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Technical note

The prevalence and molecular characterisation of *Cryptosporidium* spp. in small ruminants in Zambia

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Abstract

Although *Cryptosporidium* spp. have been associated with diarrhea and mortality in sheep and goats, this is the first study to estimate the prevalence of *Cryptosporidium* spp. in small ruminants in Southern Africa. Between August 2003 and February 2004, a cross-sectional study with single random sampling was carried out in three Zambian provinces: Central, Southern and Lusaka province. Faecal samples from lambs and goat kids less than 3 months of age were collected and examined using the TechLab *Cryptosporidium* ELISA. Faecal samples of 257 animals were collected: 152 lambs on 18 farms and 105 goat kids on 13 farms. The prevalence of *Cryptosporidium* spp. in lambs and goat kids was 12.5% and 4.8%, respectively and 30% of the sheep and goat farms had at least one positive animal at the time of the visit. A subset of the positive samples was withheld for molecular characterisation. Based on sequences obtained after amplification of the 70 kDa heat shock protein and the *Cryptosporidium* 18SrRNA gene, *C. parvum* was identified in all but one of the animals. In this lamb *C. suis* was identified. The results of the present study indicate that the animal prevalence in lambs and goats kids is rather low. However, the identification of *C. parvum* illustrates that small ruminants might be potential reservoir for human infection in Zambia.
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Keywords: *Cryptosporidium*; Sheep; Goats; Prevalence; Genotyping; Zambia

1. Introduction

Cryptosporidium parvum is considered one of the major gastrointestinal pathogens in young ruminants (de Graaf et al., 1999) and has been associated with diarrhea and death in both natural and experimental infections in goat kids and lambs (Angus et al., 1982; Koudela and Jiri, 1997; Johnson et al., 1999; Castro-Hermida et al., 2002). Most data on cryptosporidiosis in farm animals concern calves and only a few cross-sectional studies have been performed to estimate the prevalence of *C. parvum* in small ruminants. In sheep prevalences between 10.1% and 68.3% have been described in young animals (Olson et al., 1997; Abd-El-Wahed, 1999; Majewska et al., 2000; Causape et al., 2002) and in goats between 11.0% and 35.2% (Rossanigo et al., 1987; Matos-Fernandez et al., 1993; Noordeen et al., 2000; Watanabe et al., 2005), indicating that *C. parvum* might be equally prevalent in small ruminants as in calves. Not only in ruminants, but also in man *Cryptosporidium* is reported on a regular basis, especially in sub-Saharan Africa. In Zambia, the prevalence of *Cryptosporidium* in man ranges from 18 to 32% (Conlon et al., 1990; Nchito et al., 1998;

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Amadi et al., 2001). Zoonotic transmission can occur through direct contact or through contamination of drinking water. Small ruminants are considered as a potential reservoir for human infection, although recent findings in Western Australia indicate that the public health risk of sheep-derived *Cryptosporidium* spp. might be overestimated (Ryan et al., 2005). In this study, the prevalence of *Cryptosporidium* in small ruminants on Zambian farms was estimated and a subset of the positive samples was sequenced to establish the zoonotic potential of the isolates.

2. Materials and methods

Between August 2003 and February 2004, a cross-sectional study on 18 randomly selected sheep farms and 13 goat farms was carried out in three Zambian provinces: Central, Southern and Lusaka province. Faecal samples from all animals less than 3 months of age at the time of the visit were collected. The samples were stored at −20°C for ELISA (TechLab *Cryptosporidium* test, Blacksburg, Va.) and subsequent genotyping. The monoclonal antibody based ELISA was used as instructed by the manufacturer. DNA was extracted using the QIAamp® Stool Mini Kit (Qiagen) according to the manufacturer's instructions, incorporating an initial step of three freeze-thaw cycles (freezing in liquid nitrogen for 5 min and heating at 95 °C for 5 min) in the protocol to maximise oocyst lesion. The eluted DNA was dissolved in 15 μl ultra-pure water. Previously described nested PCR protocols for the characterisation of the HSP-70 gene (Morgan et al., 2001) and for the 18S rDNA gene (Ryan et al., 2003) were used in this study. Amplification products were subsequently visualized on 1.5% agarose gels with ethidium bromide. A positive (Plasmid DNA) and negative (PCR water) control sample was included in each PCR reaction. PCR products were purified using the Qiaquick® purification kit (Qiagen) and fully sequenced using the Big Dye Terminator V3.1 Cycle sequencing Kit (Applied Biosystems). Sequencing reactions were analysed on a 3100 Genetic Analyzer (Applied Biosystems) and assembled with the program Seqman II (DNASTAR, Madison WI, USA). The calf prevalence is calculated as the number of animals with a positive test result on ELISA divided by the total number of animals. The farm prevalence is the number of farms with at least one positive animal at the time of the visit divided by the total number of farms. Determination of the correlation between age or faecal consistency and infection was performed using χ^2 or Fisher's exact test. Analyses were carried out using computer software SPSS (Statistical Package for Social Sciences). Results were considered to be significant at $P < 0.05$.

