Prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* in pigs in Lusaka, Zambia

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This study was aimed at determining the prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* in pigs which were being raised in intensive management systems. Faecal samples were collected from pigs of all age groups from three different piggery units. Samples were collected directly from the rectum for piglets and weaners and from the floor within 2 min – 5 min of excretion for sows and boars. At the time of collection, faecal consistency was noted as being normal, pasty or diarrhoeic. Samples were analysed further using the Merifluor® *Cryptosporidium*/*Giardia* immunofluorescence assay. All piggeries had at least one pig infected with either parasite. From a total 217 samples collected, 96 (44.2%; confidence interval [CI] = 37.6% – 50.9%) were positive for *Cryptosporidium* spp., whilst 26 (12%; CI = 7.6% – 16.3%) had *G. duodenalis* parasites. Of all the pigs, 6.9% (15/217) harboured both parasites. With regard to *Cryptosporidium* spp. infection, statistically significant differences were observed amongst the three units (p = 0.001), whereas no significant differences were observed for *G. duodenalis* infection (p = 0.13). Prevalence was higher in weaners as compared to other pig classes for both parasites, with significant differences being observed for *G. duodenalis* infection (p = 0.013). There was, however, no difference in infection between male and female pigs for both parasites. Furthermore, most infections were asymptomatic. From the study results it was clear that *Cryptosporidium* spp. and *G. duodenalis* infections were prevalent amongst pigs in the piggeries evaluated and, as such, may act as a source of infection for persons who come into contact with them.

Introduction

Cryptosporidium spp. and *Giardia duodenalis* are protozoan parasites infecting a wide range of domestic animals including livestock, dogs and cats (Fayer 2004; Thompson 2004; Xiao & Fayer 2008). Both cryptosporidiosis and giardiosis, the respective diseases which these parasites cause, are reported to be zoonoses, although the status of giardiosis as a zoonosis is still debatable (Monis & Thompson 2003; Smith *et al*. 2006). Each genus comprises a complex of species and genotypes, some of which are pathogenic and some specific to particular hosts (Caccio *et al*. 2005; Smith *et al*. 2006; Xiao *et al*. 2004). Of the eight known assemblages, only two assemblages of *G. duodenalis*, A and B, are considered to be zoonotic (Feng & Xiao 2011).

Transmission occurs directly or indirectly by the ingestion of either oocysts or cysts from infected individuals, via contaminated water, food and pasture (Castro-Hermida *et al*. 2005; Graczyk, Fayer & Cranfield 1997; Preiser, Preiser & Madeo 2003; Xiao, Herd & Bowman 1994; Xiao, Herd & Rings 1993). With cryptosporidiosis, clinical manifestations vary and depend on the age and health status of the host, the infective dose and the genetic background of the parasite (Xiao & Fayer 2008), with stressed and immunologically compromised animals being more susceptible (Enemark *et al*. 2002; Ramirez, Ward & Sreevatsan 2004). In young livestock, the disease can be debilitating and cause severe illness and/or death (De Graaf *et al*. 1999). In calves, cryptosporidiosis is often characterised by profuse watery diarrhoea of acute onset, anorexia, dehydration and weight loss (De Graaf *et al*. 1999; Fayer *et al*. 1998). For naturally infected pigs, the infection is typically asymptomatic even in young animals (Maddox-Hyttel *et al*. 2006; Ramirez *et al*. 2004). However, symptomatic infections do occur in young piglets less than three weeks of age, with inappetance, depression, vomiting, diarrhoea and mortality being the most common signs (Enemark *et al*. 2002; Enemark *et al*. 2003; Rotkiewicz *et al*. 2001).

