Difference in prevalence of gastrointestinal protozoa in pigs kept in a forest, on hobby farms and on organic farms

R.J. Leeuwis, BSc

Faculty of Veterinary Science, Utrecht University, Utrecht, The Netherlands

July – October 2011

Difference in prevalence of gastronintestinal protozoa in pigs kept in a forest, on hobby farms and on organic farms

R.J. Leeuwis

Faculty of Veterinary Science, Utrecht University, Utrecht, The Netherlands.

TABLE OF CONTENTS

ABSTRACT

The prevalence of gastrointestinal protozoa was studied in suckling piglets, fatteners and sows coming from 8 organic farms, 19 hobby farms and 3 forest/nature farms. On the hobby farms and the forest/nature farms the pigs were of the Bentheim Black Pied breed. The prevalence was determined using faecal microscopic examination of pooled samples. Infections with *Balantidium coli, Giardia duodenalis* and *Cryptosporidium* spp were not found. Infections with coccidia were found on 75% of the organic farms, on 33% of the forest/nature farms and on 53% of the hobby farms. In the fatteners, the highest prevalence was on the hobby farms (75%). In the sows, the highest prevalence was found on the organic farms (86%). The number of examined suckling piglets was very low, so percentages from 43% up to 100% (1 sample) were found.

The farms which had 2 pigs present (Bentheim Black Pied) had a significantly lower prevalence of coccidial infections, compared to the farms with 3-9 animals and farms with >100 animals present. The farms with over 100 pigs present were the organic farms.

No clinical signs were reported.

INTRODUCTION

The animal husbandry industry is changing rapidly in the last years. In response to the creation of large farms with over a thousand animals, there is an increasing interest in small scale farming. The emphasis of these small farms is on the welfare of the animals and the more 'natural' way of farming. This small-scale farming is also found in the pig farming industry. An example of this can be found at the Bentheim Black Pied pigs. The Bentheim Black Pied is a breed of pigs which originated at the Dutch-German Border (a region called *Bad Bentheim*), this breed was used frequently from the 1900's to 1950. After this period the breed almost became extinct due to the unfavorable fat-protein rate of the meat. The Dutch organization for the Bentheim Black Pied pig tried to reintegrate this breed in the Dutch animal husbandry.

The Bentheim Black Pied pig is sometimes kept in forests; the pig roots and thus creates more biodiversity. Young trees have more opportunities due to the rooting of natural compost[1]. The pigs also cause a wider spreading of seeds and plants.

Keeping pigs to shape the forests also has disadvantages, such as competing with birds and other forest animals for food and the damaging of the growth of certain types of plants in older, more mature forests[2].

The organization of the Bentheim Black Pied wants to promote the keeping of pigs in forests and other nature conservation areas and wanted to document the possible risks of keeping the pigs in these areas. The risks which are important to map beforehand are the risks for the environment, recreational areas (such as swimming areas), other animals, the pigs themselves and the possible zoönotic risks. A start of documenting these risks can be made by investigating

the parasite burden of the pigs and the possible zoönotic risks they carry. This article is aimed at the protozoa burden in the Bentheim Black Pied pigs. Organic farms were used as a comparison to estimate the risks of keeping pigs in nature conservation areas.
The protozoa examined in this study are coccidia (*Eimeria* spp. and *Isospora* spp.), *Cryptosporidium* spp., *Balantidium coli* and *Giardia duodenalis* , according to the literature, these are the most common gastrointestinal protozoa of pigs [3],[4].

BALANTIDIUM COLI

Balantidium is a protozoa which can be found in different species. Morphological similarities are found in rats, chimpanzees, orangutans and sometimes in dogs and cats. The type of *Balantidium* which can be found in pigs (*Balantidium suis*) might be similar to the type of *Balantidium* found in humans (*Balantidium coli*). This is not determined yet, further DNA analysis is needed. It is known that pigs are the main reservoir for Balantidiosis, although under laboratory circumstances humans are not easily infected with *Balantidium suis*. There is also the fact that *Balantidium suis* is smaller that *Balantidium coli*, although this can be accredited to different growth factors between ciliates.
In this study, we use the term *Balantidium coli*, for in most literature this is regarded as both *Balantidium coli* itself and *Balantidium suis*[5].

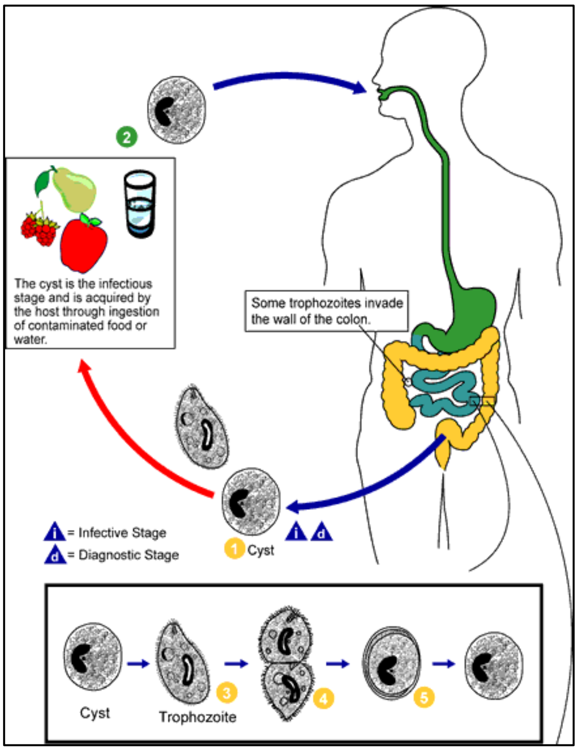

FIGURE 1. LIFE CYCLE OF *BALANTIDIUM COLI*. COURTESY OF CDC, USA

Balantidium coli is a protozoa with zoonotic potential which can affect humans via a fecal-oral infection route. In pigs, balantidiosis is mainly asymptomatic. The lifecycle of *Balantidium coli* is simple; trophozoites are found in the colon were they form cysts. These cysts are excreted in the environment where they can stay infective for up to two weeks[6] (see Figure 1). Infection occurs by ingesting trophozoits or cysts, for example the ingestion of water or food contaminated with pig faeces [8]. The cyst is able to survive the acidic pH in the stomach and the environment of the small intestine. In the colon excystation occurs, which produces a trophozoite[7].
Normally the parasite is not pathogenic in pigs and is symptomless. Sometimes (for unclear reasons) mucosal ulceration an dysentery occurs. This might be related to a secondary invasion of *Balantidium coli*.

