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## Occurrence, clinical involvement and zoonotic potential of endoparasites infecting Swiss pigs



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### ABSTRACT

In order to estimate the diversity, clinical involvement and zoonotic potential of parasites in pigs submitted for diagnosis to the PathoPig project of the Swiss Federal Food Safety and Veterinary Office, faeces ( $n = 125$ ) from suckling piglets ( $n = 39$ ), weaners ( $n = 60$ ) and piglets beginning fattening ( $n = 26$ ) from 74 Swiss farms were examined by 3 coproscopical methods (i.e. sedimentation/zinc chloride-flotation; SAFC and Ziehl-Neelsen staining). Samples microscopically positive for *Cryptosporidium* were further tested by PCR/sequencing for species assessment. The most frequently detected parasite was *Balantidium coli*, a facultative pathogenic ciliate with zoonotic potential, in 5.1, 36.7 and 50.0% of suckling, weaners and fatteners and 43.2% of farms; however, no association with disease was observed. *Isospora* (syn. *Cystoisospora*) *suis* infections were detected in 13.3 and 11.1% of suckling piglets with and without diarrhoea, and in 10.0 and 13.3% of weaners and fatteners with diarrhoea, respectively, and were significant associated with emaciation. *Cryptosporidium* infections were detected in 10.3, 15.0 and 19.2% of sucklings, weaners and fatteners, respectively, and in 18.9% of the farms. Interestingly, two age-related species were identified: *C. suis* in younger piglets (2 to 6 weeks) and *C. scrofarum* in older ones (6 to 17 weeks). None of the pigs infected with *C. scrofarum* ( $n = 8$ ), but 3 of 4 piglets infected with *C. suis* (co-infection with *I. suis* in 2 cases) had diarrhoea. The zoonotic species *C. parvum* was not detected, nevertheless, sporadic cases of human infection with the porcine-adapted species have been reported. *Ascaris suum*, *Trichuris suis* and Strongylida were rarely detected (<4%) in all age categories.

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### 1. Introduction

Gastrointestinal disorders represent a major cause of economic losses in the Swiss pig production [1]. Several bacterial, viral and parasitic pathogens have been described as cause of infectious diarrhoea in pigs, having often an age-related occurrence. In suckling piglets, enterotoxigenic *Escherichia coli* (ETEC), *Isospora* (syn. *Cystoisospora*) *suis*, Rotavirus, Coronavirus and *Clostridium perfringens* type C are regarded as the most relevant causes of diarrhoea worldwide [2]. Under certain management conditions (e.g. outdoor-housing), the nematode *Strongyloides ransomi* may also play an important role as cause of diarrhoea in suckling piglets [3,4]. In weaned and fattening piglets, gastrointestinal diseases are commonly caused by *E. coli*, *Lawsonia intracellularis*, *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli*, Porcine Circovirus Type 2, Coronavirus and nematodes such as *Ascaris suum*, *Oesophagostomum* spp. and *Trichuris suis* [2,4]. Gastrointestinal parasites

can be cause of economic losses by production of diarrhoea but also by organ condemnation (e.g. “milk spots” in the liver caused by *A. suum*), reduction of carcass quality, reduced feed conversion and daily weight gain and by potentiating other pathogens [2,4]. Parasites commonly detected in suckling piglets include mainly those species with short life cycles such as *I. suis* and *S. ransomi*. While *I. suis* is currently recognized as an important cause of diarrhoea in suckling piglets worldwide [5–9], *S. ransomi* seems to be less important in modern intensive pig production [10–12]. Weaners and fatteners are less frequently infected with *I. suis* [13–15]. In those age categories, *Oesophagostomum* spp., *A. suum* and *T. suis* appear to play a more important role, especially when pigs are housed outdoors [11,12,16]. *Cryptosporidium* spp. were described in pigs of all ages worldwide [17–22]. *Cryptosporidium* infections in pigs are usually subclinical but sometimes they are cause of non-haemorrhagic diarrhoea. Moreover, some *Cryptosporidium* spp. are important due to their zoonotic potential [23]. Amoebae and *Balantidium coli* seem to be common parasites in all age groups but they have a low clinical relevance [4,13,24].