On the other hand, *G. duodenalis* infections in domestic animals are often asymptomatic, although clinical disease does occur in young animals (Geurden *et al*. 2006; O'Handley *et al*. 1999; Robertson *et al*. 2000). In calves, *G. duodenalis* mostly affects those between five and ten weeks of age (Thompson 2000), but infections may also occur in calves as young as four days (Xiao & Herd 1994) and in adult cows (Maddox-Hyttel *et al*. 2006). Infection may result in numerous episodes of diarrhoea, which, in turn, adversely affects production and results in economic loses for producers (Xiao 1994). Other clinical signs may include chronic pasty diarrhoea, lethargy, weight

loss and poor condition (Thompson 2000). The occurrence of *G. duodenalis* in pigs has been reported in all age groups, from piglets to sows and boars, from Europe and North America (Langkjaer *et al*. 2007; Maddox-Hyttel *et al*. 2006; Olson *et al*. 1997; Xiao *et al*. 1994), with age specific prevalence ranging from 7% – 84%.

Little is known about the prevalence of *Cryptosporidium* spp. and *G. duodenalis* in pigs in Africa and in particular Zambia. The aim of the present study was to determine the prevalence of *Cryptosporidium* spp. and *G. duodenalis* in pigs of all age groups from three piggeries in Zambia.

Materials and methods

Study area, sample collection and analysis

The University of Zambia, located in the country's capital, Lusaka, has three piggery units that are run by three different departments of the university: the Schools of Veterinary Medicine (Ve) and Agricultural Sciences (Ag), located within the campus premises, and on Liempe Farm (Li), located in a neighbouring district about 20 km away from the main campus. The piggery units are used for teaching and commercial purposes. The present study was carried out in these units to determine the infection status of the pigs with *Cryptosporidium* spp. and *G. duodenalis.* Management is intensive and similar on all the three units. Weaned piglets were housed in groups of 10–20 depending on the size of the pen, whilst sows and boars were housed individually. Piglets were housed together with their dam. All pig houses had solid floors and all the pigs received piped water.

The study was carried out from March to June 2011. Visits were made to each unit and a single faecal sample was collected from each pig. The faecal samples were collected rectally using a gloved hand from piglets and weaners. Efforts were made to collect the faecal samples from the rectum of sows and boars and, where not possible, the samples were picked from the floor within 2 min – 5 min of being dropped. These latter samples were taken from the upper area of the droppings to ensure that the sample had not been potentially contaminated through contact with the floor. Faecal consistence was noted and categorised as either normal (hard, formed stool), pasty (pasty, unformed) or diarrhoeic (soft, unformed) at the time of collection. The samples were transported immediately to the laboratory in cool containers packed with ice packs, where they were placed in clean stool specimen containers containing 10% formalin. One part of stool was mixed with three parts of 10% formalin and fixed at room temperature for at least 1 h before further analysis.

A commercial immunofluorescence assay (Merifluor® *Cryptosporidium/Giardia* IFA) (Meridian Diagnostics Inc., Cincinnati, USA) for the detection of *Cryptosporidium* spp. and *G. duodenalis* was used to identify *Cryptosporidium* spp. oocysts and *G. duodenalis* cysts according to manufacturer's instructions. (Oo)cysts were visualised using an immunofluorescence microscope. A sample was identified as positive if at least one (oo)cyst was identified under the microscope.

Statistical analysis

Data were entered in an Excel spreadsheet and verified for accuracy before being pasted into STATA data editor. Statistical analysis was carried out using computer software STATA Version 10.1 (2007). Proportions, differences in infections amongst the piggeries, age groups and gender were determined by chi-square test and Fisher's exact test, where appropriate. Data were reported as absolute values or as percentages. All results were considered significant at $p < 0.05$.

Ethical considerations

Permission to conduct the study in the piggery units was sought from the respective piggery managers. Faecal samples were collected by trained veterinarians adhering to the Zambian regulations and guidelines on animal husbandry.

Results

Overall prevalence

A total of 217 faecal samples were collected from the three piggery units, comprising samples from suckling piglets aged 2–5 weeks (n = 32), weaned piglets aged 7 weeks – 6 months (n = 163), gilts or sows (n = 15) and boars (n = 7) both aged more than 6 months. Of the total samples collected, 112 were from female pigs and 105 from male pigs. The overall prevalence of *Cryptosporidium* spp. was 44.2% (96/217; confidence interval [CI] = 37.6% – 50.9%), whilst that of *G. duodenalis* was 12.0% (26/217; CI = 7.6% – 16.3%). Of all the pigs, 6.9% (15/217) harboured both parasites. All farms had at least one pig infected with either parasite.