Zoönotic risk

It is still unclear whether *Balantidium suis* is identical to *Balantidium coli*, so the zoönotic risk of *Balantidium suis* is also still unclear. Experiments have tried to prove the capability of

Balantidium derived from pigs to infect a human, but have as yet failed to do so. In another experiment, volunteers received a capsule which contained 250 trophozoites and 250 cysts derived from humans. These volunteers were followed for ten years, but no proof of infection was found.
Another experiment used a different approach: a human with proven Balantidiosis was used as a source of *Balantidium coli.* A homogenate was produced with high levels of cysts ($1.2x10^4$ to $4.8x10^4$). This homegenate was used to infect piglets. Of these piglets, 40% piglets developed severe diarrhea, 60% developed a moderate diarrhea.
There are proven cases of outbreaks of pig-derived balantidiosis in humans, most commonly in the Philippines, Papua New Guinea, West Irian and regions of Latin America. This fact clearly shows that is it possible for *Balantidium* to cross the species border from pigs to human, although the circumstances for this transmission have to be optimal. These circumstances are proximity to pigs and pig faeces, a persistent exposure, unhygienic circumstances (e.g. no appropriate waste disposal) and a hot and humid climate. Worldwide prevalence is estimated at 0.02% to 1%, but differs greatly per region. The regions named earlier have a higher prevalence. *Balantidium coli* does not always lead to clinical Balantidiosis. The host gets infected, but there may be no apparent clinical signs. Clinical Balantidiosis mostly occurs in humans who suffer from malnutrition, alcoholism or have a compromised immune system. Acute Balantidiosis expresses as explosive diarrhea and abdominal pain, leading to bloody stools and dehydration [6].

Balantidium coli is a opportunistic protozoa, which secondary can cause lesions in the intestine. The Dutch Health Organization says the prevalence of *Balantidium coli* is unknown, although it is acknowledged that pigs kept outdoors had a higher prevalence compared to pigs kept indoors [5].

GIARDIA DUODENALIS

Giardia duodenalis (*Giardia lamblia*) is a fairly common parasite of pigs. Cysts of *Giardia* were found in 3.8% of the suckling piglets, in 9.8% up to 15% of fattening pigs, in 5.7% of boars and in 4.1% of sows[8].
Giardia duodenalis is found in the duodenum, jejunum and ileum of weaned pigs. Most infected animals did not show clinical signs [8] .
Giardia has a simple cycle. Trophozoites live in the small intestine of the pigs and excretes both trophozoits and cysts (see Figure 2). The trophozoites do not survive in the environment. The cysts can survive for months in a watery environment. Ingestion of the cysts causes infection, this can happen by ingesting contaminated water, food or direct fecal-oral contact.
When the cysts arrives in the small intestine, excystation takes place and trophozoits are released, which multiply. When the trophozoites reach the colon, encystations takes place[9].

Zoönotic risk

Giardia duodenalis knows a variety of genotypes, all which have different hosts. These genotypes differ in the host-specificity but more different genotypes can be found within one species[10]. *Giardia* genotypes are allocated in different assemblages. The most important assemblages are assemblage AI, AII and assemblage B. All three of these assemblages are found commonly in the environment, humans, farm animals, cats and dogs and a few wild animal species. All three assemblages are possibly zoönotic. Yet, it is still unclear if animals act as a reservoir for humans or if humans act as a reservoir for animals[11].
Genotypes of assemblage AI can be found in humans, livestock, beavers, cats and dogs, genotypes of assemblage AII is found exclusively in humans. Genotypes of assemblage B can be found in humans, beavers, dogs and muskrats.
The most important route of infection is through fecal contamination of (recreational) water[12].
Person-to-person infection is of great importance within the human population. These infections occurs mostly in the homosexual population and in children who stay in day care centers.
Most infections in humans occur symptomless. If symptoms do occur, it is mostly abdominal distention, flatulence and diarrhea without blood or mucus. Other symptoms include anorexia, nausea, abdominal cramps and vomiting. These vague symptoms often cause a wrong diagnosis.
The most common symptom in children is not the abdominal problems but a retarded growth [13].

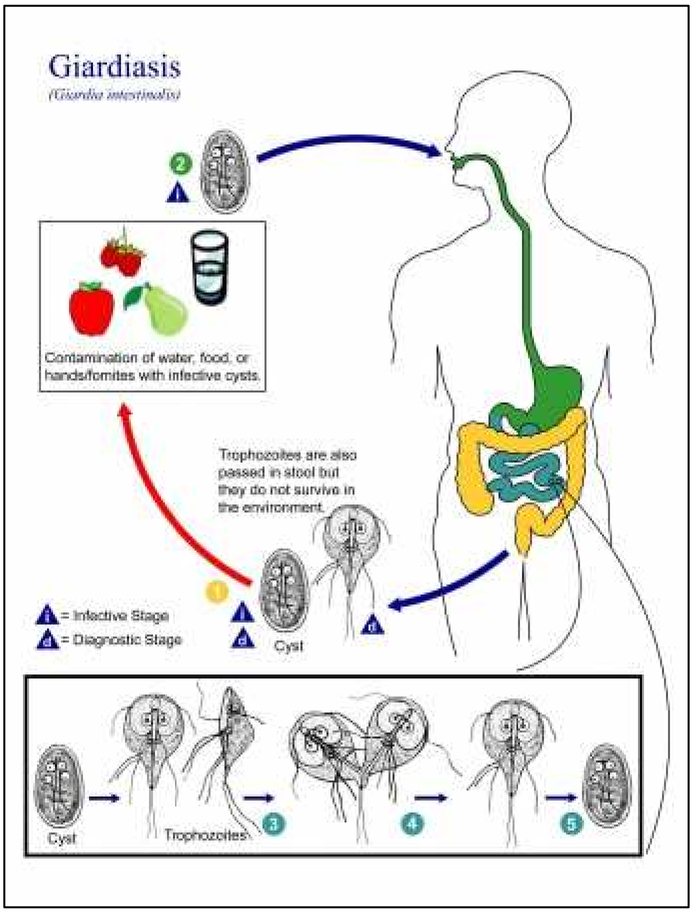

FIGURE 2. LIFE CYCLE OF *GIARDIA DUODENALI.* COURTESY OF CDC, USA

Xiao et al. found that pigs kept in lacking hygienic circumstances had a higher prevalence of *Giardia duodenalis* (also calles *Giardia lamblia* or *Giardia intestinalis*). This is due to the fact that in theory these pigs have more contact with their own faeces and thus create a vicious cycle.