In Switzerland, the conventional pig husbandry consists in indoor housing, with an optional outdoor area, generally with concrete floor,

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with or without a perforated area. A complete slatted floor is just allowed until 31st August 2018, therefore the farms are changing to partially slatted floor. Concerning the animal welfare, straw, hay, plastic- or chain-toys are obligatory in all pig pens. The higher percentage of non-perforated concrete floor leads to a higher accumulation of faecal rests in the pens, enhancing the likelihood of parasite infection. Moreover, the straw, hay or green fodder may be also potentially contaminated with parasite stages. There are only few data about the occurrence of endoparasites in Swiss piglets and their importance as cause of disease. Mundt et al. detected *I. suis* infections in 2–3 week-old piglets in 69% of 13 Swiss farms, most of them reporting diarrhoea in this age group [25]. Eichhorn investigated weaned piglets and fatteners from 90 conventional and 20 free-range farms in Switzerland by the sedimentation/zinc-chloride flotation method and observed that 32.2% of the conventional farms were positive for *T. suis*, 13.3% for *A. suum*, 3.3% for Strongylida and 1.1% for *S. ransomi* [26]. Besides, *T. suis* was detected in 60% of 20 tested free-range pig farms, *A. suum* in 35%, *Metastrongylus* sp. in 30%, Strongylida in 20% and *S. ransomi* in 5% of those farms. Interestingly, diarrhoea was present in only 9.4% (3 of 32) conventional farms with positive parasitological diagnosis and in none of the free-range farms, but in 32.8% (19 of 58) of the farms with negative parasitological results [26].

The Swiss Federal Food Safety and Veterinary Office (FSVO) launched in 2014 the *PathoPig* project together with project partners (i.e. Swine Health Service (SGD); Swiss Association for Swine Medicine (SVSM); Institute of Virology and Immunology (IVI); Institutes of Veterinary Pathology and Divisions of Swine Medicine of the Universities of Zurich and Bern), aiming to strengthen the early detection of epizootic diseases and zoonosis and improving the health status of Swiss pigs through subsidized pathological diagnosis. Farms fulfilling determined criteria (i.e. recurrent or therapy-resistant diseases of unknown origin, atypical clinical signs, increased morbidity or mortality rates and increased use of antibiotics) were encouraged to submit affected animals (1 to max. 3/farm) for subsidized diagnosis. Based on necropsy findings, selected laboratory investigations (e.g. histopathological, bacteriological, virological and parasitological studies) were performed to assess the cause of the problem. Necropsies and further laboratory diagnosis were subsidized by FSVO (up to CHF 200 per submitted animal or up to CHF 500 per farm submitting three animals) and thereby in most of the cases free of charge for the farmer [1].

The aims of this study were to determine the diversity of parasites in pigs submitted for diagnosis to the *PathoPig* project during their first months of life, to estimate their clinical involvement as cause of gastrointestinal disorders and emaciation (alone or in combination with other pathogens) and their zoonotic potential.

## 2. Materials and methods

### 2.1. Animals

A total of 125 pigs (39 suckling piglets, 60 weaned piglets and 26 pigs at the beginning of the fattening period) submitted for diagnosis to the *PathoPig* project between January and December 2014 were included in this study. These animals derived from 74 conventional farms from 14 Swiss Cantons (Suppl. Fig. 1) fulfilling one or more of the criteria to be included in the project, and represented 20% of the total of pigs of all ages analysed in the different participating laboratories in the whole Country during 2014 [27]. Gastrointestinal disorders were the reason for submission of 68% of the analysed pigs in this study.

The necropsies of the included group were performed at the Institute of Veterinary Pathology of the University of Zurich ( $n = 113$ ) and at the Institute of Animal Pathology of the University of Bern ( $n = 12$ ). Due to the fact that most parasite infections are age-related, the piglets were classified in three groups according to their age:  $\leq 4$  weeks old (“suckling piglets”),  $> 4$  to 12 weeks old (“weaned piglets”) and  $> 12$  to 24 weeks old (mean 16 weeks) (“fatteners”). When

the age was unknown, the body weight was considered: Piglets up to 8 kg were classified as “suckling piglets”, between 8.1 and 25 kg as “weaners” and pigs  $> 25$  kg as “fatteners”.

### 2.2. Faecal samples and coproscopical methods

Individual faecal samples were taken from the rectum of the piglets at necropsy and stored at 4 °C until coproscopical analyses were performed. All faecal samples were analysed by three different coproscopical methods: flotation/zinc chloride sedimentation technique; SAFC (sodium acetate - acetic acid - formalin - concentration) technique and Ziehl-Neelsen staining.

#### 2.2.1. Sedimentation/flotation technique

A combined sedimentation/flotation technique using zinc chloride solution (specific gravity 1.45) was employed for detection of coccidian oocysts and helminths eggs as described by Deplazes et al. [4]. When unsporulated coccidian oocysts were found, a small amount of filtered faeces was mixed with 2.5% potassium dichromate solution and incubated for at least one week at room temperature, mixing the faecal suspension every one to two days for aeration, for the microscopical identification of the oocysts after sporulation as *I. suis* or *Eimeria* spp.

