Differences in prevalence of gastrointestinal parasitic helminths of Spotted Bentheimer hobby farms, nature conservation farms and organic farms.

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1 INDEX

ABSTRACT

The pressure from society, government and also amongst farmers has resulted in a transition of systems with outdoor pig production systems in the Netherlands. Most of these outdoor pig production systems in the Netherlands are organic farms, but there is a growing group of small scale of agricultural farms that focus on care, education or processing of regional quality products. The breed that is often used for these purposes is the Spotted Bentheimer. Besides serving for human consumption or education, these pigs are kept in several hectares of forest with the purpose of nature conservation (project ''Pig landscape developer'', ''varkens als landschapsontwikkelaar''). This is one of the reasons that the Association of the Spotted Bentheimer requested to conduct a risk analyses for humans and animals in this group of farms. The occurrence of gastrointestinal parasitic helminths and the prevalence of these parasites on the population of the Spotted Bentheimer in suckling pigs, weaners/fattening pigs and sows were studied. Organic farms were used as a comparison to estimate the risks of keeping pigs as nature developers. 8 organic farms (OF), 16 Spotted Bentheimer hobby farms (HF) and 3 nature conservation farms (NC) participated in the questionnaire and fecal examinations. Each farm was visited once or the farmers sent faeces by mail. Infections with strongyle-type, *Ascaris suum* and *Trichuris suis* were found and no clinical cases were observed. Infections with strongyle-type eggs were found on 13 % of the OF, 43 % of the HF and 33 % of the NC . Weaners/fatteners and sows had the highest prevalence. Weaners/fatteners had a prevalence of 50 % of the NC and 63 % of the HF, sows a prevalence of 14 % of the OF, 39 % of the HF and 33 % of the NC. Few positive samples for *Ascaris suum* and *Trichuris suis* were seen. *Ascaris suum* was present on 25 % of the OF, 11 % of the HF and absent on NC. Weaners/fatteners had a prevalence of 25 % of the HF and sows had 29 % of the OF. *Trichuris suis* was present on 26 % of the HF and only seen in weaners/fatteners with a prevalence of 50 %. *Trichuris suis* was absent on the OF and NC. This study showed that pigs on Spotted Bentheimer farms have significantly higher infections with strongyle-type eggs than pigs on organic farms.

2 INTRODUCTION

The industrialization of swine production in the Netherlands the last 50 years resulted in a high productivity, a rise in work efficiency, a high bio security and an elimination of various ''infections agents'', especially some helminth species and a decrease in the worm loads in domestic pigs[1 2] .

But awareness is growing to consider reduction and extensification of swine production, to mitigate its global impact on the environment and to improve animal welfare. Examples of this are initiatives of citizen ('Citizen initiative mega stables-no', 'Burgerinitiatief megastallen-néé', environmental groups (Milieudefensie Campaign Soy: 'The drama behind cheap meat', 'Het drama achter goedkoop vlees') and policy makers (Commission van Doorn ''Only sustainable meat, a breakthrough to a healthy, safe and respected livestock production in 2020'', 'Al *het vlees duurzaam, een doorbraak naar een gezonde, veilige en gewaardeerde veehouderij in 2020').*

The pressure from society, government and also amongst farmers has resulted in a transition of systems with outdoor pig productions in the Netherlands. Most of these outdoor pig production systems in the Netherlands are organic farms. The reintroduction of outdoor pig production implies a risk of increased infection levels with various micro-organisms, especially parasitic infections[1 3]. These parasites could lead to growth retardation in fatteners or even clinical infections in pigs. They are not only of economic importance to the farmer and a health risk for the pig but some of these parasites could be a risk for human's health.

That's why several surveys in Europe have been conducted to determine the occurrence of internal parasites in pigs in different housing systems[1 3 4]. Usually these surveys involve three different types of pig housing systems, namely conventional (indoor) housing systems, the organic farms and the free ranging farms.

However, there is a growing group of small other farms. These pigs are kept on a small scale as a business unit of agricultural farms that focus on care, education or processing of regional quality products. The breed that is often used for these purposes is the Spotted Bentheimer, a rare authentic Dutch-German breed, from the border of Twente and Bentheim. They are known for their peaceful and social character, low maintenance demands and they have a strong urge to burrow. Besides serving for human consumption or education, these pigs are kept in several acres of forest with the purpose of nature conservation (project ''Pig landscape developer'', ''varkens als landschapsontwikkelaar''[5]). The pigs may come into contact with recreational areas and surrounding livestock farms. This is one of the reasons that the Association of the Spotted Bentheimer [6] requested to conduct a study after the risks for human and animal in this group of farms.

The aim of this study was to find out which gastrointestinal parasitic helminths occur, the prevalence of these parasites in the population of the Spotted Bentheimer and to look for differences between age groups (suckling pigs, weaners/fattening pigs and sows). Organic farms were used as comparison in the fecal samples.

2.1 Helminths

The worms which are generally important in pigs in the Netherlands are *Ascaris Suum, Oesophagostomum spp. (*mainly *O. dentatum), Hyostrogylus rubidus, Trichurus Suis, Strongyloides ransomi and Metastrongylus spp.* [3]

2.1.1 Ascaris Suum

Ascaris suum is one of the most common swine parasites and is the largest nematodes of the pig, the white rigid females range from 25 to 40 cm in length. The adult worms reside in the small intestine [7]. A female worm has a great reproductive potential, she is able to produce 250.000 or more eggs per day[8]. The egg is thick-shelled with multilayer, sticky and able to survive up to 4-6 years on the ground. Although embryonation and development to the infective second stage larvae may, in ideal conditions of temperature, humidity, and oxygenation, occur in 10 to 14 days, usually the development is much slower especially in winter[9]. Obviously development will be faster in heated confinement facilities and in regions with mild or warm winters.

Life cycle

Ascaris suum has a direct or indirect life cycle (see Figure 1) with paratenic host [7], Beetles and earthworms are paratenic or transport hosts of *Ascaris suum* they ingest the eggs and the L2 larvae can remain fully infective for pigs.