CRYPTOSPORIDIUM SPP.

Cryptosporidium is a small protozo which resides in the small intestine of both humans and animals. *Cryptosporidium parvum* is the name which is used the most. In 1912 the first infection with *Cryptospiridium parvum* was confirmed, it was found in the intestine of the common mouse. Later research of other animals confirmed a presence of oöcysts which resembled *C. parvum*. Without further evidence and research, *C. parvum* was named as the default organism. In time, over 150 species were reported which belong to 12 mammalian Orders. The twelve species and their primary host can be found in Table 1. [14]

***Cryptosporidium* name**	**Host**
Cryptosporidium muris	Mouse
Cryptosporidium parvum	Mouse
Cryptosporidium wrairi	Guinea pig
Cryptosporidium felis	Cat
Cryptosporidium andersoni	Cattle
Cryptosporidium canis	Dog
Cryptosporidium hominis	Human
Cryptosporidium suis	Pig
Cryptosporidium bovis	Cattle
Cryptosporidium fayeri	Kangaroo
Cryptosporidium ryanae	Cattle
Cryptosporidium macropodum	Kangaroo

TABLE 1. *CRYPTOSPORIDIUM* SPECIES FOUND IN MAMMALS

For many years, the bovine genotype was called *C. parvum.* To create stability it is suggested that the name *C. parvum* is maintained for the bovine genotype, as this is done for over 20 years and in about 2500 scientific publications, while technically *C. parvum* is only found in mice and is not able to infect humans and cattle. A suggestion is made to rename *C. parvum bovine genotype* to *C. pestis* [19].
Infections with *Cryptosporidium* spp. are usually symptomless. Young and immunocompromised animals and humans may develop a severe, watery diarrhea. After these clinical signs, oöcyst excretion takes place for one to two weeks, possibly longer.
In pigs *Cryptosporidium* is mostly found in weaners, a prevalence of 10.4% was found in Canada [12]. In older pigs the infection might be symptomless.
The cycle of *Cryptosporidium* is comparable to the earlier mentioned protozoa. The cysts are excreted in the environment and are infective for up to 45 days (see Figure 3) [8].

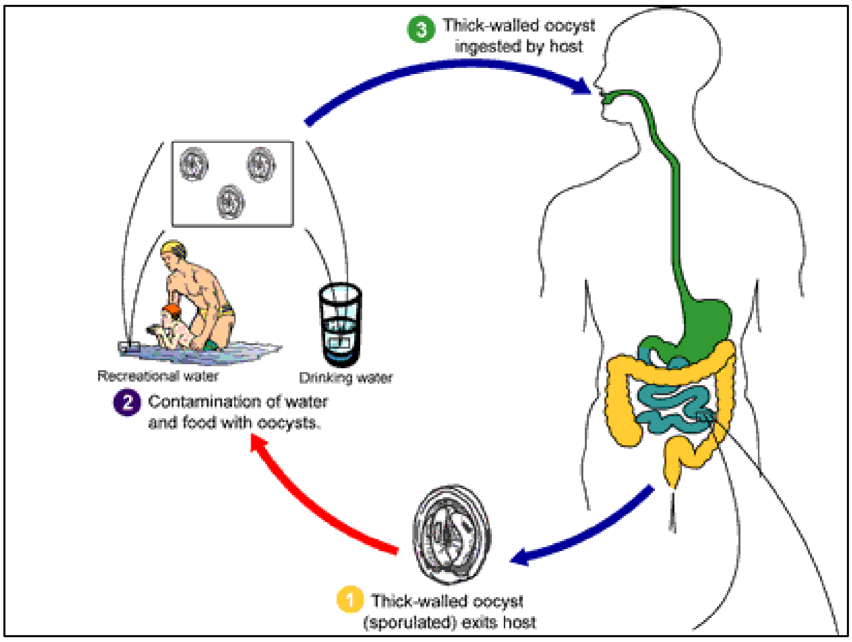

FIGURE 3. LIFE CYCLE OF *CRYPTOSPORIDIU.* COURTESY OF CDC, USA

Zoonotic risk

In pigs three genotypes of *Cryptosporidium* spp. are found:
1. *Cryptosporidium pig genotype I,* now called *Cryptosporidium suis,*
2. *Cryptosporidium pig genotype II* and 3. *Cryptosporidium parvum bovine genotype.* The last one is rarely found in pigs.
Cryptosporidium suis is zoönotic, as well as the bovine genotype*. C. suis* is found in three human patients in Peru, England and Canada [15].

Early literature stated that human cryptosporidiosis was mainly a disease of immunocompromised patients, but more recent literature discovered the disease was also found in immunocompetent humans. Cryptosporidiosis gives a cholera-like diarrhea of short duration in immunocompetent patients, while in immunocompromised patients it can give a severe diarrhea which can persist for months. Other clinical signs which are found commonly are abdominal pains, nausea, vomiting and fever. Cryptosporidiosis can give way for other (severe) opportunistic infections, such as pneumocytosis and toxoplasmosis, which can worsen the condition of a patient [14].
Transmission can occur in many ways, such as through the soil. This can result in contamination of fresh foods, drinking water and recreational water with oöcysts. Another way of infection is through ingestion of contaminated food, this is rare. Another way of food-borne transmission is ingesting contaminated (raw) vegetables. The contamination can come from using pig faeces as a fertilizer for the vegetables.
For infection to occur, humans must have had contact with contaminated food, (recreational) water or surfaces, or had close personal contact with infected people or animals [16].

Cryptosporidium has a very variable prevalence. Prevalences between 0.1% and 33% are found. Weaners and fattening pigs have the highest prevalence. Featherstone et al did not find a significant association between the presence of *Cryptosporidium*-antibodies and different housing types. There was no significant difference between pigs kept inside (conventional) and pigs kept both in- and outside (organic, free range) [4].