#### 2.2.2. SAFC

The SAFC technique, a sedimentation method using a sodium acetate - acetic acid - formalin solution, and diethyl-ether for fat extraction, was additionally performed to detect vegetative and cystic stages of protozoa, i.e. *Giardia duodenalis*, *B. coli*, and amoebae. The method was performed as described by Deplazes et al. [4].

#### 2.2.3. Ziehl-Neelsen staining

For detection of *Cryptosporidium* oocysts, a modified Ziehl-Neelsen staining was performed [28]. Briefly, a thin layer of faeces was transferred to a microscopic slide using a cotton swab, air-dried, fixed in methanol for 5 min and coloured with carbol-fuchsin for 4 min. The slide was then rinsed in tap water, decolorized with HCl-ethanol and rinsed again in water. Afterwards, the slide was counterstained with malachite green. After a final rinse in tap water, the slide was air-dried and examined microscopically with immersion oil at 50 X and 100 X magnitude.

### 2.3. Nested-PCR for *Cryptosporidium*

Faecal samples, in which putative *Cryptosporidium* oocysts were microscopically detected, were conserved at  $-20$  °C and subsequently tested by a specific nested-PCR for *Cryptosporidium* targeting the 18S ribosomal RNA gene sequence [29]. For this purpose, DNA was extracted using a commercial kit (ZR Fecal DNA MiniPrep, Zymo Research, USA) as indicated by the manufacturer. Positive samples in the PCR were further sequenced (Synergene Biotech GmbH, Schlieren, Switzerland) in order to assess the *Cryptosporidium* spp. involved in the infections.

### 2.4. Data collection

A questionnaire including data about the farm and the animals, contact data from the farmer and responsible veterinarian, health status, medical pre-treatments and reason of submission was used (“Anamneseformular”) [1]. The questionnaires had to be submitted together with the animals by the responsible veterinarians as requirement for admission.

### 2.5. Further complementary diagnostic methods

In the frame of the *PathoPig* project, further laboratory investigations (e.g. histopathological, immunohistochemical, bacteriological and virological analyses) were decided by the pathologists according to the

anamnesis, clinical data and necropsy findings. For example, a bacteriological investigation was performed to all animals with a history of diarrhoea and/or liquid faecal consistency in the large intestine, mostly directed to the identification of ETEC. The obtained *E. coli* pure isolates (isolated under aerobic conditions from Columbia blood agar with sheep blood, Oxoid AG, Pratteln, Switzerland) were further analysed by serotyping using different antisera (F4; F5; O141:K85 and O139:K82, Sifin Diagnostics GmbH, Berlin, Germany). If haemorrhagic enteritis in suckling piglets was observed, culture of *Clostridium perfringens* Type C from the faeces out of the small intestine was done (Columbia blood agar with sheep blood, Oxoid AG, Pratteln, Switzerland, incubated at 37 °C for 24 h under anaerobic conditions). Detection of the target genes for the toxins  $\alpha$ ,  $\beta$ ,  $\beta$ 2 was done using real-time multiplex PCR [30]. Diarrheic piglets with a negative bacteriological result or sometimes parallel to the bacteriological investigation were tested by immunochromatography for Rotavirus A (FASTest® ROTA Strip Megacor, Hörbranz, Austria) and porcine Coronaviruses (Anigen Rapid TGE/PED Ag Test kit BioNote, Gyeonggi-do, Korea). A histopathological examination of the intestines was performed if no signs of autolysis were present, mostly in recently euthanized animals and only sporadically in animals which died on the farm. Lesions suggestive for *Brachyspira* spp. infection were never detected at necropsy and specific bacteriological diagnosis (culture followed by subsequent PCR detection [31]) was only performed by request of the submitting veterinarian. In wasting pigs, ileum and lymphatic organs were always histopathologically investigated for lesions of infection with *L. intracellularis* and PCV2 respectively. *L. intracellularis* was intraluminally demonstrated with Warthin–Starry silver nitrate-based stain and PCV2 was detected by immunohistochemistry using the monoclonal antibody F217 [32]. Only parasitological examinations were systematically conducted to all the animals included in this study.

