachim et al., 2018). Also, faecal samples were kept in room temperature during transport which may have resulted in hatching of eggs of for example *S. ransomi* and *Oesophagostomum* spp., resulting in false negative results.

When risk factors were assessed in correlation to the results from the faecal analysis, only a few specific factors were identified as having a significant impact. A likely reason for this is the uniformity within the pig production, including that most farms had very similar management routines. As many of the potential risk factors for gastrointestinal parasite infections are mandatory by national legislation, such as bedding material and solid

floors, there were no herds raised under different conditions to compare the results with.

It should also be mentioned that organic and outdoor herds were not included in Study II and III. Given the different rearing conditions in these types of production, the prevalence of gastrointestinal parasites may have been very different.

7.5 Implications of the findings within this thesis

The findings within this thesis are of large relevance to the national pig production. However, the results are also relevant beyond the borders of Sweden. The strict animal welfare legislation in Sweden, where pigs are to always be loose-housed, rooting material must be provided and fully slatted floors cannot be used, are in many ways also considered more suitable for parasite survival, as has been discussed earlier. However, must animal welfare friendly conditions also always result in more parasite friendly conditions? When growth promoting antibiotics were banned in Sweden in the 1980s, improved biosecurity became necessary to ensure healthy pigs (Wallgren, 2009). Improved management and preventing disease hence became general practice in Swedish pig herds, instead of the routine use of antibiotics. From the work done in this thesis, it has also become evident that housing conditions that improve animal welfare does not always result in more parasites, if adequate hygiene and biosecurity practices are also implemented.

Yet the use of anthelmintic drugs was high, and one could argue whether that really should be necessary? In a Danish study conducted more than 20 years ago, it was suggested that routine use of anthelmintic drugs could be discontinued in pig herds with good management, and a low occurrence of gastrointestinal parasites (Roepstorff, 1997). Instead, parasitic monitoring could be done, and treatments only administered when deemed necessary. Such targeted treatments have already been implemented for ruminants as a response to the development of anthelmintic resistance (Charlier et al., 2014, Greer et al., 2020). Similar strategies with targeted selective treatments, where only selected individuals are treated, have been introduced for e.g., horses due to the same reason (Tydén et al., 2019). There is also the option of adapting selective non-treatments, where treatments are

withheld from certain animals based on predetermined criteria (Greer et al., 2020).

Based on the results from this thesis, similar strategies ought to be considered for pig herds as well. Especially when taking into account the new knowledge that *Oesophagostomum* spp. showing poor treatment response to IVM are present in Swedish pig herds. The aim with gastrointestinal parasite control is not to eradicate these parasites from pig herds, but instead to keep the parasitic burdens at levels where animal health and farm productivity are not negatively affected. There is subsequently no need for the routine use of antiparasitic drugs in herds where there are no real negative effects caused by gastrointestinal parasites.

Adapting new strategies will however result in new challenges such as finding ways to monitor the presence of intestinal parasites in a cost-effective manner and developing suitable guidelines for when treatment should be done. For example, there must be economic incentives for the farmer to choose the option of parasitic analysis prior to treatment given it will incur increased labour, as well as a cost for laboratory analysis.

8. Summary and concluding remarks

The five studies that are included in this thesis have vastly extended the knowledge of the occurrence, control, and treatment of gastrointestinal parasites in Swedish pig herds, which was also the overall aim. It was clear from the studies performed that there have been major changes in both the control measures, as well as of the overall occurrence of gastrointestinal parasites since this was last thoroughly investigated 30 years.

Some specific conclusions include:

- Animal welfare and more natural rearing systems do not need to be compromised in favour of parasite control if adequate hygiene and biosecurity practices can be maintained.
- High quality biosecurity and hygiene practices were commonly practiced in units for growing pigs but less so in units for dry sows.
- The strategic hygiene and biosecurity practices have likely contributed to the reduced prevalence of *A. suum* in growing pigs compared to previous studies.
- The prevalence of *Cystoisospora suis* was reduced on a sample level compared to previous studies but on a herd level the prevalence was high.
- *Oesophagostomum* spp. were the most prevalent parasites found in Swedish pig herds, with the highest prevalence in pre-partum sows.
- *Cryptosporidium spp.* were found on all sampled farms and are likely common in Swedish pig herds.
- Pigs should be considered a potential source of zoonotic cryptosporidiosis.