COCCIDIOSIS

Coccidiosis is a disease which is most important in piglets of 5 to 15 days old. In these young piglets *Isospora suis* is the main causative agent. Piglets infect themselves by ingesting faeces and contaminated food. Clinical signs are a change in fecal consistency, ranging from transient, pasty faeces, white faeces to profuse, watery diarrhea, retarded growth and a hairy coat. Mortality can be as high as 20%. Sows rarely carry *I. suis* and therefore aren't the main source of infection for the piglets. Infection occurs through contact with the faeces of other piglets and contaminated farrowing crates. Oöcysts of *I.suis* are very resistant and can survive in the environment for months. Infected animals excrete unsporulated oöcysts, which sporulate in the environment (see Figure 4).

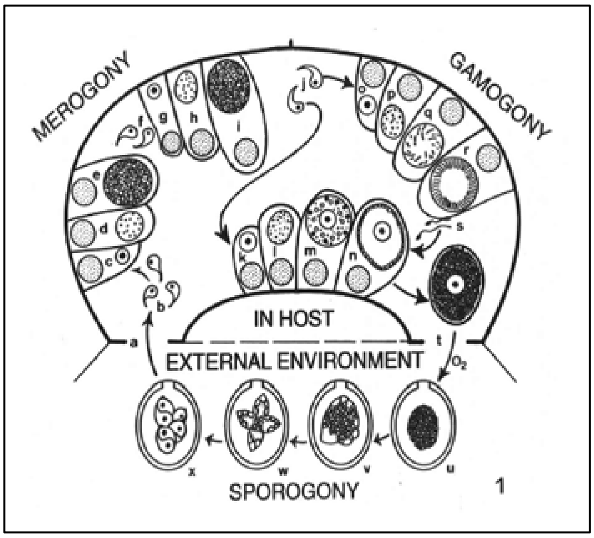

FIGURE 4. LIFE CYCLE OF COCCIDIA. COURTESY OF HTTP://WWW.ENGORMIX.COM

Weaned pigs and sows are also susceptible to coccidiosis, but *I. suis* is not the main causative agent. Eimeria species, sucht as *E.debliecki, E.suis, E.perminuta, E.neodebliecki, E. spinosa, E.porci, E.polita and E.scabra* are the main species responsible for coccidiosis in older pigs.
Clinical signs in older pigs are wasting and watery, yellow diarrhea. Sows are carriers of Eimeria species and can thus infect the piglets. Most Eimeria species are not harmful for piglets (with the exception of *E.polita*). Symptomless excreting pigs are mainly among the sows of great importance[8].

Coccidia are normally found on any type of pig farm, regardless the different types of farming. Eijck et al concluded that coccidial infections were found on organic farms, as well as on free range farms and conventional farms[17]. Sows from organic – and free range farms generally have a higher prevalence of coccidial infections compared with sows from conventional farms. Following this, we can expect that both the Bentheim Black Pied and the pigs from organic farms have a relatively high prevalence of coccidial infections.

HYPOTHESIS

There is no significant difference in prevalence of *Cryptosporidium* spp. and *Balantidium coli* between Bentheim Black Pied pigs and organic kept pigs. Coccidia and *Giardia duodenalis* will have a higher prevalence at the Bentheim Black Pied pigs compared to the pigs kept at organic farms.

We expect a low prevalence of both *Cryptosporidium* spp. and *Balantidium coli* in general and we expect no difference in prevalence between the Bentheim Black Pied pigs and organic-kept pigs, so there is no greater risk for humans in recreational areas and waters. *Giardia duodenalis* will have a higher prevalence and is zoönotic, this can cause a risk for the environment and recreational areas. Coccidia are not zoönotic and will not cause a risk for humans.

MATERIALS AND METHODS

In this study 8 organic farms participated. Organic farms were defined as farms which keep pigs on a professional base according to the directives defined by the SKAL organization. The farms all have more than 100 pigs present, either for fattening or breeding purposes. For the directives defined by the SKAL organization, see www.SKAL.com. The addresses of the organic farms were randomly selected from a list provided by SKAL[18].
A total of 22 Bentheim Black Pied pig-farms participated. 3 of these farms kept the pigs in a forest a 'environmental managers', 19 kept the pigs for hobby (4 of the 19 were kept in petting zoos). A hobby farm was defined as a small scale farm with less than 100 pigs present. These pigs are not primarily kept for fattening of breeding, although this does occur on small scale. The products derived from this slow fattening (generally the age before slaughter is over 1 year) are a niche product and are sold personally by the farmers or sold to a specialized butchery.
The pigs of nature/forest farms are kept extensively as the pigs generally have over 5000 square meters per pig available. These pigs are kept in a enclosed forest and have the freedom to shape this area to their own liking.
Keeping the Bentheim Black Pied has recreational purposes, educational purposes(petting zoos, social care farms) or are kept for the generating of special niche products.
The addresses of the Bentheim Black Pied farms were received from the pedigree record holder of the Dutch organization of the Bentheim Black Pieds. The owners of the Bentheim Black Pieds were contacted whether they wanted to cooperate in the study.

Because of the relatively short duration of this study, the farmers were not asked to withhold treatment with anthelmintics and anticoccidia. Rather they were interviewed about their usage of anthelmintics, which later could be linked to the results from this study.

A number of farms were personally visited, this so the investigators could become familiar with the different housing systems of Bentheim Black Pied pigs. The remaining farms (both organic farms and farms with Bentheim Black Pied pigs), received a package by mail which contained all necessary items to collect and send the pooled samples to the investigators.
The farmers were asked to fill in a questionnaire send to them by e-mail. This questionnaire contained several questions about the management, number of animals and use of anthelmintics. This questionnaire was used to search for trends in the outcomes.

The questions differed a little between the organic farms and the Bentheim Black Pied farms. This is because on organic farms the different age groups have sometimes very different housing types. At most Bentheim Black Pied farms, all the pigs are kept together in the same pen or forest area.
A shortened version of the questionnaire is found in Table 2. Both questionnaires can be found in attachment A and B.

The faeces was stored in a refrigerator at 4 °C. The faeces were al processed the same way. A centrifugation – sedimentation – flotation technique was used to detect the eggs and oöcysts.