## 2.6. Statistical analyses

Associations between parasite presence and diarrhoea in the different age groups and also between presence of parasites and emaciation were analysed using Fisher's exact test (<http://graphpad.com/quickcalcs/contingency1/>). Correlations were considered significant at  $p < 0.05$ . It is to note that only animals derived from farms experiencing different sanitary problems were included in this study. Therefore, parasitological findings in animals showing specific clinical signs (e.g. diarrhoea or emaciation) were compared with these findings in animals without those signs, although other different clinical signs could have been present, (e.g. arthritis).

## 3. Results

### 3.1. Frequency of gastrointestinal parasitic infections

The frequency of parasitic infections detected in the analysed piglets at the animal level is displayed in Table 1. *I. suis*, *Cryptosporidium* spp., *B. coli* and amoebae were detected in all three age groups, however some differences in the frequency of detection were observed. In

suckling piglets, *I. suis* and *Cryptosporidium* sp. were the most frequently detected parasites. Weaners and fatteners were most commonly infected with *B. coli* and amoebae, followed by *Cryptosporidium* spp. and *I. suis*. The occurrence of *B. coli* and amoebae infections showed a significant increase with age (<8 to 50%). The frequency of *I. suis* infection seemed to decrease according to the age of the three groups and that of *Cryptosporidium* spp. to increase, however, these differences were not statistically significant. *Ascaris suum*, *T. suis* and Strongyloidea were only seldom detected (<4%) and *Giardia* and *Strongyloides* were not detected in any animal. The occurrence of parasite species at the farm level (at least one positive animal/submitted group/farm) is shown in Table 2.

### 3.2. Frequency of parasites and other enteropathogens in piglets with diarrhoea

The most commonly detected pathogen in all three piglet groups was ETEC. After *E. coli*, the most frequently detected pathogens were: in suckling piglets *I. suis* and *Clostridium perfringens* type C; in weaned piglets *B. coli* and *L. intracellularis* and in fatteners *B. coli* and *B. hyodysenteriae*. Regarding to the occurrence of co-infections, 42.4% of the piglets with diarrhoea were infected with only one pathogen and 24.7% with two or more. The combinations of pathogens detected were very variable, and no special pattern seemed to prevail. Amoebae and oedema disease-*E. coli* (EDEC) were not considered in this estimation, because they appeared to be just part of the flora without clinical significance. In 28 piglets (32.9%) no pathogen was detected. The observed frequency of parasites and other enteropathogens in piglets with diarrhoea ( $n = 85$ ) and the occurrence of co-infections are displayed in Tables 3 and 4, respectively. The detected parasite species in piglets not showing diarrhoea when submitted for diagnosis ( $n = 40$ ) are shown in Table 5. *I. suis* was present in suckling piglets with and without diarrhoea, but only in weaners and fatteners with diarrhoea, still the differences were not significant. *A. suum* and *T. suis* were detected only in pigs with diarrhoea, but in a low number of cases. *B. coli*, amoebae and *Cryptosporidium* spp. were significantly more frequent in pigs without diarrhoea.

### 3.3. Nested-PCR for *Cryptosporidium* and sequencing

Seventeen of the 18 microscopically positive *Cryptosporidium* samples were tested by PCR. Amplified products from positive PCR samples were further sequenced in order to assess the *Cryptosporidium* spp. involved in the infections. Twelve of the microscopically positive *Cryptosporidium* samples gave positive results by PCR. After sequencing, 4 samples were identified as *C. suis* and 8 as *C. scrofarum* (syn. *Cryptosporidium* pig genotype II). All *C. scrofarum* positive pigs were 6 weeks or older (6–17 weeks) whereas *C. suis* positive ones were 6 weeks or younger (2–6 weeks). None of the pigs infected with *C. scrofarum* had diarrhoea; however, diarrhoea was present in 3 of 4 of the piglets infected with *C. suis* (co-infections with *I. suis* in 2 cases and with *B. coli* in one case were detected). The zoonotic species *C. parvum* was not detected in any sample.

