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Short Communication

Cryptosporidiosis in two alpaca (*Lama pacos*) holdings in the South-West of England

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Abstract

Cryptosporidiosis was investigated on two alpaca (*Lama pacos*) holdings in the South-West of England. Diagnosis was initially confirmed in a cria with diarrhoea from each holding. Cohort faeces samples were subsequently collected and examined for presence of *Cryptosporidium* oocysts by immunofluorescence microscopy. On the first holding, 30 samples (24 adults, 6 crias) were tested, and oocysts were detected in three of the cria samples but in none of the adults. On the second holding, 14 floor faeces samples representing apparently healthy crias and one faeces sample from a cria with diarrhoea were collected. Oocysts were detected in four of the "healthy" faeces samples and the sample of diarrhoeic faeces. All isolates were confirmed as *Cryptosporidium parvum* using polymerase chain reaction restriction fragment length polymorphism of the cryptosporidium oocyst wall protein (COWP) and ssu rRNA genes. Sequence analysis of a 741 bp region of ssu rDNA was carried out on nine of these and revealed high sequence homology with previously reported *C. parvum* isolates. This investigation highlights the possibility of alpaca crias subclinically shedding oocysts, which has implications for epidemiology and transmission in animals as well as raising zoonotic concerns for human contacts. Gene sequencing of UK isolates from South American camelids is also described for the first time.

Keywords: South American camelid; Alpaca; Cria; Diarrhoea; Cryptosporidiosis; *Cryptosporidium parvum*

The genus *Cryptosporidium* is principally a gastrointestinal protozoan parasite of animals and humans, affecting a wide host range (Fayer et al., 2000). Epidemiological data in animals mostly relate to cattle, with infections being asymptomatic or resulting in clinical signs, usually neonatal diarrhoea (Casemore et al., 1997). Infection in animals also represents a potential zoonotic risk (Casemore et al., 1997; Fayer et al., 2000). *Cryptosporidium* has been reported in South American camelids (SACs), including alpacas (*Lama pacos*), llamas (*Lama glama*) and guanacos (*Lama guanicoe*). However, published diagnoses in these species are rare (a summary of those known to us is given in Table 1), with only two previous isolates confirmed as *C. parvum*. This short communication describes the investigation of cryptosporidiosis on two alpaca holdings in the South-West of England.

At the time of investigation, holding 1 had 24 adult alpacas, 1–8 years old, and seven crias, born between June and October 2005. In November 2005, a 4-week-old cria suddenly developed diarrhoea and recumbency, and died 3 days later. Holding 2 had 180 adult alpacas, approximately

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Table 1
Previous reports of *Cryptosporidium* in South American camelids

Authors	Main clinical signs	Diagnostic method	Other comments
Hovda et al. (1990)	Diarrhoea	Not given	Case report of a llama cria with post-operative diarrhoea from which *Cryptosporidium* spp. was isolated
Rickard (1994)	Diarrhoea	Faecal flotation	Review of llama parasites. The author reports cryptosporidiosis in crias with diarrhoea, but number of cases observed is not given
Spano et al. (1997, 1998); Morgan et al. (1998)	Clinical details not given	PCR, PCR-RFLP, or sequence analysis of multiple loci including COWP, poly (T), ITS1, TRAP-C1, RNR, 18 S rDNA, acetyl-CoA synthetase	These papers include the A1 alpaca isolate from Peru in comparison with isolates from other species using various molecular techniques
Bidewell and Cattell (1998)	Diarrhoea and dehydration	Modified Ziehl–Neelson stained intestinal smears	Reports cryptosporidiosis in three alpaca crias, 9–30 days old
Gómez et al. (2000)	No clinical signs associated with *Cryptosporidium* infection were identified	Modified Ziehl–Neelson stained faecal smears	Reports screening a zoo collection in Spain for *Cryptosporidium* spp. Of 18 faecal samples from three adult guanacos, six were positive, with at least one positive sample from each guanaco tested
Cebra et al. (2003)	Diarrhoea	TB auramine M stained faecal smears	Survey of potential enteric pathogens in 45 llama and alpaca crias based on faecal examination. Four (9%) were positive for *Cryptosporidium* spp. in crias between 10 and 45 days old
Ryan et al. (2003)	Clinical details not given	PCR sequencing of 18S rDNA and HSP-70 genes	Reports *Cryptosporidium parvum* from an alpaca in the Czech Republic, which showed close relationship to the cattle genotype
Shapiro et al. (2005)	Diarrhoea	Not given	Reports highlights of camelid diagnoses from necropsies at University of Guelph's Animal Health Laboratory, Canada, from 1998 to 2004. Of 93 camelids examined, cryptosporidiosis was diagnosed on one farm affecting multiple alpaca crias (exact number not given)
Stewart et al. (2005)	Clinical details not given	Not given	Report of zoonoses survey in Scotland between 1993 and 2002. Alpaca is listed as an animal species in which *Cryptosporidium* spp. was recorded
Whitehead and Anderson (2006)	Diarrhoea	Not given	Reviews causes of neonatal diarrhoea in llamas and alpacas. As well as reviewing the previous literature, this paper reports *Cryptosporidium* spp. as the cause of diarrhoea in 25.9% of 58 cases investigated at the Ohio State University between 1999 and 2004, affecting crias between 7 and 100 days old

2 years old, and 26 crias, born between October 2005 and January 2006. In January 2006, a 5-week-old cria developed diarrhoea and recumbency, and was euthanased in extremis. Following post mortem examination, cryptosporidiosis was confirmed in both crias by demonstration of characteristic oocysts in modified Ziehl–Neelson (mZN)-stained smears of intestinal contents, and histopathological examination of the small intestine demonstrated *Cryptosporidium*-like structures in the brush border of enterocytes. In the cria from holding 1, this was associated with mild crypt hyperplasia and moderate mixed infiltrates of lamina propria including eosinophils. In the cria from holding 2, there was marked villous stunting and multifocal bacterial colonisation with attaching and effacing organisms similar morphologically to *Escherichia coli* (AEEC).

Faeces samples representing cohort alpacas were subsequently collected from each holding and screened for further evidence of *Cryptosporidium* by a fluorescent antibody test (FAT) (Crypto-Cel, Cellabs), which has improved sensitivity for *Cryptosporidium* oocysts over tinctorial stains (Arrowood, 1997). On holding 1, visited in November 2005, rectal faeces were collected from the remaining alpacas (24 adults, six crias), which appeared healthy. On holding 2, visited in February 2006, apparently healthy crias were separated from their dams and penned prior to sampling. Fourteen floor faeces samples representing these crias and one faeces sample from another cria with diarrhoea were collected. All samples were concentrated by two-step sedimentation (initial brief low speed centrifugation to remove larger debris, followed by sedimentation of oocysts at 1100 *g*) prior to FAT screening.

Of the 30 samples collected from holding 1, three of the cria samples were positive by FAT; cryptosporidia were not detected in any of the adult samples. Of the 14 floor samples collected from holding 2, four were positive by FAT. The sample from a cria with diarrhoea, taken during the same visit, was also positive by FAT. The original isolates from dead crias on both holdings also tested positive by this method.