- Name
- Address and telephone number
- Type of farm (petting zoo, landscaping purposes, hobby farming)
- Number of animals present on the farm
    - Sows
    - Weaners/fattening pigs
    - Suckling pigs
- Covered area:
    - Size
    - Floor material
    - Removing of feces
    - Cleaning/disinfection
        - Active substance
- Outside area:
    - Size
    - Floor material
    - Removing of feces
    - 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 2. QUESTIONNAIRE

Two grams of faeces was weighed, mixed and diluted with plain water and was placed in a centrifuge at 3000 RPM for one minute. The supernatant was removed, the sediment was diluted with a sugar solution (relative density of 1,28 grams per milliliter). The sediment with sugar solution was further diluted until a fluid meniscus was created. A coverslip was placed, after which the test tube was placed in the centrifuge at 3000 RPM 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 there were any (oö)cysts were present, a McMaster technique was used. With the McMaster a satiated salt solution was used (relative density of 1.19 grams per milliliter).

For the McMaster technique 3 grams of faeces were weighed and diluted with either 42 ml of sugar solution or with 42 ml salt solution (relative density of 1.19 grams per milliliter) . This was mixed and placed in two McMaster counting chambers. After a waiting period of about five minutes, the counting chambers were examined at a 10 x 10 magnification. The (oö)cysts present in the grid were counted, and afterwards multiplied by factor 50 to receive the total amount of (oö)cysts per gram of faeces (OPG).

For the detection of *Cryptosporidium* oöcysts a modified Ziehl – Neelsen staining technique was used. With a swab a thin film of faeces was placed on a microslide. After drying, the sample was fixated by placing it in methanol for five minutes. Subsequently the sample was placed in carbol fuchsin for 16 minutes. The microslide was rinsed with water and decolorized by placing it in hydrochloric acid for about one minute. Next the slide was rinsed again with water and colored with malachite green for five to ten seconds. After rinsing it again with water, the microslide was dried and covered with a drop of Gürr solution, after which a coverslip was placed. The sample was examined at a 10 x 40 magnification.

The pigs which were examined were placed in one of three age groups;

- Suckling piglets, <6 weeks old
- Fatteners, 6-52 weeks old
- Sows, >52 weeks old

STATISTICS

Statistic analysis was done using the Fisher's Exact Test or the Chi-square test. All results were examined in combination with the outcomes of the questionnaire to search for possible trends.

RESULTS

PREVALENCE ON FARMS

In the search for the four earlier mentioned protozoa, only *Eimeria* spp. and *Isospora suis* were found. *Cryptosporidium*, *Balantidium coli* and *Giardia duodenalis* were not found. Detailed information on where the coccidial oöcysts were found can be found in Table 3.

Table 3 shows how many farms were examined and how many farms were found positive for oöcysts of coccidia, regardless in which age group the coccidia were found. No significance exists between the different types of farms. The P-value which was found (using the Fisher's Exact Test) is $p = 0.47$, so there can be concluded that there is no association between farming type and presence of coccidia.

Type of farm	No. of farms investigated	*Cocc* No. positive (%)
Organic Farm	8	6 (75)
Nature/Forest Farm	3	1 (33)
Hobbyfarm	19	10 (53)

TABLE 3. PREVALENCE OF COCCIDIAL INFECTIONS ON ORGANIC-, NATURE/FOREST- AND HOBBY FARMS

PREVALENCE IN DIFFERENT AGE GROUPS

The three age groups which were established were not found on each of the tested farms. This means the number of age groups is equal or lower compared to the number of tested farms in Table 3.

The results of presence of coccidia in the different age groups can be found in table 4.

Pig group	Type of farm	No of groups investigated	*Cocc.* No. Positive (%)
Suckling pigs (<6 wks)	Organic	7	3 (43)
	Nature/Forest	1	1 (100)
	Hobbyfarm	4	2 (50)
Weaned pigs /fatteners (6 wks - 52 wks)	Organic	8	1 (13) [a]
	Nature/Forest	2	1 (50)
	Hobbyfarm	8	6 (75) [b]
Sows (>52 wks)	Organic	7	6 (86) [b]
	Nature/Forest	3	1 (33)
	Hobbyfarm	18	7 (39)

TABLE 4. PREVALENCE OF COCCIDIAL INFECTIONS IN SUCKLING PIGLETS, FATTENERS AND SOWS ON ORGANIC -, NATURE/FOREST- AND HOBBY FARMS
[a,b] Different letters indicate a significant difference (p <0.05)

Coccidia were found in all three age groups. In the suckling piglets and sows, no difference was found between the different types of farms (p = 1 respectively p = 0.096, using the Fisher's Exact Test). In the fatteners, pigs found on hobby farms had a significantly higher prevalence of coccidia than fatteners on organic farms (p = 0.041). Between the organic fatteners and the forest-kept fatteners was no difference, and between the forest-kept fatteners and the hobby farm-fatteners was also no difference (p = 0.37 respectively p = 1, using the Fisher' Exact Test).

Sows from organic farms had significant higher prevalence of coccidial infections compared to the fatteners from organic farms (p =0.01, using the Fisher's Exact Test). No other differences existed between age groups originating from the same farm type.
A visual presentation of Table 4 can be found in Figure 5. In Figure 5 the 95% confidence interval was displayed.
The range in OPG was from <50 OPG up to 12200 OPG (a sow on an organic farm).