The pig ingests eggs with an L2 larvae inside (**B**) or a paratenic host with L2 larvae in their tissue (**D**) the eggs hatch in the small intestine, the L2 larvae penetrate the intestinal mucosa (**F**), enter the portal blood stream travel to the liver (**G**). The so called 'milk spots' are produced by migrating larvae, they cause intralobulair necrosis and an inflammatory reaction[9]. Milk spot livers are detected at the slaughterhouse and are an

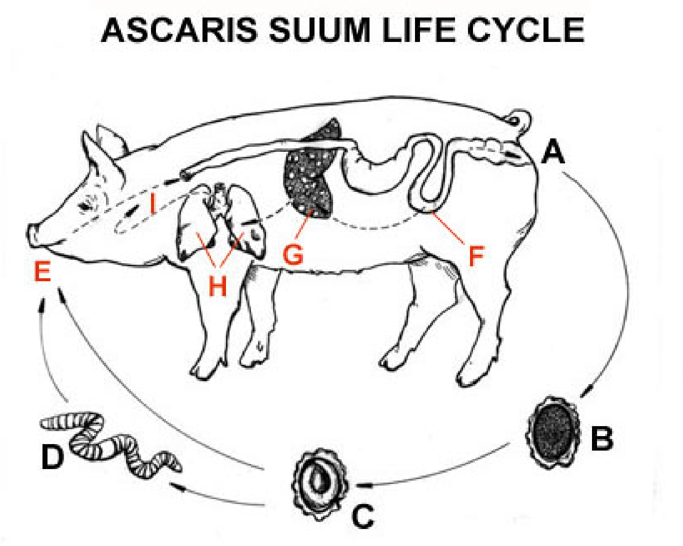

Figure 1: Life cycle of *A.suum*
source: *Parasites & Parasitic Diseases of domestic animals* Dr. Colin Johnstone (principal author)

indication of recent infection because the lesions begin to regress after the larvae leaves the liver and had usually completely disappeared by 4 to 6 weeks [10]. The larvae moult to the third stage within 36 hours, the third stage larvae migrate via the blood stream, from the liver to the lung (**H**)after 4-6 days after infection[9]. After 7-8 days of infection the larvae move from the capillaries into the alveoli, bronchi, trachea and pharynx where they may be swallowed (**I**)and reach the small intestine. In the small intestine the larvae undergo the last two final moults and the young adult worms are present by 3-4 weeks after initial ingestion of the eggs. They are sexually mature by 6-8 weeks (prepatend period), produce eggs which are excreted in the feces (**A&B**) and these eggs develop to infective stage in 3-8 weeks (**C**). The longevity of adult worms is around 6-9 months [7].

Clinical signs

Fatteners and gilts show the highest prevalence of *A. suum*[11]. An infection with *Ascaris suum* induces a strong immunity [10]. *A.suum* causes few clinical signs even in heavy infections [12]. Growth rate may be decreased by 2-10 % and food conversion by 5-13 % [12]. For these reasons the effects of *A.suum* are most evident in young pigs which are 2-5 months of age[10]. Invasion of the lungs by large numbers of larvae can cause hemorrhages, oedema, emphysema and eosinophilic infiltrations [13]. Oedema and emphysema are probably responsible for rapid shallow breathing called "thumps" and a moist cough, ''heaves''[12]. The lungs are more sensitive for infections for example *Actinobacillus pleuropneumonia* or influenza virus.

Zoonotic risk

The larvae of *A.suum* can migrate into the tissues of the pig, wild boar, cattle, rarely sheep and humans[7]. *A.suum* infection is spread from pigs to human when the embryonated eggs are ingested by man, for example when pig manure is used as a garden fertilizer and contaminates the crops. Some authors (Roepstorff L., et. al.) describe two cases of adult *Ascaris* worms which were found in children[14] , but other authors suggest that generally, the migrating larvae do not mature to the adult stage [15], so the clinical signs are because of the migrating larvae. But clinical infections with *A.suum* are rare[16]. A few cases of *A.suum* infection in man are reported, in 1970 in Canada a postgraduate student in parasitology contaminated the food of four people with *A.suum* eggs. The people manifested the clinical picture of pulmonary infiltrates with asthma and edema[17] of the lungs . In 1999 they described a case of visceral larva migrans in Japan due to an infection with *A.suum*[18].

Ascaris lumbricoides the roundworm of humans is closely related to *A. suum*, which infect 1221 million people worldwide [19]. The western part of the world is considered non-endemic for *Ascaris lumbricoides* [14]. It is difficult to determine the source of human infections, so much attention is given to the relationship between *A.suum and Ascaris lumbricoides.* Even though Ascaris in humans and pigs seems to represent two different species, cross infections definitely occur[14]. Concluding these findings define *A.suum* as a potential zoonotic risk.

2.1.2 Trichuris suis

Trichuris suis also known as whipworm has an anterior that is long, slender and whip like. The adults are whitish and 3-5 cm long. The adult worms reside in the cecum and colon, with its whip tangled in the mucosa[7]. The eggs are lemon shaped (60-25 µm) and have a thick smooth shell with a polar plug at both ends[7]. The eggs are very resistant to desiccation and can remain infective for up to 7-11 years[20].

Life cycle

The life of *T.suis* cycle is direct. The infective L_1 stage larvae within the egg develop in one or two months of being passed in the faeces. Under warm conditions development to the L_1 stage larvae will take about three weeks[21]. The pig becomes infected by ingestion of eggs with these L_1 stage larvae, the plugs of the eggs are digested in the small intestine, and cecum, after this the first stage larvae penetrate the crypts (the tubular invaginations of the epithelium around the villi) of the distal ileum, cecum and colon. The first moult starts in the submucosa of these crypts. The second stage larvae leaving the anterior end buried in the mucosa undergo three more molts. They are sexually mature approximately 6 weeks after initial infection. Longevity is around 4 to 5 months. The prepatend period is about 6-8 weeks.

Clinical signs

Fatteners around 2-4 months are most susceptible to infection, because they don't have a immunity against *T.suis.* They show the highest prevalence[7]. Few clinical signs are seen in affected pigs[12]. The parasite induces a strong protective immunity that keeps the prevalence of *T.suis* low in sows and boars. Heavily affected pigs could show swine dysentery. In heavy (experimental) infections grow rate may decrease, anemia, dehydration, emaciation and even death may be seen. The persistence of the adult worms and their inflammation of the mucosa of the cecum could be a predisposition to secondary bacterial enteritis [21], such as *Treponema hyodysenteriae* leading to watery bloody diarrhoea.

Zoonotic risk

Trichuris trichiura is the whipworm of man and simian primates, which infected 795 million people worldwide[16]. Adult worms, larvae and eggs from *Trichuris suis* and *Trichuris trichiura* are hard or impossible to distinguish morphologically. It is generally suggested that their taxonomic status is settled but Cutillas et al.(2011) and Nissen (2011) suggest that *Trichiuris suis* may be a zoonosis[14]. *T.suis* when established only short term in humans, is experimentally used in down regulating autoimmune th1 diseases like Crohn's disease in man[14]. Concluding, it is still unclear if *Trichuris suis* is a zoonosis.