- Antiparasitic drugs were used frequently and often without prior knowledge of the actual parasite status in the herd.
- Reduced efficacy of ivermectin on *Oesophagostomum* spp. was detected for the first time in three Swedish pig herds.

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9. Future perspectives

Some implications for the future have already been raised in the discussion above, and based on new insights gained from the studies within this thesis, there are some specific areas that warrants further consideration in the future:

- Updated recommendations regarding sampling for parasitic infections and for treatment should be established to ease the decision process on when, and when not to treat for gastrointestinal parasites.
- The common practice of routine anthelmintic use should be re-evaluated and strategies such as targeted treatments or selective non-treatments should be considered in appropriate pig herds instead.
- Anthelmintic efficacy should be monitored more carefully in pig herds to ensure that the anthelmintic substances remain effective, and routines for this should be implemented.
- The occurrence of gastrointestinal parasites should be evaluated in organic and outdoor herds as well, given the different rearing conditions that may warrant different control strategies compared to conventional herds.

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Popular science summary

Gastrointestinal parasites occur in pigs worldwide and in all types of production. Common parasites to be found in the gastrointestinal tract are roundworms, nodular worms, and whipworms as well as single cell parasites, also known as protozoa. These parasites may cause clinical signs of disease For example piglets infected with coccidia, a protozoan parasite, may develop diarrhoea, dehydration, and a stunted growth. It is however more common for pigs to suffer from subclinical infections, which means the animal is infected but do not show any obvious signs of illness. Still, infections may result in an inability to properly utilise the feed and poor growth. This in turn will have negative effects on the health and welfare of the pigs as well as on the sustainability and productivity of the farm, due to an increased use of feed and other resources. Some gastrointestinal parasites, for example the larvae of the pig roundworm, also cause damage to other internal organs such as the liver. The organ damage may result in condemnations at slaughter and thus additional financial losses for the farmer.

Infected pigs excrete parasite eggs in their faeces and new pigs become infected by accidentally ingesting these eggs. To control gastrointestinal parasites, as well as their negative effects in a herd, a combination of management practices and antiparasitic drugs are often used. There is however a growing problem with parasites of livestock developing resistance to the available antiparasitic drugs, that thus cease to work effectively. Hence antiparasitic drugs need to be used responsibly.

This thesis investigated gastrointestinal parasites in Swedish pigs with focus on overall parasite occurrence and control measures in five different studies. Pig parasites have not been thoroughly investigated in Sweden since the 1980s and since then there have been major changes in the national pig

production. Pig farms are now larger with more intensified production which in turn often results in more hygienic conditions, and less chance of disease spread. At the same time there are now higher demands on animal welfare compared to 30 years ago which may favour survival of the parasites.

In the first study (Study I), management practices related to parasite control measures on pig farms were documented using an online questionnaire. From that study it was evident that strategic hygiene and biosecurity practices were common and more so for growing pigs (piglets, growers, and fatteners) than for adults (sows). Study I also showed that antiparasitic drugs were used frequently, and mainly by routine as it was uncommon for farms to test if the pigs were infected with parasites before treating with the drugs.

To assess the current occurrence of gastrointestinal parasites in pig herds, three separate prevalence studies were carried out. The prevalence of coccidia in piglets was investigated in Study II. Faecal samples were collected and analysed when the piglets were two, four and six weeks of age. The results showed that more than half of the farms had the parasite present at the first sampling, but the number of infected litters was lower compared to previous national studies performed in the 1980s. In Study III, faecal samples were collected from pigs of five different age categories and analysed for both worm eggs and coccidian oocysts. The nodular worm was found to be the most common parasite and was mainly found in samples from adult sows. The prevalence of the pig roundworm, a parasite of large importance in growing pigs, was found to be lower in these age categories compared to previous national studies. A likely reason for this reduced prevalence was the improved hygiene and biosecurity practices that have been put in place in recent decades. The pig whipworm was rarely found.

The single cell parasite *Cryptosporidium* was investigated separately in Study IV. This parasite was found on each sampled farm and hence concluded to likely be common on Swedish pig farms of today. On two farms a species of this parasite that is known to cause diarrhoea in humans was also found.