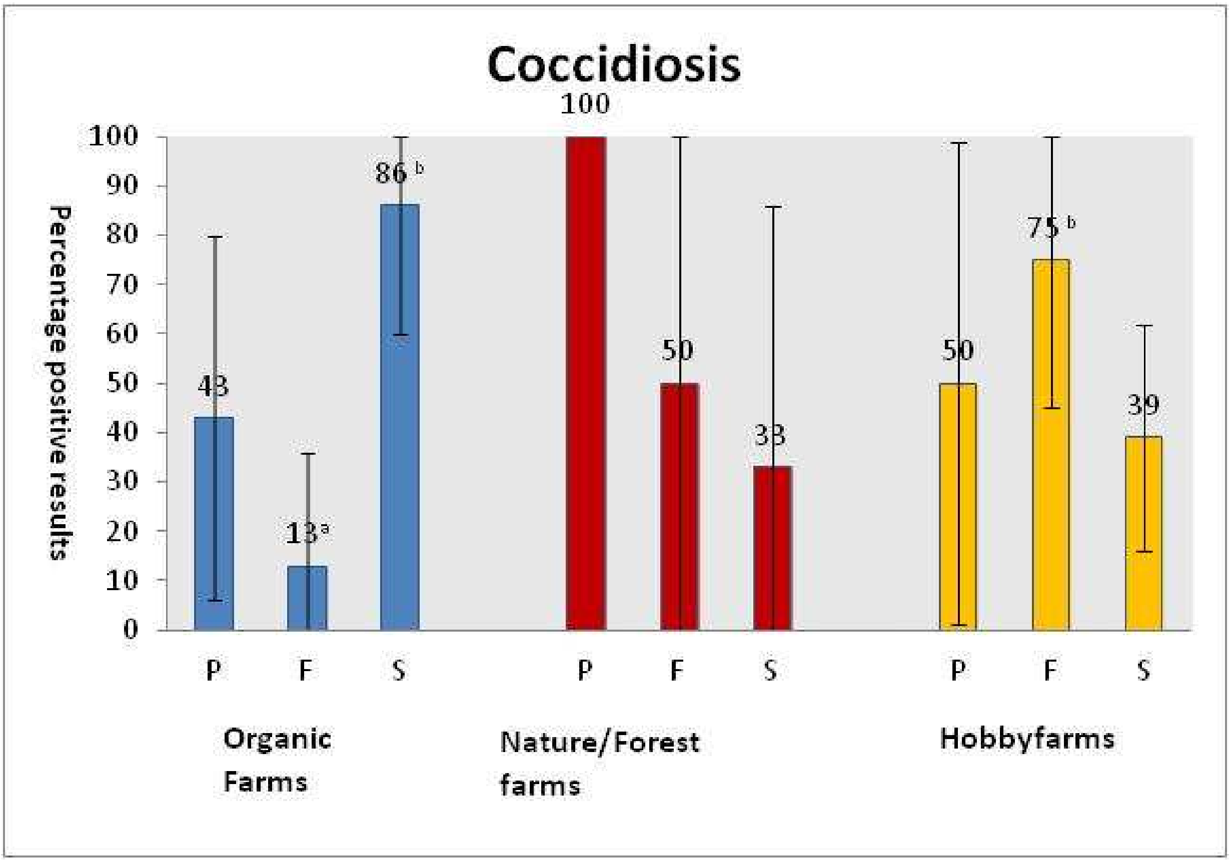

FIGURE 5. PREVALENCE OF COCCIDIOSIS IN SUCKLING PIGLETS, FATTENERS AND SOWS ON ORGANIC -, NATURE/FOREST – AND HOBBY FARMS.
P= piglet, F = Fatteners, S = Sows. [a,b,] Different superscripts indicate a significant difference (p <0.05)
Bars: 95% confidence interval

PREVALENCE IN SAMPLES

Coccidial infections were found on all types of farms and in all three aging groups. While on some farms all present age groups had coccidial infections, some other farms had the coccidia present in one or two age groups, while the other age group was negative. In Table 5 all samples per farm type are entered individually to look for differences between farming types.

Type of farm	No. of samples investigated	Cocc. No Positive (%)
Organic Farm	22	10 (45)
Nature/Forest Farm	6	3 (50)
Hobbyfarm	30	15 (50)

TABLE 5. PREVALENCE OF COCCIDIAL INFECTIONS ON ORGANIC-, NATURE/FOREST AND HOBBYFARMS

No difference between farm types was found (p = 0.93, using the Fisher's Exact Test).

QUESTIONNAIRE

The answers on the questionnaire were analyzed for possible trends. From the answers can be concluded that the Bentheim Black Pied pigs are kept in varying circumstances. The area per pig varied from 12 m^2 up to 5000 m^2, the mean area is 435 m^2 per pig. As the Bentheim Black Pied pigs mainly are kept together regardless of the age, the area per pig can't be specified for one age group, as is the case with the organic kept pigs. The organic kept pigs had a area per pig which is consistent with the standards established by the SKAL organization. Those standards can be found in Table 6 [27].

Age group	Indoor area in m^2	Outdoor area in m^2(grassland not included)	Total area in m^2 (grassland not included)
Fattener, <50 kg	0.8	0.6	1.4
Fattener, 50-85 kg	1.1	0.8	1.9
Fattener, 85-110 kg	1.3	1.0	2.3
Piglet, >40 days, <30 kg	0.6	0.4	1.0
Nursing sow with piglets	7.5	2.5	10.0
Breeding sow	2.5	1.9	4.4
Breeding boar	6.0	8.0	14.0

TABLE 6. AREA PER PIG (IN M^2) ON ORGANIC FARMS

The organic kept pigs all had a area per pig which is below 10 m^2 (breeding boars did not participate in this study). Out of those 8 organic farms, 6 were found positive for coccidia (75%). 6 Bentheim Black Pied pig farms had an area per pig which was below 50 m^2, of these farms 3 were negative and 3 had oöcysts present (50%). 5 Bentheim Black Pied farms had an area per pig which was between 50 m^2 and 100 m^2, 4 farms were negative (80%) and one was positive for oöcysts (20%). The other seven farms had an area per pig which was larger than 100 m^2. 2 farms were negative (29%) and the other 5 were positive for oöcysts (71%) (see Table 7).
No significant difference existed ($p = 0.26$, using the Fisher's Exact Test).

The number of animals varies between the farms. 9 farms had two pigs present, 8 were negative (89%) and one was positive (11%). 6 farms had 3 to 9 animals present, one was negative (17%) and the other five were positive for oöcysts (83%). 4 farms had 10 or more pigs present (maximum is 65 pigs per farms), out of those 4 farms, one was negative (25%) and the other 3 were positive (75%). The organic farms each had more than 100 pigs present, 6 out of 8 farms were positive for coccidia (75%).
A significant difference excisted between the farms with 2 animals present and the farms with 3-9 animals present ($p = 0.011$, using the Fisher's Exact Test). A significant difference also existed between the farms with 2 animals present and the farms with more than 100 animals present ($p = 0.015$, using the Fisher's Exact Test) (see Table 8).

Two Bentheim Black Pied farms had wild boars in the environment. One of those farms was negative, the other had oöcysts. Due to the low number of farms, this was not further analyzed.

Use of anthelmintics was questioned in the questionnaire, but not the use of anticoccidial treatments. One organic farm and one Bentheim Black Pied farm used fenbendazole, which is an anthelmintic but also eliminates *Giardia* infections.