2.1.3 OESOPHAGOSTOMUM *SPP.*

Oesophagostomum spp. also known as the nodular worm are a complex of four species *O. dentatum*, *O. brevicaudum*, *O. georgianum*, and *O. quadrispinulatum*. *O. dentatum* is the most common of these species[3]. The adult worms are white and male are 8 to 16mm long and females are 10 to 21 mm long[7]. Adult female worms lay thin shelled, Strongylie-type eggs in the murula stage. The larvae of *O. dentatum* are susceptible for hot, dry and freezing conditions and desiccation. The infective larvae could survive up to 8 to 10 months on the ground if there is a absent of persistent freezing[9].

Life cycle

Oesophagostomum spp. have a direct life cycle. Third stage larvae protected by a sheath are ingested by pigs. In the stomach the sheaths disappear and the third stage larvae enter the colon or caecum where they penetrate the mucosa[9]. After 6 to 7 days the larvae molt to the fourth stage in the submucosa. In the mucosa they create a nodule, where the fourths stage larvae can stay arrested up to 2 months. Larvae return to the lumen of the large intestine, molt and become to sexually mature adults in another 17-21 days. The adult female worms lay eggs which are shedded out in the feces. The morula stage eggs develop after 24-40 hours to eggs with first stage larvae. The first stage larvae molt after 24 hours, the second stage larvae grow and feed for about three days, and third stage larvae will be produced. The prepatent period is 6-7 weeks[7].

Clinical signs

Highest infection rates have been seen among the sows and in fatteners. The clinical sign is soft stools or diarrhea[12]. Usually there are few clinical signs in affected pigs. High levels of worms up to 5000 could usually have subclinical effects like poor milk yields, reduced litter size, reduction in weight, the so called ‘thin sow syndrome’[9]. In fatteners, it may result in growth depression and a raise of feed conversion by 15 %[7].

Zoonotic risk

Oesophagostomum spp. do not pose a zoonotic risk.

2.1.4 HYOSTRONGYLUS RUBIDUS

Hyostrongylus rubidus also known as the stomach worm is slender and reddish when fresh. The adult males are about 5-7 mm and females 6-10 mm in length [7]. The typical strongylie-type eggs are thin shelled. The larvae of *Hyostrongylus rubidus* are susceptible for hot, dry and freezing conditions. The infective larvae could survive up to 8 to 10 months on the ground if there is no persistent freezing[9.]

Life cycle

Hyostrongylus rubidus has a direct life cycle. The prepatent period is around 15-22 days [23]. The third stage larvae are ingested by pigs; they enter the pits of the gastric glands where they stay for about 13 to 14 days. After this they have two molts into developing young adults. Some larvae will return to the lumen of the stomach, other fourth stage larvae go into hypobiosis, they remain in the glands as fourth stage larvae. They provide a reservoir of replacement worms that help to maintain a stable adult population[9]. These hypobiotic worms are often seen in older animals, for example during the periparturient period while the immunity of the sow is decreased and a periparturient egg rise occurs [9]. The adult female worms in the stomach lay eggs which are driven out in the faeces. The eggs with morula stage develop after 24-40 hours to first stage larvae. The first stage larvae molt after 24 hours, the second stage larvae grow and feed for about three days, and third stage larvae will be produced[7].

Clinical signs

Highest infection rates have been seen in (lactating) sows where loss of body condition is noticed. This weight loss continues after the litter is weaned, despite adequate feeding. The sow is thin, has anaemia and in chronic cases a lost of appetite and they become dull and lethargic. Heavy infections in fatteners can lead to a strong declined grow rate [12].

Zoonotic risk

Hyostrongylus rubidus do not pose a zoonotic risk.

2.1.5 STRONGYLOIDES RANSOMI

Strongyloides ransomi also known as the threadworm are slender, hair like worms. The parasitic females are 3.3 to 4.5 mm long, and 45 to 62 um wide and found in the small intestine[7]. The eggs are thin shelled (26-35 µm), ellipsoidal and contain a larva[7].

Life cycle

Strongyloides rasomi has a direct life cycle. The prepatent period is about 4-7 days[23]. The infective third stage enters through the oral way or skin. Percutaneously they could travel via the circulatory system to the lungs, and then up to the trachea in the oral cavity, or they migrate through the subcutaneous tissue until they reach the oral cavity [9]. In the oral cavity they are swallowed and enter the intestine. A part of the third stage larvae migrate to the mammary glands of the sow, where they arrest in the adipose tissue[9]. The third stage infective larvae are passed through the colostrum into the suckling piglets, where they directly go to the small intestine of the piglet. The suckling piglets could show clinical signs within 4 days of birth[9]. In the intestine of the adult pig the third stage larvae molt twice, and become parthenogenetic females in 7-9 days. These parthenogenetic females lay eggs which are expelled in the faeces. The eggs embryonate and hatch to first stage larvae. These first stage larvae could either develop into third stage infective larvae or to sexually mature free living male and female worms. These free living adults produce eggs that produce infective third stage larvae.

Clinical signs

The clinical importance of threadworms is primarily in suckling piglets, but they can be found in all age classes of swine [9]. It may appear as an acute dysentery, the diarrhoea containing blood. In neonatal piglets, 75 per cent could die because of an infection. Also anorexia, anaemia and listlessness may be seen [12.].They induce a strong immunity, after recovery from infection the pigs show a high immunity to re-infection [9].

Zoonotic risk

Strongyloides ransomi do not pose a zoonotic risk.

2.1.6 *METASTRONGYLUS SPP.*

Metastrongylus spp. also known as the lungworm, of which is *M. elongatus* is the most common[9]. The adult worms are white and males are up to 25-mm and females up to 58 mm. They live in the bronchi or bronchioles of the posterior part of the diaphragmatic lobes of the lungs [12]. The larvated eggs with a rough thick shell are capable of surviving in the soil and in earthworms for a long time; the eggs can survive over 1 year in soil[7]. The longevity of the third stage larvae in the earthworm may be up to 4 years[9.]

Life cycle

The life cycle of the lungworm is indirect, it requires an earthworm intermediate host[9]. Prepatent period is about 24 days [7]. The pig ingests earthworms containing the third stage larvae. After digestion of the earthworm the third stage larvae penetrate the gut wall and travel to the mesenteric lymp nodes where they molt to the fourth stage. After this they reach the right heart and lungs by the lymphaticvasculair route, where their final molt into young adults occurs. The eggs laid by the female are coughed up and swallowed by the pig and excreted in the faeces. The eggs are ingested by an earthworm where they hatch and develop to the third stage larvae in about 10 days [7].