In Study V, the efficacy of the deworming drugs that are used on Swedish pig farms were investigated. For the first time in Sweden, a reduced treatment efficacy to ivermectin on the nodular worm was recognised in three separate pig herds. This highlights the need for a more responsible use of these remedies as well as emphasised that we should revise the common routine

use of deworming drugs on pig farms. Especially in the light of the other findings within this thesis, such as a low prevalence of parasites in growing pigs and the overall good hygiene and biosecurity on pig farms. In Sweden there are only two registered substances to use when deworming pigs and hence it is essential their efficacies are preserved. The question on whether pigs should be dewormed without knowing the status regarding gastrointestinal parasites, when there is a growing global concern about the development of resistance to these drugs, was raised.

In conclusion, several changes in both the occurrence and the control of gastrointestinal parasites were identified in this thesis, for example that improved animal welfare does not always results in more parasites. This new knowledge can in turn contribute to healthier pigs and a more sustainable and profitable pig production.

Populärvetenskaplig sammanfattning

Mag-tarmparasiter är vanliga hos grisar världen över och i alla typer av uppfödning. Grisar smittas i huvudsak av spolmask, knutmask och piskmask, men även av encelliga parasiter så som koccidier. Mag-tarmparasiter kan orsaka klinisk sjukdom, till exempel smågrisar som smittats av koccidier kan få diarré med uttorkning och minskad tillväxttakt som följd. Vanligare är det dock att grisar som smittas med mag-tarmparasiter får så kallade subkliniska infektioner där inga uppenbara tecken på sjukdom kan ses. Trots detta kan parasiterna påverka foderomvandlingsförmågan vilket gör att grisarna inte utnyttjar det foder de äter fullt ut och då växer sämre. Detta i sin tur påverkar grisens hälsa och välbefinnande och även gårdens produktion. Indirekt påverkas även gårdens hållbarhet eftersom parasitinfektioner resulterar i att ökade resurser, så som foder och läkemedel krävs. Spolmaskens larver kan också orsaka skador på andra inre organ så som levern när de vandrar genom kroppen. Detta resulterar i att levern kasseras vid slakt och orsakar ytterligare ekonomiskt bortfall för lantbrukaren.

Grisar smittas av mag-tarmparasiter genom att infekterade djur urskiljer parasitägg i avföringen som nya djur sedan kan få i sig ifrån sin omgivning. För att minska förekomsten av dessa parasiter, och även deras negativa effekter, så tillämpas ofta en kombination av olika hygienåtgärder och behandling med avmaskningsmedel. Ökade rapporter om resistens mot avmaskningsmedel hos lantbrukets djur innebär dock att dessa läkemedel måste användas på ett ansvarsfullt sätt.

Den här avhandlingen har undersökt mag-tarmparasiter hos grisar avseende förekomst och kontroll i fem olika studier. Detta hade inte undersökts i Sverige på över 30 år och mycket har ändrats i grisproduktionen sedan dess. Besättningarna är större och har bättre rutiner för att minska risken för smittsamma sjukdomar. Samtidigt har strängare krav på god

djurvälfärd införts, vilket kan gynna vissa parasiter som trivs bra i de förhållanden som nu äger rum i svenska grisbesättningar.

I den första studien (Studie I) dokumenterades åtgärder relaterade till besättningens parasitkontroll med hjälp av en enkätundersökning. Studien visade att strategiska hygien– och biosäkerhetsrutiner var vanliga åtgärder för växande grisar (smågrisar, tillväxtgrisar och slaktgrisar), men mindre vanliga hos vuxna djur (suggor). Studien visade också att avmaskningsmedel ofta användes rutinmässigt.

För att ta reda på förekomsten av mag-tarmparasiter genomfördes tre separata studier. Inledningsvis undersöktes förekomsten av koccidier hos smågrisar (Studie II). Avföringsprover samlades in och analyserades för parasitförekomst när grisarna var två, fyra och sex veckor gamla. Resultaten visade att mer än hälften av de provtagna gårdarna hade parasiten i besättningen men att andelen positiva prover var lägre jämfört med de senaste studierna som genomfördes på 80-talet. I Studie III undersöktes förekomsten av både maskägg och oocystor från koccidier i avföringsprover från fem olika ålderskategorier av grisar. Knutmask visade sig vara vanligast och återfanns huvudsakligen hos suggor. Förekomsten av spolmask som framförallt har betydelse hos växande grisar, hade minskat hos dessa jämfört med tidigare studier. En trolig orsak till minskningen är förbättrade hygien– och smittskyddsrutiner som nu används i de allra flesta besättningar. Piskmask hittades sällan.