Area per pig (in m²)	No. of farms investigated	Cocc. No. Positive (%)
<10	8	6 (75)
10-50	6	3 (50)
50-100	5	1 (20)
>100	7	5 (71)

TABLE 7. PREVALENCE OF COCCIDIAL INFECTIONS ON FARMS WITH AN AREA PER PIG OF <10 M², 10-50 M², 50-100 M² AND >100 M²

Animals present per farm	No. of farms investigated	Cocc. No. Positive (%)
2	9	1 (11) [a]
3-9	6	5 (83) [b]
10-99	4	3 (75)
>100	8	6 (75) [b]

TABLE 8. PREVALENCE OF COCCIDIAL INFECTIONS ON FARMS WITH 2, 3-9, 10-99 AND >100 ANIMALS PRESENT
[a],[b] Different letters indicate a significant difference (p <0.05)

CONCLUSIONS

This study shows that coccidial infections are present in all three farm types and in all three age groups. The fatteners from organic farm have a significant lower prevalence compared to the fatteners from hobby farms. Fatteners from organic farms also have a significant lower prevalence compared to sows from the organic farms. Pigs coming from a farm with 2 pigs present have a significant lower prevalence of coccidial infections compared with farms with 3-9 pigs present and compared with farms with >100 pigs present.
No clinical cases were known in this study.
We did not find infections with *Cryptosporidium* spp., *Balantidium coli* and *Giardia duodenalis*.
The hypothesis was that there was no difference between the organic farms and the Bentheim Black Pied pigs for infections with *Cryptosporidium* and *Balantidium coli*. Since we did not find both, we can confirm this part of the hypothesis. We expected a higher coccidial infection rate in the Bentheim Black Pied pigs. This can be partly confirmed, as we did find a significant difference between the organic kept fatteners and the hobby farm fatteners. Between the other groups we did not find any significant differences.
Giardia duodenalis was not found, and thus we have to reject the hypothesis that the Bentheim Black Pied pigs have a higher prevalence of *Giardia* infections.
No zoönotic protozoa was found in this study.

DISCUSSION

The results showed that of the four protozoa searched for (Coccidia, *Balantidium coli, Giardia lamblia* and *Cryptosporidium*), only the coccidia were found. The hypothesis was that all four of these protozoa would be found, yet only this one was found. Coccidia are the most common protozoa in pigs, so this was no surprise. The prevalence of *Balantidium coli* in the Netherlands is unknown[19]. According to Danish research, *Giardia lamblia* is mostly found in weaners (84%), and less in piglets and sows (22% respectively 18%)[20]. *Cryptosporidium* is mostly found in pigs of 4 to 13 weeks old [21]. Prevelences found in other studies varied from 0.1% up to 33% [22,23,24]. The reason that these three protozoa were not found can be numerous.
Infections with coccidia are common in all age groups and in all three types of management. Differentiation between *Eimeria* spp. and *Isospora* spp (mainly *suis*) by sporulation was tried but not successful, as the sporulation did not occur.
Eijck and colleagues (2005) found a prevalence of 70% in the organic kept suckling piglets, this is more than the prevalence found in this study (43%)[4]. The 95% confidence interval in this study is 6% up to 80% (see Figure 5). Eijck and colleagues examined piglets of almost four weeks old. In this study, the age of the piglets varied from one week to almost six weeks. Infections with *Isospora suis* are most common in pigs of two weeks [25]. The range in age in this study is much wider than in the study performed by Eijk and colleagues.

Eijck and colleagues found a prevalence of 80% in the organic kept sows. This is comparable with the prevalence found in this study (86%). The 95% confidence interval in this study is 60% up to 100%. The pigs in the age of six weeks up to 52 weeks were all entered into one group, the fatteners. In most other studies, these pigs are divided into two or three groups; weaners, fatteners and finishers. The range in age in this study is much wider than in other studies.

Coccidial infections are generally symptomless, except in young piglets. A high infection rate of young piglets in combination with clinical signs can indicate a need of anticoccidial treatment.

The pigs which were examined all had an outside area (both the Bentheim Black Pied and the organic kept pigs), so the possibility of passengers also exists. The pigs can have contact with the soil, faeces of birds and possibly other animals. This way they can ingest, for example, coccidial oöcysts of crows, which they pass unchanged in their faeces and can't be distinguished from pig oöcysts in the CSF technique.

The reason *Giardia lamblia* and *Cryptosporidium* were not found can also be that *Giardia* is very small (about 10 to 20 um) and is excreted intermittently. In this study an one-day sample was investigated, so the lack of cysts does not mean the pig is not infected with *Giardia*. The lack of findings could also be due to the fact that the researchers were not professionals but veterinary students.

Detection of *Cryptosporidium* can be difficult due to the staining technique and the similarities between oöcysts of *Cryptosporidium* and, for example, yeast. There was one farm which had sent in three faeces samples (one of each age group), where *Cryptosporidium* oöcysts were found in all three samples after the modified Ziehl-Neelsen staining technique. Because detecting oöcysts of *Cryptosporidium* can be extremely difficult, there was a snap test used to confirm the finding. The snap test used was the Bodia test for diarrhea of calves, produced by Fassisi. This test detects Rotavirus, Coronovirus, *E.coli* K 99 and *Cryptosporidium parvum.* This test was performed 1.5 weeks after receiving the faeces, which was stored in a refrigerator at 4 °C. Two

tests were performed, which were both negative. The reason for this negative test can be the faeces was too old, the number of oöcysts was beneath the detection limit, the test does only detect *Cryptosporidium parvum* and not other species, such as *Cryptosporidium suis*. According to the manufacturer, the snap test is cross reactive to other *Cryptosporidium* genotypes, yet no studies were performed to confirm this.
Due to the fact that microscopic examination of *Cryptosporidium* oöcysts is difficult and the oöcysts can be mistaken for numerous other items, we considered the snap test to act as a control. If the snap test was negative, so was the sample.

In the Materials and Methods section, is was decided that this study would use pooled samples for analysis. Yet, sometimes we received a sample which was collected from one or two animals. The reason for this can be that there only was one animal per age group present (mostly in the population of the Bentheim Black Pied pigs) or the sample was wrongly taken by the farmer himself. Contamination of the samples could also have occurred, this could have happened by the sampling of old faeces or by accidently sampling more age groups as one pooled sample.