Clinical signs

Lungworm causes few clinical signs in pigs, except in heavy infections. A infection may be the cause of coughing, depression of growth and emphesemia and pneumonia [12]. Lungworm could also predispose to other respiratory infections such as influenza, *Actinobacillus pleuropneumonia* or *Mycoplasma*[9] .

Zoonotic risk

Metastrongylus spp. do not pose a zoonotic risk.

3 MATERIALS EN METHODS

3.1.1 FARMS

Eight organic farms participated in this study. Nineteen farms with Spotted Bentheimers participated in the study. Two types of Spotted Bentheimer farms were classified. The Spotted Bentheimers, kept on a small scale as a business unit of agricultural farms that focus on care, education or processing of regional quality products, were classified as hobby farms (HF). Sixteen hobby farms participated in the study. The Spotted Bentheimers kept in several hectares of forest with the purpose of nature conservation were classified as nature conservation farms (NC). Three nature conservation farms participated in the study. The organic farms did not have Spotted Bentheimer pigs, but Yorkshire, Duroc or Pietrain pigs. These organic farms kept 100 or more pigs, with a commercial purpose. They are regulated by EU regulation (EEC) 2091/92. They were all SKAL certified. The addresses for these organic farms where given by Platform Biologica, the organization for organic farmers in the Netherlands. The addresses for the Spotted Bentheimer farms where given by the Association of the Spotted Bentheimer. Because of the relatively short duration of this study, the farmers of the three groups were not asked to withhold treatment with anthelmintics or to change their management.

3.1.2 GROUPS OF PIGS ON THE FARM

Three age groups of pigs which were present on the hobby farms, nature conservation and organic farms were sampled were sampled once: Suckling piglets of less then 6 weeks old, weaned pigs or fattening pigs of 6-52 weeks old and sows (mainly pregnant sows). It was not possible to sample all age groups on every farm, because some farms had only sows or only fattening pigs.

3.1.3 ''SAMPLING''

A few organic farms were visited once. One nature conservation farm and a few hobby farms were actually visited. The other farmers were send a packet to collect the faeces on a proper way and they were asked to send the faeces to the Faculty of Utrecht.
At the organic farms 10 faecal samples per age group were collected. At the Spotted Bentheimer farms 2-4 faecal samples per age group were collected, if there were only five pigs or less in an age group faecal samples were collected from all the pigs.
The faeces were collected from freshly deposited stools. Transport of faeces to the Faculty of Utrecht took place by car or by mail, where they were stored in a refrigerator at 7 degrees until examination within one week took place.

3.2 Laboratory techniques

3.2.1 Faecal analyses

In the laboratory a pooled sample of a individual sample was prepared by mixing the faeces well of each age group. For the Centrifuge Sedimentation Flotation (SCF) technique two gram of each pooled sample was used. The faeces was mixed with water in the mortar and after the faecal suspension was poured through a tea strainer and poured into a test tube. It was centrifuged at 3000 R.P.M. for 1 minute. Subsequently the supernatant was discarded and sugar solution (spec. gravity of 1.23mg/ml-1.28 mg/ml) was added to the sediment until a positive fluid meniscus was created. A coverslip was placed on the test tube and the test tube was placed in the centrifuge at 3000 R.P.M. for two minutes. The coverslip was removed and placed on a microslide for microscopic examination. The sample was examined at a 10 x 10 magnification. If the CSF technique was positive for helminth eggs, faecal egg counts were determined by the concentration Mc Master technique with a sensitivity of 50 eggs per gram of faeces.

3.2.2 Larval cultures

Samples positive for strongylie-type type eggs were differentiated using larval cultivation, about 15 gram of faeces was held for 10 days at room temperature (20-25 degrees), subsequently feces was added, the jar with suspended faeces were turned on a Petri dish with a bit of water for 1 day, hereafter the larvae were harvested and if possible 100 infective larvae from the culture were identified as *Hyostrongylus rubidus* or *Oesophagostomum spp with* a light microscope at 10 x 10 magnification, using the descriptions by the FAO/RVC guide to veterinary parasitology[22].

3.3 Questionnaire

A questionnaire was used to gather information about housing, management, number of animals, purpose of the pigs, contact with other animals including wild boars and use of anthelmintics. Through a survey we tried to give insight in the population of the Spotted Bentheimer and the way of housing of the Spotted Bentheimers. Based on the answers from the survey links could be made with the results of the faeces samples.
The Spotted Bentheimer farmers and the organic farmers were asked to fill in a different questionnaire because the management of these farms could be very different.
Of the 42 Spotted Bentheimer farms who were contacted, 45 per cent (19 farms) agreed to participate in the survey. They filled in the questionnaire on the internet (and sent the faeces by

mail to the University of Utrecht). Of the 20 organic farms that were contacted 8 organic farms agreed to participated in the survey. A shortened version of the survey is found at table 1.

Organic and Spotted Bentheimer questionnaire

(the question marked in green were only asked to Spotted Bentheimer farmers)

- Name
- Address and telephone number
- Type of farm (nature conservation, hobby farming)
- Number of animals present on the farm
    - Sows
    - Weaners/fattening pigs
    - Suckling pigs
- Covered area : (organic farms were asked to fill this for each age category)
        - Size
        - Floor material
        - Removing of faeces
        - Cleaning/disinfection
            - Active substance
    - Outside area:
        - Size
        - Floor material
        - Removing of faeces
        - Cleaning/disinfection
            - Active substance
    - Use of anthelmintics:
        - Schedule
        - Product
            - Active substance
        - Last use of anthelmintics (date)
- Environment:
    - Contact with other species
    - Contact with wild hogs

Table 1: Survey for Organic and Spotted Bentheimer farms

3.4 Statistics

Statistic analyses of the data were done with the *Fisher's exact test*. All results of the fecal samples and the survey were studied for possible trends.

4 RESULTS

4.1 PREVALENCE ON FARMS

Eggs of *Ascaris suum*, strongyle-type eggs and *Trichuris suis* were found. No infections with *Salmonella spp*. were detected.
Table 1 shows how many farms were examined and the prevalence of *A.suum*, strongyle-type eggs and *Trichuris suis* positive samples. *A.suum* was found in hobby and organic farms, not in nature conservation farms. *Trichuris suis* was only found in hobby farms, while strongylie-type eggs were found in all farm types.