**Table 1**  
Frequency of parasite species detected in piglets of the PathoPig project (animal level).

Parasite species	Suckling piglets ( $n = 39$ )			Weaned piglets ( $n = 60$ )			Fatteners ( $n = 26$ )			Total pigs ( $n = 125$ )		
	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)
<i>Balantidium coli</i>	2	5.1	(0–12)	22	36.7	(24.5–48.9)	13	50	(30.8–69.2)	37	29.6	(21.6–37.6)
Amoebae	3	7.7	(0–16.1)	16	26.7	(15.5–37.9)	13	50	(30.8–69.2)	32	25.6	(18.0–33.3)
<i>Isospora suis</i>	5	12.8	(1.8–22.2)	4	6.7	(0.4–13.3)	2	7.7	(0–18.0)	11	8.8	(3.8–13.8)
<i>Ascaris suum</i>	0	0		0	0		1	3.8	(0–11.2)	1	0.8	(0–2.4)
<i>Trichuris suis</i>	1	2.6 <sup>a</sup>	(0–7.6)	1	1.7	(0–4.9)	0	0		2	1.6	(0–3.8)
Strongyloidea	0	0		1	1.7	(0–4.9)	1	3.8	(0–11.2)	2	1.6	(0–3.8)
<i>Cryptosporidium</i> spp.	4	10.3	(0.8–19.9)	9	15	(6.0–24.1)	5	19.2	(4.1–34.4)	18	14.4	(8.2–20.5)

<sup>a</sup> Intestinal passage.



**Table 2**  
Frequency of parasite species at the farm level.

Parasite species	Farms (n = 74)		
	n	%	(95% CI)
<i>Balantidium coli</i>	32	43.2	(31.9–54.5)
Amoebae	24	32.4	(21.7–43.1)
<i>Isospora suis</i>	6	8.1	(1.9–14.3)
<i>Ascaris suum</i>	1	1.4	(0–4.1)
<i>Trichuris suis</i>	2	2.7	(0–6.4)
Strongylida	2	2.7	(0–6.4)
<i>Cryptosporidium</i> spp.	14	18.9	(10.0–27.8)

### 3.4. Frequency of parasites and body condition

Parasitic infections can lead to poor weight gain. Therefore, the piglets were classified at necropsy in four different groups according to their body condition: i.e. well-fed, slightly, moderate or high-grade emaciated. *B. coli* and amoebae were often detected in all of the groups. *I. suis* was found significantly more frequently in moderately and high-grade emaciated pigs than in well-fed ones. *A. suum*, *T. suis* and Strongylida were detected only in emaciated pigs, still they were not common enough for a significant difference. *Cryptosporidium* were detected in well-fed as well as in moderately emaciated piglets. Apart from *I. suis*, the differences were not significant (Suppl. Fig. 2).

## 4. Discussion

In this study, *I. suis* infections were detected in 13.3% of the suckling piglets with diarrhoea and in 11.1% of the piglets without diarrhoea, and also in 10.0% and 13.3% of the weaner and fatterer pigs showing diarrhoea, respectively, but in no pig without diarrhoea within these categories. Probably due to the small number of samples from pigs without diarrhoea, this difference was not significant. While several studies in suckling piglets involving a large amount of litters or farms reported a significant association between the presence of *I. suis* oocysts in the faeces and diarrhoea [5,7,8], experimental infections of piglets with *I. suis* showed a great individual variation in oocyst shedding and clinical outcome [33–35]. Interestingly, it was observed that the peaks of oocysts excretion and diarrhoea frequently do not occur at the same time [33–35]. In the current study, we had the limitation, that individual faecal samples from 1 to 3 animals/farm were examined instead of faecal pools from whole litters or several litters/farm as in most epidemiological studies; therefore, we can assume that the chances to diagnose *I. suis*-associated diarrhoea in a farm were lower than in other studies.

**Table 3**  
Frequency of parasites and other enteropathogens in pigs with diarrhoea.

Microorganism	Suckling piglets (n = 30)			Weaned piglets (n = 40)			Fatteners (n = 15)			Total (n = 85)		
	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)
<i>Balantidium coli</i>	2	6.7	(0–15.7)	13	32.5	(18.0–47.0)	4	26.7	(4.3–49.1)	19	22.4	(13.5–31.3)
Amoebae	3	10	(0–20.7)	10	25	(11.6–38.4)	4	26.7	(4.3–49.1)	17	20	(11.5–28.5)
<i>Isospora suis</i>	4	13.3	(1.2–25.5)	4	10	(0.7–19.3)	2	13.3	(0–30.5)	10	11.8	(4.9–18.7)
<i>Ascaris suum</i>	0	0		0	0		1	6.7	(0–19.4)	1	1.2	(0–3.5)
<i>Trichuris suis</i>	1	3.3 <sup>a</sup>	(0–9.7)	1	2.5	(0–7.3)	0	0		2	2.4	(0–5.7)
Strongylida	0	0		1	2.5	(0–7.3)	0	0		1	1.2	(0–3.5)
<i>Cryptosporidium</i> spp.	2	6.7	(0–15.7)	3	7.5	(0–15.7)	1	6.7	(0–19.4)	6	7.1	(1.6–12.6)
<i>Escherichia coli</i> (ETEC)	8	26.7	(10.9–42.5)	17	42.5	(27.2–57.8)	4	26.7	(4.3–49.1)	29	34.1	(19.4–38.7)
<i>Escherichia coli</i> (EDEC)	9	30	(13.6–46.4)	5	12.5	(2.3–22.8)	2	13.3	(0–30.5)	16	18.8	(10.5–27.1)
<i>Lawsonia intracellularis</i>	0	0		5	12.5	(2.3–22.8)	0	0		5	5.9	(0.9–10.9)
<i>Clostridium perfringens</i> type C	3	10	(0–20.7)	0	0		0	0		3	3.5	(0–7.4)
<i>Clostridium perfringens</i>	1	3.3	(0–9.7)	0	0		0	0		1	1.2	(0–3.5)
Rotavirus	1	3.3	(0–9.7)	0	0		0	0		1	1.2	(0–3.5)
<i>Brachyspira pilosicoli</i>	0	0		1	2.5	(0–7.4)	0	0		1	1.2	(0–3.5)
<i>Brachyspira hyodysenteriae</i>	0	0		0	0		3	20	(0–40.2)	3	3.5	(0–7.4)
Porcine Circovirus type 2	0	0		0	0		1	6.7	(0–19.4)	1	1.2	(0–3.5)