Prevalence by farm and pig class

Prevalence amongst the three farms or locations in the different age groups and pig classes is indicated in Table 1. On all the farms, the number tested was higher for weaners and so was the number of positive animals. With regard to *Cryptosporidium* spp. infection, statistically significant differences were observed amongst the three farms (p = 0.0010). Between farm differences were also observed for Li and Ve (p = 0.0110) and between Ag and Ve ($p < 0.0001$) but not for Li and Ag (p = 0.0770). On the other hand, no significant differences were observed amongst the farms for *G. duodenalis* infection (p = 0.1300), as was the case for between farms; Li and Ag (p = 0.3100), Li and Ve (p = 0.2600). However, differences were observed between Ve and Ag (p = 0.0430).

The overall prevalence of *Cryptosporidium* spp. in the different pig classes was 31.3% (10/32) in piglets, 46.6% (76/163) in weaners, 53.3% (8/15) in sows and 28.6% (2/7) in boars. On the other hand, the overall prevalence of *G. duodenalis* in the pig classes was 6.3% (2/32) in piglets, 10.4% (17/163) in weaners, 40.0% (6/15) in sows and 14.3% (1/7) in boars. Significant differences were further observed amongst the pig classes for *G. duodenalis* (p = 0.0130), whilst no difference was observed for *Cryptosporidium* spp. infection (p = 0.3000).

TABLE 1: Prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* arranged by pig class, sampled from three piggeries in Lusaka, Zambia over the study period from March to June 2011.

Farm	Pig class	Number tested	*Cryptosporidium* spp.			*Giardia duodenalis*		
			Number + ve†	%	95% CI‡	Number + ve†	%	95% CI‡
Ag	Piglets	2	2	-	-	0	-	-
	Weaners	26	18	-	-	5	-	-
	Gilts or sows	3	2	-	-	1	-	-
	Boars	4	2	-	-	1	-	-
Subtotal	-	**35**	**24**	**68.6**	**52.4–84.8**	**7**	**20.0**	**6.1–33.9**
Li	Piglets	8	2	-	-	1	-	-
	Weaners	103	46	-	-	10	-	-
	Gilts or sows	7	5	-	-	4	-	-
	Boars	2	0	-	-	0	-	-
Subtotal	-	**120**	**53**	**44.2**	**35.2–53.2**	**15**	**12.5**	**6.5–18.5**
Ve	Piglets	22	6	-	-	1	-	-
	Weaners	34	12	-	-	2	-	-
	Gilts or sows	5	1	-	-	1	-	-
	Boars	1	0	-	-	0	-	-
Subtotal	-	**62**	**19**	**30.6**	**18.8–42.4**	**4**	**6.5**	**0.2–12.7**
Total	**All**	**217**	**96**	**44.2**	**37.6–50.9**	**26**	**12.0**	**7.6–16.3**

Ag, School of Agricultural Sciences piggery unit; Li, Liempe farm piggery unit; Ve, School of Veterinary Medicine piggery unit.
†, Number of animals positive for *Cryptosporidium* spp. or *Giardia duodenalis.*
‡, 95% Confidence Interval.

Prevalence by sex

The prevalence was similar in both male pigs (41.9%; 44/105) and female pigs (46.4%; 52/112) for *Cryptosporidium* spp. (p = 0.50). This was also the case for *G. duodenalis*, with prevalences of 9.5% (10/105) and 14.3% (16/112) in male and female pigs, respectively (p = 0.28).

Association between infection and faecal consistency

Most of the faecal samples collected were formed and had a normal consistency. Only four (1.8%) of the samples were diarrhoeic and nine (4.2%) were pasty. Half of the diarrhoeic faeces contained *Cryptosporidium* spp. oocysts, but the parasites were absent in the pasty samples. Almost half (46.1%; 94/204) of the normally formed faeces had *Cryptosporidium* spp. parasites. The difference amongst the three categories was significant (p = 0.024).