Type of farm	No. of farm Investigated	*Ascaris suum* positive (%)	Strongyle-type positive (%)	*Trichuris suis* positive (%)
Organic	8	2 (25)	1 (13)^{a}	0 (0)
Nature conservation	3	0 (0)	1 (33)^{b}	0 (0)
Hobby	19	2 (11)	9 (47)^{b}	5 (26)

Table 1: prevalence of A.suum, strongyle-type and Trichuris suis positive samples on organic, nature conservation and hobby farms.
Different letter(a,b) in the same column indicates a significant difference. (p<0.05)

Organic farms had a significantly lower prevalence of strongylid type compared to the hobby farms and nature conservation farms (p<0.05), while nature conservation farms do not significantly differ in prevalence of strongylid type compare to hobby farms. No statistic difference between the farm types was found for Trichuris suis (p>0.05). No statistical difference between the farm types was found for *Ascaris suum* (p>0.05).

4.2 PREVALENCE ON AGE GROUPS

It was not possible to sample all the three age groups all of the farms; therefore the number of age groups differ per farm. The results of the fecal analysis in the different age groups are shown in table 2.

Pig group	Type of farm	No of groups investigated	*Ascaris suum* No. Positive (%)	*Strongyle-type* No. Positive (%)	*Trichuris suis* No. Positive (%)
Suckling pigs	Organic	7	0	0	0
(<6 wks)	Nature conservation	1	0	1 (100)	0
	Hobby	4	0	0	1 (25)
Weaners/fatteners	Organic	8	0	0[a]	0
(6 wks - 52 wks)	Nature conservation	2	0	1 (50)[b]	0
	Hobby	8	2 (25)	5 (63)[b]	4 (50)
Sows	Organic	7	2 (29)	1 (14)	0
(>52 wks)	Nature conservation	3	0	1 (33)	0
	Hobby	18	0	7 (39)	0

Table 2: prevalence of positive samples in suckling pigs, weaners/fatteners and sows on organic, nature conservation, petting zoos and hobby pig farms.
Different letter(a,b) in the same column indicates a significant difference. ($p<0.05$)

Weaners/fatteners on organic farms have a significantly lower prevalence of Strongylid type eggs compared to weaners on hobby farms and nature conservation farms. There are no significant differences for *A.suum* between age groups of the different farms. There are no significant differences for *T.suis* between age groups of the different farms.

4.2.1 *ASCARIS SUUM*

This species was absent in the suckling piglets. In the weaners/fatteners two samples scored positive on the hobby farms and the sows in organic farms also scored two positive samples. The lowest egg count during the study was <50 epg in sows of an organic farm, the highest egg count was 2200 epg in weaners/fatteners on a Spotted Bentheimer hobby farm.

4.2.2 STRONGYLE-TYPE EGGS

In suckling piglets on a nature conservation farm only one sample scored positive. There is a high prevalence in weaners/fatteners on hobby farms and nature conservation farms, respectively 63 % and 50 %. There is a high prevalence of 39 % in sows on a hobby farm. Faecal egg counts differ in age groups and farm type. The highest egg count was 3700 epg in weaners/fatteners of a hobby farm; the highest prevalence in sows on a nature conservation farm was 550 epg.

4.2.3 TRICHURIS SUIS

This species was absent in the sows. In the suckling piglet only one sample on the hobby farms scored positive. In the weaners/fatteners four samples scored positive on the hobby farms, this resulted in a 50 % prevalence. The lowest egg count was <50 epg in suckling piglets and weaners/fatteners on a hobby farm, and the highest egg count was 250 epg in weaners/fatteners on a hobby farm.

A visual presentation of table 2 can be found at Figure 3-6.

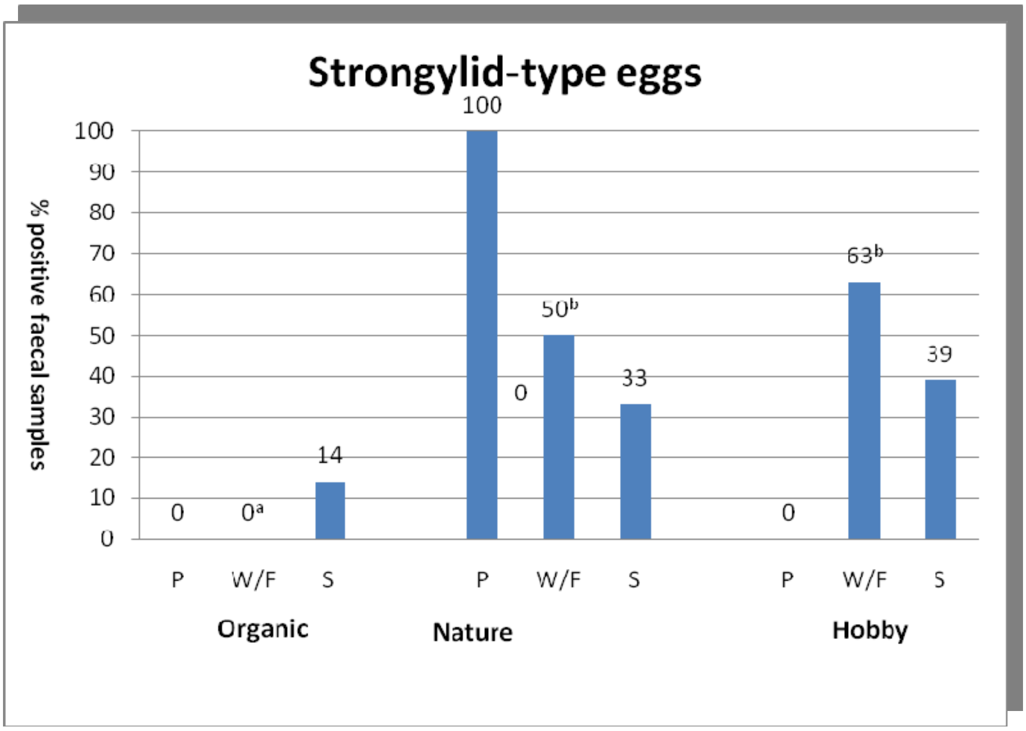

Fig. 3 Prevalence of strongylid type eggs.(% positive animals) on the three farm types Signature on x-axis: P= piglets, W/F= weaners/fatteners, S= sows.