<sup>a</sup> Intestinal passage.**Table 4**  
Frequency of co-infections detected in pigs with diarrhoea.

Combinations of pathogens detected	Number of piglets
<i>Isospora suis</i> & <i>Cryptosporidium</i>	3 <sup>a</sup>
<i>Isospora suis</i> & <i>Balantidium coli</i>	3
<i>Isospora suis</i> & ETEC	1
<i>Isospora suis</i> & <i>Balantidium coli</i> & ETEC	1
<i>Isospora suis</i> & <i>Cryptosporidium</i> & ETEC	1
<i>Cryptosporidium</i> & <i>Balantidium coli</i>	1 <sup>b</sup>
<i>Cryptosporidium</i> & <i>Trichuris suis</i> & ETEC	1
<i>Balantidium coli</i> & ETEC	3
<i>Balantidium coli</i> & ETEC & <i>Clostridium perfringens</i> type C	1
<i>Balantidium coli</i> & <i>Brachyspira hyodysenteriae</i>	2
<i>Ascaris suum</i> & ETEC	1
<i>Brachyspira pilosicoli</i> & ETEC	1
<i>Brachyspira hyodysenteriae</i> & ETEC	1
Rotavirus & <i>Clostridium perfringens</i> type C	1

<sup>a</sup> PCR allowed the identification of *C. suis* in two of three cases.<sup>b</sup> PCR allowed the identification of *C. suis*.

It is possible that in some of the animals submitted for diagnosis the peak of oocysts excretion had occurred before the onset of diarrhoea or that the diarrhoea had impaired the detection of oocysts due to the dilution of the faeces [24,36]. Considering all examined pigs (with and without diarrhoea) in the current study, the prevalence for *I. suis* was 12.8% in suckling piglets, 6.7% in weaners and 7.7% in fatterers. In Europe, some estimated prevalences at the animal level for *I. suis* infection in piglets were: Germany: 10% [13]; Denmark: 19.5%; Finland: 4.5%; Iceland: 31.8%; Norway: 0.3%; Sweden: 20.1% [12]. When considering the results at the farm level, it has to be taken into account that a large number of farms, but only up to three animals/farm were analysed in this study. Therefore, we can assume that the real prevalences might be even higher. However, despite of the small amount of animals/farm analysed, the occurrence of *I. suis* was revealed in 8.1% of the farms, showing the high frequency of occurrence in Switzerland. In nationwide surveys carried out in Germany, Austria and Switzerland one decade ago, *I. suis* was detected in 77.8% of 135 herds with diarrhoea and in 37.5% of 8 herds without diarrhoea (at least 5 different pooled litters/farm were tested) [25]. [8]. In the Netherlands, *I. suis* was detected in suckling piglets from 41 of 113 (53%) litters from 17 of 25 farms (68%) [10]. Further, *I. suis* infections are also known to cause decreased daily weight gain and uneven weaning weights [24,25,36]. Accordingly, in this study it was observed that *I. suis* infections were significantly associated with emaciation and were less frequent in well-fed animals.