3. Results and discussion

To the authors knowledge this is the first study to estimate the prevalence of *Cryptosporidium* spp. in small ruminants in Southern Africa. Faecal samples of 257 animals were collected: 152 lambs on 18 farms and 105 goat kids on 13 farms. The lambs (58% male and 42% female) were Blackheaded Dorper and had a mean age of 22 (2–49) days. The goat kids (39% male and 61% female) were African dwarf goats and had a mean age of 20 (7–56) days. There was an average number of 8.4 (1–28) lambs and 7.9 (1–17) goat kids on these farms. The number of positive lambs was 19 (prevalence (p) = 12.5%) and the number of positive goat kids was 5 (p = 4.8%). Both in lambs (Olson et al., 1997; Abd-El-Wahed, 1999; Majewska et al., 2000; Causape et al., 2002) and in goat kids (Rossanigo et al., 1987; Matos-Fernandez et al., 1993; Noordeen et al., 2000; Watanabe et al., 2005) the prevalence was rather low compared to prevalence studies in other parts of the world, although both for sheep and for goats 30% of the farms had at least one positive animal at the time of the visit. In Africa similar prevalences were found in extensively reared calves (Mtambo et al., 1997; Geurden et al., 2006) altough a higher calf prevalence was described in Uganda (Nizeyi et al., 2002). The low prevalence in small ruminats might be imputed to differences in climate and husbandry system. Most sheep and goats in Zambia are managed extensively, in large outdoor paddocks or on pasture, where oocysts are dispersed on a large surface, and stocking rates are low, resulting in a low infection pressure. Furthermore, the sampling in this study took place from August to February, which includes the hot season. Exposure of infective oocysts to heat and direct sunlight (Walker et al., 2001), might reduce the oocyst viability, resulting in a reduced infection pressure and prevalence. Similar reflections were made for extensive cattle husbandry systems in Zambia (Geurden et al., 2006). The higher prevalence in small ruminants in the dry agroclimatic zone in Sri Lanka (Noordeen et al., 2000), was probably due to poor hygienic standards and nutrional stress, impairing the development of acquired immunity. In this study, there was no significant correlation between age and oocyst excretion or between faecal consistency and oocyst excretion.

Sequences were obtained for seven isolates: six from lambs, one from each positive farm and from one goat kid (Table 1). One sample did not amplify on the 18S rRNA gene. In five lambs and in the goat kid sample *C. parvum* (genotype 2) was identified, based on a high homology (>96%) with previously published sequences, both for the 18SrRNA gene (Ryan et al., 2003) and for the HSP-70 gene (Sulaiman et al., 2000; Ryan et al., 2003). In one lamb the pig genotype of *C. parvum* or *Cryptosporidium suis* (Ryan et al., 2004) was identified (homology > 97%). Although there is little information on the genotypes found in sheep and goats, both *C.*

Table 1
The results of the genotyping for sheep and one goat using the 70 kDa heat shock protein (HSP-70) and the small subunit rRNA gene (18SrRNA)

Sample	HSP-70	18SrRNA	Animal
J21	*C. parvum*	*C. parvum*	Goat
J26	*C. suis*	*C. suis*	Sheep
48	*C. parvum*	*C. parvum*	Sheep
49	*C. parvum*	*C. parvum*	Sheep
50	*C. parvum*	*C. parvum*	Sheep
51	*C. parvum*	*C. parvum*	Sheep
54	*C. parvum*	NA	Sheep

One sample did not amplify (NA) on the 18SrRNA gene.

parvum (Chalmers et al., 2002) and *C. suis* (Ryan et al., 2005) have previously been reported in sheep. In goats only *C. parvum* (Chalmers et al., 2002) has been identified. Despite the low prevalence of oocysts found in lambs and goats, the identification of the zoonotic *C. parvum* in these samples indicates that sheep and possibly goats should be considered as reservoirs for human infection in Zambia, especially with the high HIV prevalence (Conlon et al., 1990) and intensive contact between animals and man. Although in contrast with findings in Western Australia where the cervid and the new bovine B genotype were predominantly identified and where *C. parvum* was not identified (Ryan et al., 2005), the genotyping results in the present study are in accordance with the genotyping results in sheep and goats in the UK where *C. parvum* was identified (Chalmers et al., 2002), indicating regional differences in the occurence of zoonotic *Cryptospridium* genotypes in sheep and goats.

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