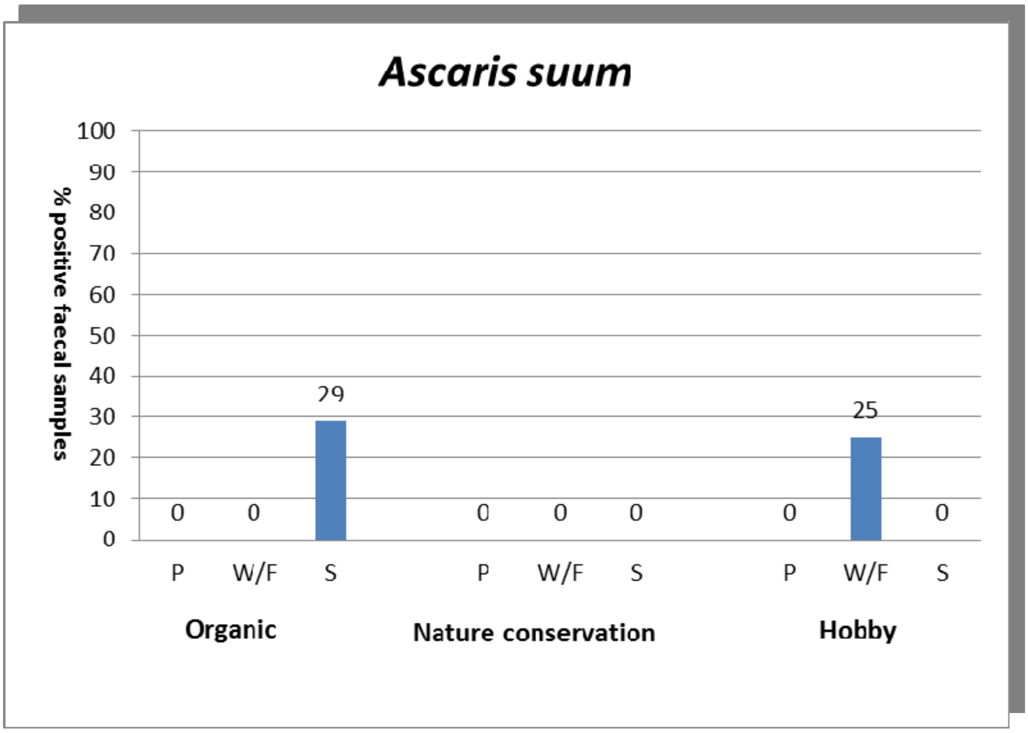

Fig.4 : Prevalence of A.suum eggs. (% positive animals) on the three farm types

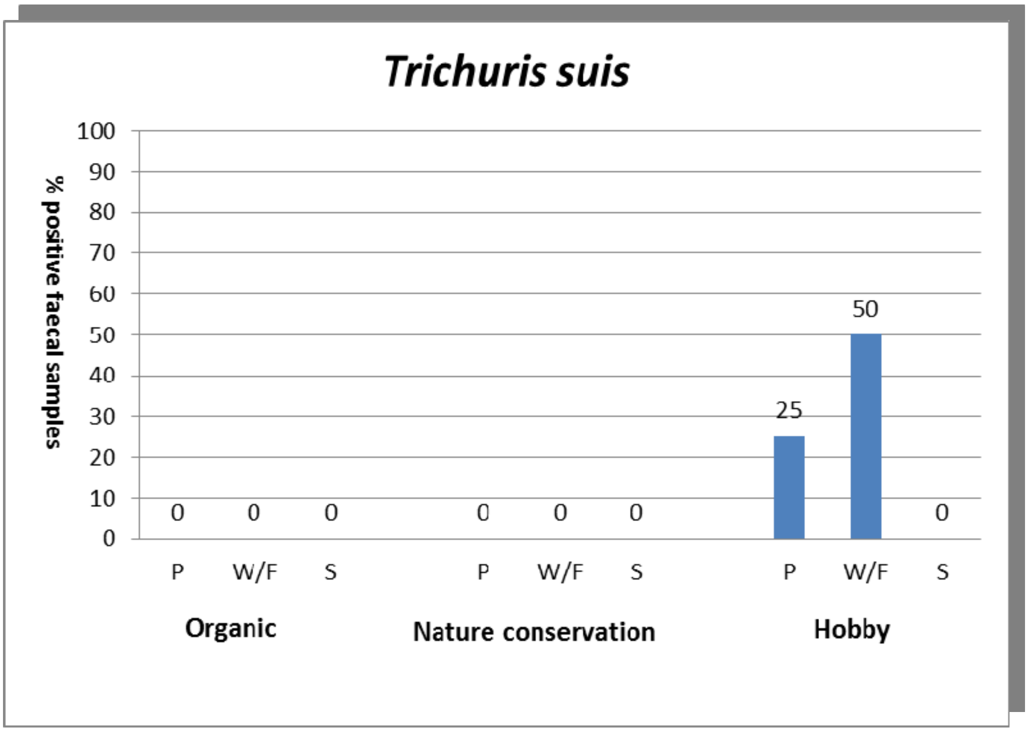

Fig. 5 : Prevalence T.suis type eggs. (% positive animals) on the three farm types

5 QUESTIONNAIRE

The limited number of Spotted Bentheimer farms allows us only to look for trends.

5.1 HOUSING

The Spotted Bentheimer are kept in great diversity, for example the living space varied from 15 m2 to 5000 m2 per pig. The average total living space is 435 m2/pig.
Table 3 shows the relation between the prevalence in helminth eggs and the total living space of one pig on a farm.

Area per pig (m2)	No. of farms investigated	No. pos. helminth eggs(%)	No. Strongylid type pos.(%)	No. A. suum pos.(%)	No. *T. suis* pos.%
<10	8	3 (38)	1(13)	2(25)	0(0)
10-50	6	3 (50)	2(33)	0(0)	1(17)
50-100	5	2(40)	2(40)	1(20)	1(20)
>100	7	5(71)	4(57)	1(14)	3(43)

Table 3: the relation between the results of the faecal analyses and the total living space of one pig on a hobby, nature conservation and organic farm.

On the 8 organic farms pigs have a total living space less than 10 m2. Of these 8 farms, 38 % was positive for helminth eggs, 13 % was positive for strongylus type eggs, 25 % for *A.suum. T.suis* was absent.
On 6 Spotted Bentheimer farms swine have a total living space between 10- 50 m2 (inside and outside). Of these 6 farms 50 % were positive for helminth eggs, 33 % were positive for strongylid type eggs, 17 % of *Trichuris suis* and 0 % of *A.suum*. On 5 Spotted Bentheimer farms the pigs have a total living space between 50-100 m2 (inside and outside). Of these 5 farms 40 % was positive for helminth eggs, 40 % were positive for strongylid type, 20 % for *Trichuris suis* and 20 % for *A.suum*.On 7 Spotted Bentheimer farms swine have a total living space of more than 100 m2 (inside and outside). Of these 7 farms 71 % were positive for helminth eggs, , 57% were positive for strongylid type eggs, 43 % for *Trichuris suis* , en 14 % for *A.suum*.
On the organic farms the pregnant sows had entrance to pasture. The suckling piglets and weaners/fatteners did have entrance to a concrete floor and straw but not to pasture. On the Spotted Bentheimer farms most were kept in great diversity, usually they had entrance to pasture or forest.