Statistic differences were sporadically found (see the Results section), this is due to the low number of participating farms. The Bentheim Black Pied population is fairly small in the Netherlands, especially the pigs which are kept in forests as nature conservers. All Bentheim Black Pied farmers which are found in the pedigree record were contacted. Not every farmer contacted (both organic and Bentheim Black Pied) was interested to participate in this study.

The farmers who were participating send in a sample of the faeces, yet we did not receive all samples from interested farms. The packages were send by mail, which sometimes took as much as five days to reach the laboratory. The sample was not cooled during this time, it was also unknown how fresh the faeces was when the farmers collected the samples. Ideally, every farm should be visited personally by the researchers to collect a pooled sample of fresh faeces, which should be cooled immediately.

All participating farmers received the questionnaire by e-mail. Most farmers did complete it but a few farmers did not fill in the questionnaire completely or left blanks. This made it difficult to search for trends and make comparisons between farms. Some farms left blanks at the question about the size of the area, therefore it was not possible to include all farms in the search for a correlation between area per pig and prevalence. Only the farms who did answer this question were entered in Table 7.

RECOMMENDATIONS

The low numbers of participating farms in this study impairs the statistically significant differences which could be found. It is therefore recommended to perform a larger study with more participating farms. A possibility would be to look beyond the population of the Bentheim Black Pied and try to form a larger group of forest kept pigs.

REFERENCES

[1] *Het varken als landschapsontwikkelaar* (1). 18-01-2011 http://www.groenkennisnet.nl/Pages/Hetvarkenalslandschapsbeheerder.aspx

[2] *Het varken als landschapsontwikkelaar*(2). Veehouder – Dierenarts. Achtergrond. November 2010 http://edepot.wur.nl/156733

[3] http://www.thepigsite.com/pighealth/article/425/protozoan-diseases. Last seen: 05-10-2011

[4] Featherstone, A., Marshall, J. A., Giles, M., Sayers, A.R., Pritchard, G.C. *Cryptosporidium species infection in pigs in East Anglia.* Veterinary Record 2010 166: 51-52

[5] Schuster, F.L., Ramirez-Avila, L. *Current World Status of* Balantidium coli. Clinical Microbiology Reviews, October 2008. P 626-638

[6] Taylor, D.J., *Pig Diseases*. Eight edition. Published by D.J. Tayloir, Glasgow, Great Britain. 2006

[7] Author unknown. *Life Cycle of* Balantidium coli*.* *http://www.stanford.edu/class/humbio103/ParaSites2003/Balantidium/Life_Cycle.htm.* (Last seen: 25-09-2011)

[8] Olson, M.E. , Guselle, N. *Are Pig Parasites a Human Health Risk?* Advances in Pork Production (2000) Volume 11, pg. 153

[9] Unknown author. *Parasites and Health. Giardiasis.* Laboratory Identification of Parasites of Public Health Concern. http://dpd.cdc.gov/dpdx/html/Giardiasis.htm. Last seen: 25-09-2011

[10] Thompson, *R.C.A., Giardiasis as a re-emerging infectious disease and its zoonotic potential*. International Journal for Parasitology 30 (2000) 1259±1267

[11] Keulen, H. van, Macechko, P.T., Wade, S., Schaaf, S., Wallis, P.M. Erlandsen, S.L. *Presence of human Giardia in domestic, farm and wild animals, and environmental samples suggests a zoonotic potential for giardiasis.* Veterinary Parasitology 108 (2002) 97–107

[12] Fayer, R., Dubey, J.P., Lindsay, D. S. *Zoonotic protozoa: from land to sea.* TRENDS in Parasitology Vol.20 No.11 November 2004

[13] Sun, T. *Color atlas and textbook of diagnostic parasitology.* IGAKU-SHOIN Medical Publishers, Inc., New York. 1988

[14] Fayer, R. *Taxonomy and species delimitation in Cryptosporidium*. Experimental Parasitology 124 (2010) 90–97

[15] Xiao, Lihua & Feng, Yaoyu. *Zoonotic cryptosporidiosis*. FEMS Immunol Med Microbiol 52 (2008) 309–323 December 2007

[16] Fayer, R., Morgan, U., Upton, S.J. *Epidemiology of* Cryptosporidium*: transmission, detection and identification.* International Journal for Parasitology 30 (2000) 1305-1322

[17] Eijck, F.H.M. Borgsteede, *A Survey of Gastrointestinal Pig Parasites on Free-range, Organic and Conventional Pig Farms in The Netherlands,* ASG Wageningen University, Veterinairy Research Communications 2005

[18] www.SKAL.nl (last seen: 26-09-2011)

[19] Vermaas, M. *GD: Behandeling B.coli niet noodzakelijk.* 10-05-2010

[20] Maddox-Hyttel, C., Langkjær, R.B., Enemark, H.L., Vigre, H. Cryptosporidium *and* Giardia *in different age groups of Danish cattle and pigs—Occurrence and management associated risk factor.* Veterinary Parasitology 141 48–59, 2006

[21] Guselle, N.J., Appelbee, A.J., Olson, M.E. B*iology of* cryptosporidium parvum *in pigs: from weaning to market*. Veterinary parasitology 113, 7-18, 2003

[22]Quilez, J., Sanchez-Acedo, C., Clavel, A., Delcacho, E. & Lopezbernad, F. *Prevalence of* cryptosporidium *infections in pigs in Aragon (northeastern Spain).* Veterinary parasitology 67, 83-88, 1996

[23] Izumiyama, S., Furukawa, I., Kuroki, T., Yamai, S., Sugiyama, H., Yagita, K. & Endo, T. Prevalence of cryptosporidium parvum infections in weaned piglets and fattening porkers in Kanagawa Prefecture, japan. Japanese Journal of Infectious diseases 54, 23-26, 2001

[24] Epe, C., Coati, N. & Schneider, T. *Results of parasitological examination of faecal samples from horses, ruminants, pigs, cats, hedgehogs and rabbits between 1998 and 2002*. Deutsche Tierärztliche Wochenschrift 111, 243-247, 2004

[25] Greve. E., Isopora suis *species in a Danish SPF-herd.* Nordisk Veterinaermedicin 37, 440-444. 1985