Trustworthiness

The test used in the present study (Merifluor® *Cryptosporidium*/*Giardia*) is reliable and has been used in many reported studies. Parasite identification, however, requires an experienced technician or researcher. The samples in the present study were analysed by an experienced researcher and verified independently by another. The study was conducted on a small scale as a result of limited funds. A larger sample size with more piggeries included would have helped to make more generalised conclusions.

Discussion

In the present study, the prevalence of *Cryptosporidium* spp. and *G. duodenalis* was determined for three university farms in Zambia. This is the first report of the two protozoan parasites in Zambian pigs. The overall prevalence of *Cryptosporidium* spp. was 44.2%, a result that is significantly higher than what has been reported elsewhere. For example, Olson *et al*. (1997) reported a prevalence of 11.0% from 236 Canadian pigs, whilst Fiuza *et al*. (2011) reported a prevalence of 2.2% from 91 pigs in Rio de Janeiro, Brazil. In addition, Kváč *et al*. (2009) reported a prevalence of 21.1% from 413 samples in the Czech Republic, whilst in Spain, Quílez *et al*. (1996) reported a prevalence of 21.9% from 620 pigs. Comparable results for *Cryptosporidium* spp. have, however, been reported in pig manure from different farms in Canada in which 44.3% of the samples were positive for *Cryptosporidium* spp. and 50.8% had *G. duodenalis* parasites (Farzan *et al*. 2011); the *G. duodenalis* percentage being much higher than what we report in the present study.

The overall prevalence of *G. duodenalis* in the present study was 12%. Our results are in agreement with those reported by Olson *et al*. (1997) in which they found a prevalence of 9% in pigs from Canadian farms and that by Xiao *et al*. (1994) (7%) from pigs in Ohio, USA. Nevertheless, higher prevalence rates (44%) have been reported elsewhere (Koudela *et al*. 1991). The differences in the prevalence of both *Cryptosporidium* spp. and *G. duodenalis* in the present study and that from other studies highlighted above can be attributed to differences in sample sizes, as well as sample processing and detection methods.

The results in the present study are reported with caution. The prevalences for both *Cryptosporidium* spp. and *G. duodenalis* are probably underestimated because only one sample was collected and tested. It has been shown that parasite excretion can be intermittent (Buret *et al*. 1990) and therefore it is suggested that three samples be collected consecutively and tested (Van Gool *et al*. 2003). The triple sample collection was not conducted in the present study because of limited resources.

In all the three piggeries, prevalence of *Cryptosporidium* spp. was higher than that of *G. duodenalis* for all pig classes. The prevalence was higher in weaners than in any other pig class

for both parasites, but significant differences amongst the three units was only observed for *G. duodenalis*. This finding of higher infection rates in weaners corroborates reports by other authors (Maddox-Hyttel *et al*. 2006; Quílez *et al*. 1996) in which they found pigs aged one to six months to be infected with *Cryptosporidium* spp., an indication that infection is more common in younger animals. Establishment of infection is reported to be delayed until weaning (Quílez *et al*. 1996). However, Olson *et al*. (1997) reported higher prevalence in animals older than six months compared to younger animals.

Of the infected pigs, 98% were asymptomatic, whilst only two were symptomatic. This is in agreement with previous reports that indicated largely asymptomatic infections in most pigs (Quílez *et al*. 1996; Ramirez *et al*. 2004) for both *Cryptosporidium* spp. and *G. duodenalis*.

Conclusion

The present study has demonstrated that *Cryptosporidium* spp. and *G. duodenalis* are prevalent in pigs and most infections are asymptomatic. These pigs may act as a source of infection for humans through direct contact with faeces and faecal contaminated materials. It is therefore important that control measures be considered and put in place to prevent such infections. Further studies are warranted to determine the infection status of other pigs in other areas of the country, including free range pigs which are common in rural areas.

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Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this paper.

Authors' contributions

J.S. (University of Zambia) and K.E.M. (University of Zambia) were responsible for the design of the study and the sample collection. J.S. (University of Zambia) performed the laboratory analysis of the samples and the statistical analysis of data. J.S. (University of Zambia) wrote the first draft of the manuscript. J.S. (University of Zambia) and K.E.M. (University of Zambia) wrote subsequent drafts of the manuscript.

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