Trends
It could be concluded that more space per pig results in less negative samples, the more space per pig results in more positive samples of strongylid type eggs and the more space per pig results in more positive samples of *Trichuris suis*.

5.2 Number of swine

Table 4 shows the relation between prevalence in helminth eggs and the number of pigs on a farm.

Pigs present on a farm	No. of farms investigated	No. pos. helminth eggs(%)	No. Strongylid type pos.(%)	No. A. suum pos.*(%)*	No. *T. suis* pos.%
2	9	2 (22)	2(22)	1(11)	1(11)
3-9	6	3 (50)	4(67)	1(17)	1(17)
10-99	4	2(50)	2(50)	0(0)	3(75)[a]
>100	8	3(37)	1(13)	2(25)	2(25)[b]

Table 4: the relation between the results of the faecal analyses and the number of a pig on a hobby, nature conservation and organic farm.

There were 9 farms only kept two pigs on a farm. Of these nine farms 22 % was positive for helminth eggs, 22 % was positive for strongylid type eggs, 11 % of *Trichuris suis* eggs and 11 % of *Ascaris suum.*
There were 6 farms which kept three to nine pigs, of these farms, of these 6 farms 50 % was positive for helminth eggs, 67 % was positive for strongylid type eggs, 17 % of *Trichuris suis* and 17 % *Ascaris suum*.
For farmers kept between 10-99 pigs on their farms, of these farms 50 % was positive for helminth eggs, 50 % was positive for strongylid type eggs, 75 % *Trichuris suis* and negative for *Ascaris suum*.
The 8 organic farms kept more than 100 pigs on a farm. On these organic farms 37 % was positive for helminth eggs, 13 % for strongylus type, 25 % for *Ascaris suum* and 25 % was positive for *Trichuris suis.*

Trends
Farms with 10-99 pigs have a significantly higher prevalence of *T.suis* than farms with more than 100 pigs.
Farms with two pigs, most samples were found negative (78%).

5.3 CONTACT WITH WILD BOARS

Wild boars lived less than 1 km from Two Spotted Bentheimer farms. One farm was negative for helminth eggs and the other farm was positive for strongylid type eggs and *Trichuris suis*. No trend was found in these results. Wild boars lived less than 1 km from one of the eight organic farms, this farm was found negative for helminth eggs.

5.4 USE OF ANTHELMINTICS

Figure 6 shows a overview of the use of anthelmintics on Spotted Benheimer farms.

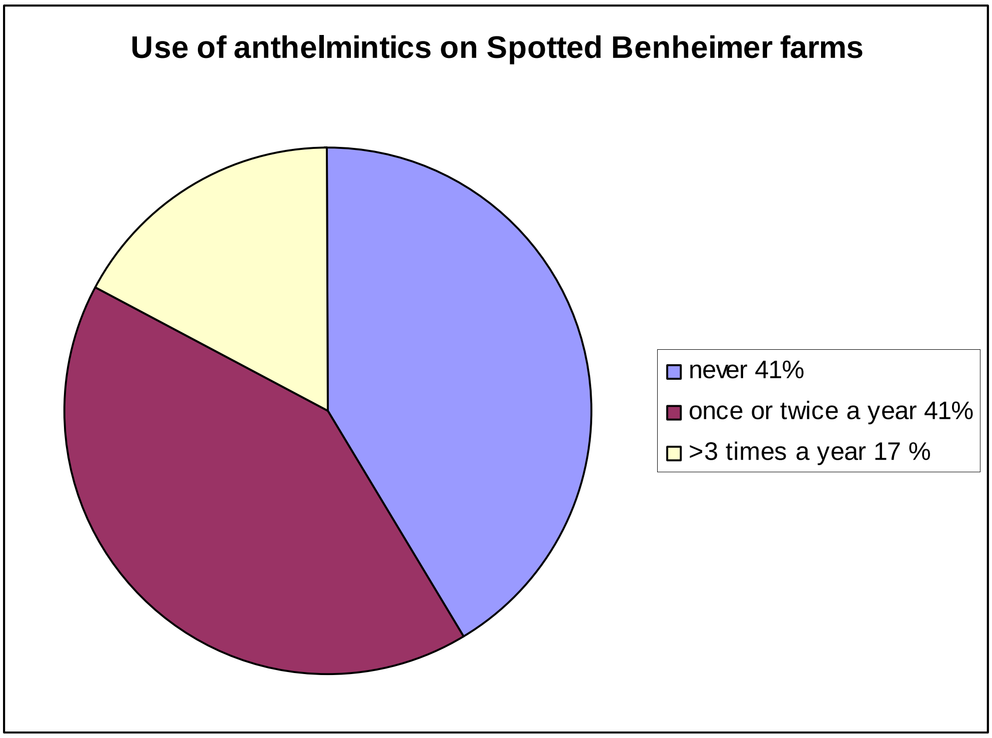

Figure 6: use of anthelmintics on Spotted Bentheimer farms

On 7 of the 17 (41 %) farms antiparasitic products were never used. Of these farms 29 % was negative for helminth eggs, of the remaining 71 %, 57 % was positive for strongylid type eggs, 29 % for *Trichuris suis* and 14 % for *Ascaris suum*. On 7 farms once or twice a year antihelmintics were used. Of these farms 29 % was negative, the remaining 71 %, 57 % was positive for strongylid eggs, 43 % for *Trichuris suis* and 14 % for *Ascaris suum*.

On 5 of the 8 organic farms 3-4 times a year anthelmintics for the sows were used, 2 farms used twice a year anthelmintics for the sows. Of these two farms one farm was negative for helminths and one farm was positive for *A.suum* with a low epg of 200. One organic farm did not have sows. On 7 of the organic farms every 4 weeks up to 55 kg anthelmintics were used for the weaners/fatteners. The 8 organic farms never used anthelmintics for the suckling piglets.

Trends Spotted Bentheimer

Three farms used three times a year or more anthelmintics. These were all negative for helminth eggs. There is no difference in the results if the farmers never use antihelmintics or once or twice a year.

6 CONCLUSION

In this study the occurrence of gastrointestinal parasitic helminths, the prevalence of these parasites in the population of the Spotted Bentheimer and differences between age groups (suckling pigs, weaners/fattening pigs and sows) have been subject of this research. Organic farms were used as comparison in the fecal samples.