*Balantidium coli* is a facultative pathogen. Most of the *B. coli* infections in pigs are subclinical, and usually limited to the intestinal lumen

**Table 5**  
Frequency of parasite species detected in piglets not showing diarrhoea (n = 40).

Parasite species	Suckling piglets (n = 9)			Weaned piglets (n = 20)			Fatteners (n = 11)			Total (n = 40)		
	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)
<i>Balantidium coli</i>	0	0		9	45	(23.2–66.8)	9	81.8	(59.0–104.6)	18	45	(29.6–60.4)
Amoebae	0	0		6	30	(9.9–50.1)	9	81.8	(59.0–104.6)	15	37.5	(22.5–52.5)
<i>Isospora suis</i>	1	11.1	(0–31.6)	0	0		0	0		1	2.5	(0–7.3)
<i>Ascaris suum</i>	0	0		0	0		0	0		0	0	
<i>Trichuris suis</i>	0	0		0	0		0	0		0	0	
Strongylida	0	0		0	0		1	9.1	(0–26.1)	1	2.5	(0–7.3)
<i>Cryptosporidium</i> spp.	2	22.2	(0–39.4)	6	30	(9.9–50.1)	4	36.4	(7.9–64.8)	12	30	(15.8–44.2)

but in some cases, usually after injuries of the intestinal wall by other agents, the trophozoites can penetrate in the mucosa causing necrosis and haemorrhages mostly in colon and caecum resulting in diarrhoea, bloody faeces, tenesmus, and even death [4,37]. Moreover, *B. coli* has zoonotic potential and several cases of human infection transmitted by pigs were reported worldwide [38]. In this study, *B. coli* was commonly observed by coproscopy, however a histopathological examination is needed in order to enable any clinical association. In 10 animals with positive coproscopy, from which histopathological examination of the large intestine was performed, no association of this parasites with intestinal lesions could be observed. However, as in the frame of the *PathoPig* project the histopathological examination of the large intestine was not systematically performed to all animals, the involvement of *B. coli* in some clinical cases cannot be completely excluded.

Infections with amoebae in pigs (i.e. *Entamoeba* spp., *Iodamoeba buetschlii*, *Chilomastix mesnili*) are generally harmless and in middle Europe epidemiologically irrelevant [37]. Moreover, pigs are not considered a reservoir of *Entamoeba histolytica* for humans [24].

In this study, *B. coli* and amoebae were detected in all age groups and the frequency of infection increased with age significantly. Interestingly, both *B. coli* and amoebae were significant more frequently detected in pigs without diarrhoea. The lower frequency of detections in pigs with diarrhoea might be the result of a dilution effect on the parasite burden caused by the high water content of diarrhoeic faeces. Damriyasa and Bauer also reported an age-related increasing of *B. coli* and amoebae infections [13]. They examined 514 suckling and weaned piglets (age up to 7 weeks) in Germany, and 21% were positive for *B. coli*, 14% for *Entamoeba* sp. and 10% for *Iodamoeba* sp. In contrast, Wieler et al. [14] investigating 287 suckling and weaned piglets with diarrhoea in Germany found *B. coli* in only 0.7% of the animals. This difference might have been at least in part, due to the different diagnostic sensitivity of the detection methods used: Wieler et al. [14] used MIF (merthiolate iodine formalin concentration technique) whereas Damriyasa and Bauer [13] and the current study used the SAFC method.

*Cryptosporidium* infections in pigs have been reported worldwide [39]. In this study, *Cryptosporidium* oocysts were found in 10.3% of the suckling piglets, 15.0% of the weaned pigs and in 19.2% of the fatteners. According to several authors, the most common *Cryptosporidium* spp. in pigs are the host-specific genotypes *C. suis* and *C. scrofarum* (previously *Cryptosporidium* pig genotype II), however the zoonotic species *C. parvum* has been also occasionally found [17,19,23,24,39,40]. In this study, only two species of *Cryptosporidium* could be identified by molecular analysis: *C. suis* in piglets aged 6 weeks or younger and *C. scrofarum* in animals aged 6 weeks or older. This age-related difference was also observed in recent epidemiological studies, in which *C. suis* was predominantly found in young or pre-weaned piglets and *C. scrofarum* (or *Cryptosporidium* pig genotype II) in older animals [19,21–23,41]. Experimentally, piglets inoculated with *C. scrofarum* at 4-weeks of age remained uninfected, whereas only 5-week-old and older animals were fully susceptible to infection [42]. Cryptosporidiosis in pigs does not always result in clinical signs and the species of *Cryptosporidium* involved could play a significant role. While in experimental studies, piglets inoculated with *Cryptosporidium*-oocysts derived from calves (most likely *C. parvum*) developed depression, anorexia and gastrointestinal

signs [43,44], infections with *Cryptosporidium hominis*, *C. suis*, or *Cryptosporidium* pig genotype II were shown to be less severe [43–46].