Det encelliga parasitsläktet *Cryptosporidium,* med arter som även kan orsaka sjukdom hos människor, undersöktes i Studie IV. Det visade sig att alla provtagna besättningar var infekterade och *Cryptosporidium* är därmed sannolikt vanligt förekommande i svenska grisbesättningar. Även en av de arter som kan orsaka sjukdom hos människor hittades.

Avslutningsvis i Studie V undersöktes effekten av de avmaskningsmedel som finns registrerade till svenska grisar. För första gången hittades knutmask som inte svarade på behandling med avmaskningsmedlet ivermektin i tre av nio undersökta besättningar. Detta fynd visar på vikten av att avmaskningsmedel används på ett ansvarsfullt sätt. Den rutinmässiga användningen som sker idag behöver därför ses över. Speciellt mot bakgrund av de övriga fynden i avhandlingen, så som den låga förekomsten av spolmask hos växande grisar och de över lag goda rutinerna för hygien och smittskydd i besättningarna. Frågan är om grisar ska avmaskas rutinmässigt utan vetskap om deras infektionsstatus, särskilt som det finns en globalt

växande oro för att parasiter kan utveckla motståndskraft (resistens) mot dessa läkemedel.

Sammantaget kunde flera förändringar gällande både parasitförekomst och kontroll identifieras i den här avhandlingen, till exempel att ökade krav på god djurvälfärd inte måste resultera i en ökad parasitförekomst. Den nya kunskapen kan i sin tur bidra till friskare grisar och en mer hållbar och lönsam grisproduktion.

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Appendix

English translation of the web-based questionnaire “Avmaskningsrutiner i svenska grisbesättningar”.

Parasite control routines in Swedish pig herds

The aim with this study is to document the herd structures and management routines that can be related to parasite control on Swedish pig farms. The parasite status of Swedish pigs has not been investigated since the 1980s, and a lot has changed in Swedish pig production since then. Today we know very little of the actual parasite status of Swedish pigs and there is also hardly any information on how herds control and manage parasites. The goal with this questionnaire, and a subsequent prevalence study, is to gain information and knowledge that can form a base for new and updated recommendations on parasite control, suitable for modern pig production.

It takes approximately 20 minutes to fill out this questionnaire.

1) What type of herd do you have?

- ○ Farrow-to-finish
- ○ Specialised piglet producer
- ○ Specialised fattener producer
- ○ Central unit in a sow pool
- ○ Satellite in a sow pool, farrow-to-finish
- ○ Satellite in a sow pool, specialised piglet producer

2) What type of production do you have?

- ○ Conventional
- ○ KRAV-certified or organic
- ○ EU-organic
- ○ Specific Pathogen Free (SPF)
- ○ Outdoor
- ○ Other

3) What herd size do you have?

- ○ No sows, the herd is a specialised fattening herd
- ○ Less than 100 sows, or a satellite in a sow pool with less than 220 farrowings/year
- ○ 100 - 400 sows, or a satellite with 220-880 farrowings/year
- ○ More than 400 sows, or a satellite with more than 880 farrowings/year

4) How many fatteners are produced each year?

- ○ 0 (I do not have fatteners)
- ○ Less than 1500
- ○ 1 500 - 5 000
- ○ 5 000 - 10 000
- ○ 10 000 - 20 000
- ○ More than 20 000

5) What type of pens do you have?

- ☐ Conventional farrowing pens
- ☐ Conventional grower pens
- ☐ Unit pens (weaned piglets remain in the farrowing pens)
- ☐ Multi-litter pens for growers
- ☐ Family-pens (several sows with their piglets)
- ☐ Farrow-to-finish pens
- ☐ Conventional fattening pens
- ☐ Deep litter straw pens (fatteners)
- ☐ Unspecified fattening pens
- ☐ Deep litter straw pens (dry sows)
- ☐ Conventional dry sow pens

6) Do you practice batch wise production?