Eggs of three helminth species were found, strongylid type eggs, *Trichuris suis* and *Ascaris suum*. It showed that Spotted Bentheimer farms have significantly higher infections with strongylid type eggs than organic farms. Weaners/fatteners on organic farms have a significantly lower prevalence of strongylid type eggs compared to weaners/fatteners on hobby farms and nature conservation farms. The prevalence for *Trichuris suis* en *Ascaris suum* was low. The faecal egg counts found were generally low and no clinical cases were known.

7 DISCUSSION

The results showed that three helminth species were found in this study, strongyle-type, *Trichuris suis* and *Ascaris suum*. *Strongyloides ransom*i and *Metastrongylus* spp. were absent. Absent of *Strongyloides ransom*i and *Metastrongylus* spp. in organic farms was also noted by *Eijck* and colleagues (2005*), and also by Carstensen and collegues* in Denmark*. Hyostrongylus rubidus* and *Metastrongylus* spp*. were also absent in Roepstorff and collegues (1998)* in Denmark.

7.1 BETWEEN FARMS

The organic farms had a significantly lower prevalence of strongylid type infections compared to Spotted Bentheimer hobby farms and nature conservation farms. *Trichuris suis* infections were significantly more prevalent on hobby farms compared to organic farms, while no statistic difference between organic and nature conservation farms was found.
This could be explained by the differences in management, housing, use of anthelmintics between organic and Spotted Bentheimer farms (questionnaire) and lack of uniformity in the selected criteria. Suckling piglets and weaners/ fatteners on organic farms have no entry to pasture; they are kept on a concrete floor with straw. It is possible to clean and disinfect, a

concrete floor, but not pasture or forest. The risk of infection in organic farms could be lower due to cleaning the pen (indoor and outdoor). So if it is not possible to clean the living area of the Spotted Bentheimer it increases the risk of infection and the prevalence of parasitic helminths.

7.2 Between age groups

7.2.1 Trichuris suis

T.suis infections were rare, also reported by Joachim et al.[4] and it is well in accordance with the induction of a strong immunity against this parasite[21]. It is remarkable that one sample in the suckling piglets on a hobby farm scored positive for *T.suis,* because of the prepatent period of 6-8 weeks. The sows on this hobby farm scored positive for *T.suis* so this could be due to contamination of the sample by the sows. This sample could also be positive because feces of other animals that passed by, for example foxes or dogs, that lied in the living area of the pigs.

7.2.2 Strongyle-type eggs

Strongyle-type infections were seen the most. Infections showed an increasing infection level with age, this is commonly seen[23]. One sample in the suckling piglets on a nature conservation farm scored positive. However there was only one sample collected in this group, so therefore it gave a 100 % positive percentage. The sows on this hobby farm also scored positive for strongyle-type with 550 epg so the positive result for suckling piglets could be due to contamination during sampling by the sows.
It was not possible to differentiate 100 larvae for 50 per cent (6) of the positive samples for strongylid type egg. Sometimes no larvae were found at all, even the epg of that sample was quite high (450 epg). So it was not possible to claim with certainty which strongylid type eggs were found. The other fifty percent was found to be *Oesophagostomum* spp., other studies in the Netherlands and Denmark also found *Oesophagostomum* spp., not *Hyostrongylus rubidus*. Probably *Oesophagostomum* spp. were found in this study.

8.2.3 Ascaris suum

A.suum infections were rare, in contrast with other studies [3] [1], but it may be well in accordance with the induction of a strong immunity against this parasite. *A.suum* infections showed the same pattern (weaners/fatteners) in age distribution as is commonly seen[23]. Except for one positive sample in sows on an organic farm, this could be a coincidence. This could be in accordance with a low percentage of adult excretes.

7.3 Population

Despite approaching all the members of the Spotted Bentheimer association to participate, only half of them took part in the study, of which a small part were nature conservation farms. This resulted in a small number of samples per farms and even more per age group, so it was difficult to draw significant conclusions from the results In certain situations this gave a distorted picture, like the one positive sample for strongylid type eggs in the suckling piglets on a nature conservation farm. This immediately caused a 100 % score for this group because there was only one sample in this group. There was also a great diversity in the population of the Spotted Bentheimer, the age groups were not equal in number so there were much more sows than suckling piglets. On the organic farms all age groups were present, except for one farm. Most of the conclusions of the faecal samples and survey are not significant but trends.

7.3.1 Management

The Spotted Bentheimer farms have big differences in management. It is proven that prevalence and faecal egg counts of infection are influenced by management factors such as hygiene and housing systems [23 24]. Hygiene measures can reduce the infection rate[4] . For example the use of separate clean and non-clean zones, the use of different clothes and boots before entering the pig's living area, the way and frequency of cleaning the pig houses and the use of pasture rotation. Despite the questionnaire it was hard to distinguish different groups with different management, big differences in management systems existed between the Spotted Bentheimer farms even within the same farm type.
The farmers were not asked to withhold treatment with anthelmintics, so it happened that we received a sample of which the pigs were treated with anthelminitics one week ago or one year ago. The use of anthelmintics was not standardized.

7.4 Study design

The design was chosen to collect a single faeces sample off an age group per farm in the period of August and September; this was a pooled sample per age group on the farm.

Possible seasonal effects have not been considered. The development of *A. suum* eggs is highest during the summer period, which leads to high prevalences in young pigs in autumn [25]. But other articles claim that there were no seasonal differences[3], and the immunity by older pigs overrule this seasonality[23].
The obtained percentage of positive swine may be overestimated due to the fact that uninfected pigs may have low egg counts per gram faeces (epg) due to coprophagia of truly infected pigs' egg-containing faeces[26]. This is most likely to occur for helminths of which the eggs do not hatch in the external environment, i.e. *A.suum* and *T.suis* of the present study.
We avoided this problem of false positive egg counts by pooling of samples per age group.
A pooled sample per age group was collected, therefore it may occur that a sample with pigs did not give a representative image of a farm. Ideally, every pig on a farm should be studied for

helminth eggs.
Not all the farms were visited by the students, so a lot of farmers sent the faeces by mail. Sometimes this took about three to five days to reach the University of Utrecht. The sample was not cooled during this time, so strongyle-type eggs could develop into larvae. It is also unknown if the farmer sent fresh faeces or faeces from the ground. These larvae could have been missed during the examination of the helminth eggs.
At the start of the study the researchers were not experienced in performing laboratory techniques. This could resulted in a few false positive and false negative outcomes.

8 RECOMMENDATIONS

Despite the low number of positive samples of helminths eggs, there were two positive samples for Ascaris suum. Because of the zoonotic potential of this parasite, we recommend the Spotted Bentheimer farms to monitor their pigs regularly for excretion of helminth eggs. Furthermore we recommend a follow-up study if the population of the Spotted Bentheimer, especially when the nature conservation farms has increased.

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