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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.

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: ≤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-fuchsine 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

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