	Always	Mostly	No	n/a
For piglets	○	○	○	○
For growers	○	○	○	○
For fatteners	○	○	○	○

7) What type of bedding material is used? More than one option can be selected.

	n/a	Straw	Peat	Wood shavings	Deep litter	Other
For piglets	☐	☐	☐	☐	☐	☐
For growers	☐	☐	☐	☐	☐	☐
For fatteners	☐	☐	☐	☐	☐	☐
For dry sows	☐	☐	☐	☐	☐	☐

8) Do you use dry or wet feed?

	n/a	Dry	Wet	Both
For growers	☐	☐	☐	☐
For fatteners	☐	☐	☐	☐
For dry sows	☐	☐	☐	☐

9) How is the water supplied?

	n/a	Automatic waterers	Nipple drinkers	Both	Other
For piglets	○	○	○	○	◉
For growers	◉	○	○	○	○
For fatteners	◉	○	○	○	○
For dry sows	◉	○	○	○	○

10) Where is the water facility placed? More than one option can be selected.

	n/a	Over the slats	Over the solid floor	Over feed trough
For piglets	☐	☐	☐	☐
For growers	☐	☐	☐	☐
For fatteners	☐	☐	☐	☐
For dry sows	☐	☐	☐	☐

11) At what age are the piglets weaned?

- ○ n/a
- ○ 4–5 weeks
- ○ 5–6 weeks
- ○ 6–7 weeks
- ○ Older than 7 weeks

12) For how long do the piglets stay in the farrowing pens?

- We do not have piglets
- We have unit pens
- Moved directly at weaning
- Moved 1 week after weaning
- Moved 2 weeks after weaning
- Moved 3 weeks after weaning

13) At what age are the growers moved to the fattening units, or sold?

- n/a
- 8 weeks or younger
- 9 weeks
- 10 weeks
- 11 weeks
- 12 weeks
- 13 weeks
- 14 weeks or older

14) What is the estimated weight when the growers are moved to the fattening units, or are sold?

- n/a
- Less than 23kg
- 23-26kg
- 27-29kg
- 30-32kg
- 33-35kg
- More than 35kg

15) After how many weeks in the fattening units are the first animals sent to slaughter?

- n/a
- 10 weeks or earlier
- 11 weeks
- 12 weeks
- 13 weeks
- 14 weeks
- 15 weeks
- More than 15 weeks

16) After how many weeks in the fattening units are the last animals sent to slaughter?

- n/a
- 13 weeks or earlier
- 14 weeks
- 15 weeks
- 16 weeks
- 17 weeks
- 18 weeks
- More than 18 weeks

17) What is the estimated slaughter weight?

- n/a
- 75kg or less (100kg or less live weight)
- 75-79kg (100-105kg live weight)
- 80-82kg (106-110kg live weight)
- 83-86kg (111-115kg live weight)
- 87-90kg (116-120kg live weight)
- 91-93kg (121-125kg live weight)
- 94-96kg (126-130kg live weight)
- More than 96kg (more than130kg live weight)
- Do not know

18) Do any pigs have outdoor access?

	n/a	Yes	No
Nursing piglets			
Growers			
Fatteners			
Dry sows			

19) How often are the following pens washed?

	n/a	Before every new batch	Before every second batch	Before every third batch	Less frequently	Never
Farrowing pens	○	○	●	○	○	○
Grower pens	○	○	●	○	○	○
Family pens	○	○	●	○	○	○
Farrow-to-finish pens	○	○	●	○	○	○
Fattening pens	○	○	○	○	○	○
Deep litter straw beds	○	○	○	○	○	○
Conventional dry sow pens	○	○	●	○	○	○

20) How often are the following pens disinfected?

	n/a	Before every new batch	Before every second batch	Before every third batch	Less frequently	Never
Farrowing pens	○	○	○	○	○	○
Grower pens	○	○	○	○	○	○
Family pens	○	○	○	○	○	○
Farrow-to-finish pens	○	○	○	○	○	○
Fattening pens	○	○	○	○	○	○
Deep litter straw beds	○	○	○	○	○	○
Conventional dry sow pens	○	○	○	○	○	○

21) What disinfectant(s) do you use? If you do not disinfect you can leave this space blank.

22) How long downtime period do the following pens have?

	n/a	0	1–3 days	4-6 days	7-10 days	More than 10d	Varies
Farrowing pens	○	○	○	○	○	○	○
Grower pens	○	○	○	○	○	○	○
Family pens	○	○	○	○	○	○	○
Farrow-to-finish pens	○	○	○	○	○	○	○
Fattening pens	○	○	○	○	○	○	○
Deep litter straw beds	○	○	○	○	○	○	○
Conventional dry sow pens	○	○	○	○	○	○	○

The antiparasitic drugs available in Sweden are:

A) Avermectins such as Ivomec, Noromectin and Bimectin

B) Benzimidazoles such as Axilur, Panacur and Zerofen

C) Triazine compounds such as Baycox and Zorabel

23) Are any of the sows treated with any antiparasitic drugs? If yes, what drug is used, and how is it administered?