In this study, *Cryptosporidium* infections (when considered at the genus level) were significantly more frequent in pigs without diarrhoea (30%) than in pigs with diarrhoea (7.1%). A lack of association between diarrhoea and *Cryptosporidium* oocysts shedding in pigs was observed in several studies [19,39,41,47]. However, other studies observed an association between diarrhoea and *Cryptosporidium* infection in suckling piglets [48] or between the occurrence of diarrhoea and the level of oocysts shedding within infected pig groups [20]. In addition, co-infections with *I. suis* or Rotavirus can increase the severity of clinical signs [24,43]. After molecular characterization of the *Cryptosporidium* isolates, we observed that none of the pigs infected with *C. scrofarum* (n = 8) had diarrhoea; whilst diarrhoea was present in three out of four piglets infected with *C. suis*. However, two of these piglets were co-infected with *I. suis* and one with *B. coli*. Although the number of analysed samples is small, this would be in agreement with the study from Hamnes et al. [48] in Norway, which suggested that *C. suis* infections may contribute to diarrhoea in suckling piglets whilst animals infected with *Cryptosporidium* pig genotype II (syn. *C. scrofarum*) may not develop clinical signs. Regarding the zoonotic risk, the relevance of pigs as a source for *Cryptosporidium* infections for humans is apparently limited [24]. The zoonotic species *C. parvum* is not highly prevalent in pigs and the pig-adapted species are not frequently detected in humans. Nevertheless, sporadic cases of *C. suis* infections have been reported both in immunocompromised [49–51] and in immunocompetent humans worldwide [29,52]. Moreover, infections with *C. scrofarum* have been also detected in immunocompetent humans [53].

*Strongyloides ransomi* was not found in this study. This is consistent with other studies from Europe in which *S. ransomi* was not detected, i.e. the Netherlands [10]; Denmark, Finland and Norway [12] or Germany [14]; or it was rare, i.e. in 1 of 18 farms in Germany [8]; in 1 of 90 conventional pig farms and 1 of 20 free-range farms in Switzerland [26]. Thus, *S. ransomi* seems not to play an important role in European swine production nowadays.

Interestingly, *Giardia* was not found in any of the analysed pigs in the present study, although adequate diagnostic methods such as SAFC were used. *Giardia* infections in pigs were reported worldwide with very variable prevalences, however they did not seem to be associated with clinical illness [24,48].

*A. suum* and *T. suis* eggs were not expected in faecal samples from suckling piglets due to the relatively long prepatent periods (~6 weeks) of these parasites [4], thus the detection of *T. suis* eggs in one suckling piglet (Tables 1 and 3) has to be considered as intestinal passage (e.g. through accidental ingestion of eggs attached to the skin of the sow during suckling or while foraging in a contaminated pen). However, *A. suum* and *T. suis* were also very rarely detected in weaners and fatteners, appearing not to be common parasites in the set of pigs analysed in this study. Also Strongylida-eggs were seldom found in the examined pigs. This could be due to a low infection pressure or to a good response to routine anthelmintic treatments, as weaners are often dewormed before entering fattening.

Analyses of the results obtained after the first year (2014) of the *PathoPig* project showed that the cause of disease or death could be

assessed for 84% of all submitted cases (a total of 623 pigs of all age groups from 371 farms were analysed during 2014; one case was defined by 1 to 3 submitted animals/farm showing the same clinical signs) and gastrointestinal problems represented the main cause of submission (i.e. 56% of the cases). ETEC infections were found to be involved in around 44% of the cases with gastrointestinal problems. However, in the frame of *PathoPig* project not all animals were systematically tested against all possible infectious agents. Complementary tests were decided by the pathologists based on anamnesis data and/or necropsy findings and parasites were a priori only seldom suspected as cause of disease at necropsy, therefore, coproscopical examinations were only rarely ordered as complementary diagnostic methods by the pathologists. According to the 2014 year's report, parasites seemed to be negligible as cause of gastrointestinal problems in Swiss pigs. Only in two farms each, *I. suis* and *T. suis* infections were recognized as cause of disease [27]. However, in the frame of this parallel study on a subset of those samples, all piglets were systematically tested by three coproscopical methods independently of the initial suspicion of parasitic disease, revealing a higher frequency of parasitic infections that in some cases could have contributed to the observed clinical signs.

## 5. Conclusion

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.parint.2016.09.005>.

## Conflict of interest

The authors declare that they have no conflict of interest.

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