	Drug class (see above)						Administration route				
	n/a	No treatment	A	B	C	Other	n/a	In feed	In water	Drench	Injection
Before insemination	○	○	○	○	○	○	○	○	○	○	○
During pregnancy	○	○	○	○	○	○	○	○	○	○	○
Before farrowing	○	○	○	○	○	○	○	○	○	○	○
During suckling	○	○	○	○	○	○	○	○	○	○	○

24) Are pigs of any other ages treated with antiparasitic drugs? If yes, what drug is used, and how is it administered?

	Drug class (see above)					Administration route				
	n/a	No treatment	A	B	C	n/a	In feed	In water	Drench	Injection
Nursing piglets	○	○	○	○	○	○	○	○	○	○
Growers	○	○	○	○	○	○	○	○	○	○
Fatteners	○	○	○	○	○	○	○	○	○	○
Replacement animals older than 6 months	○	○	○	○	○	○	○	○	○	○

25) Is faecal analysis carried out to check for parasites?

	n/a	Never	Sometimes	Often
Sows	○	○	○	○
Nursing piglets	○	○	○	○
Growers	○	○	○	○
Fatteners	○	○	○	○
Replacement animals older than 6 months	○	○	○	○

26) If sarcoptic mange has not been eradicated in the herd, how often to treat for this?

- ○ Never
- ○ Twice or more per year
- ○ 1 time per year
- ○ More than 1–3 years ago
- ○ More than 3–5 years ago
- ○ More than 5 years ago

27) How often does diarrhoea occur in the following pigs?

	n/a	Always	Most batches	Occasional batches	Never	Do not know
Piglets during the first week	☐	☐	☐	☐	☐	☐
1-3 week old piglets	☐	☐	☐	☐	☐	☐
Piglets at weaning	☐	☐	☐	☐	☐	☐
Growers	☐	☐	☐	☐	☐	☐
Fatteners	☐	☐	☐	☐	☐	☐
Adults	☐	☐	☐	☐	☐	☐

28) Is zinc oxide used in the feed?

- Never
- Not the past 12 months
- Sometimes
- Always at weaning

The larvae of the pig roundworm migrate through the body of the pig and may then cause damage to the liver and the lungs. This damage may be noted at slaughter.

29) What proportion (mean value over the past year) of livers are condemned at slaughter due to parasitic liver damage/white spots (code 83/84)?

- Do not have fatteners
- Less than 5%
- 5 - 10%
- 10 - 20%
- 20 - 35%
- 35 - 50%
- More than 50%
- Do not know

30) What proportion (mean value over the past year) of pneumonic lesions are registered at slaughter (code 61/62)?

- Do not have fatteners
- Less than 5%
- 5 - 10%
- 10 - 20%
- 20 - 35%

- 35 - 50%
- More than 50%
- Do not know

Information and a question regarding part two of this project

In a second part of this project, we will be collecting faecal samples from pigs of different age categories to look for gastrointestinal parasites

31) Would you be interested in participating in part two of this study and send in faecal samples?

- Yes
- No

All of you who have answered yes on question 31 and are interested in participating in part two of this study, please fill in your contact details (name, address, phone number and email address) so that we are able to contact you. If you are selected to be in the second part of the study, sampling material will be sent out to you together with detailed information about sampling. The samples will be analysed at the National Veterinary Institute and you will of course be notified of the results from your herd. For you this means up to 50 free faecal samples, In the final report the results will however be anonymous.

Results will be disseminated through Grisföretagaren.

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Doctoral Thesis No. 2021:34

Gastrointestinal parasites are common in pigs and have a negative effect on their health and on farm productivity. This thesis investigated the prevalence and the control strategies of gastrointestinal parasites in Swedish pig herds. It was evident that good hygiene and biosecurity were commonly practiced, with a positive effect on the occurrence of several different parasites. Anthelmintics were commonly used and for the first time in Sweden, a reduced efficacy to the drug ivermectin was found in pigs.

Emelie Pettersson received her graduate education at the Department of Clinical Sciences, Swedish University of Agricultural Science, Uppsala, Sweden. Her undergraduate degree in veterinary medicine was obtained at the University of Melbourne, Australia in 2008.

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