

Molecular identification of *Cryptosporidium* spp. in alpacas (*Vicugna pacos*) in China

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ABSTRACT

Cryptosporidium is a ubiquitous protozoan in human and animals. To investigate the genetic diversity of *Cryptosporidium* spp. in alpaca (*Vicugna pacos*) in China, 484 fecal samples from alpacas were collected at nine sites, and *Cryptosporidium* spp. were screened with PCR amplification of the small subunit ribosome RNA (SSU rRNA) locus. *Cryptosporidium* spp. infected 2.9% (14/484) of the alpacas. Of the nine collection sites, two were positive for *Cryptosporidium*, Wensu (3.0%, 3/100) and Qinghe (31.4%, 11/35). Three *Cryptosporidium* species were identified: *C. parvum* (n = 2), *C. ubiquitum* (n = 1), and *C. occultus* (n = 11). *Cryptosporidium parvum* and *C. ubiquitum* were further subtyped with the 60-kDa glycoprotein locus (*gp60*). The two *C. parvum* isolates were subtype IIdA15G1, but the one *C. ubiquitum* isolate was not subtyped successfully. A phylogenetic analysis indicated that the *Cryptosporidium* isolates clustered with previously identified species. To our knowledge, this is the first report of *Cryptosporidium* infections in alpacas in China and provides baseline data for the study of *Cryptosporidium* in alpacas in China.

1. Introduction

Cryptosporidium is a ubiquitous apicomplexan parasite that mainly infects the gastrointestinal epithelium of a wide range of human and animal hosts (Checkley et al., 2015; Ryan et al., 2018). *Cryptosporidium* infections are recognized as one of the major causes of moderate to severe diarrhea in developing countries (Ryan et al., 2014). The pathogenicity of *Cryptosporidium* infection varies with the species of parasite involved and the type, age, and immune status of the host (Thomson et al., 2017; Feng et al., 2018). At least 38 *Cryptosporidium* species have been identified, and zoonotic *Cryptosporidium* infections play an important role in both developed and developing countries (Xiao, 2010; Feng et al., 2018). Since the first report of *Cryptosporidium* spp. infections in young alpacas in 1998 (Bidewell and Cattell, 1998), cryptosporidiosis has been recognized as a common cause of diarrhea in young (preweaned) alpacas (Starkey et al., 2007; Waitt et al., 2008).

Several studies have demonstrated natural infections of *Cryptosporidium* spp. in South American camelids, including alpacas (*Vicugna pacos*), llamas (*Lama glama*), and guanacos (*L. guanicoe*) in the past decades (Rulofson et al., 2001; Whitehead and Anderson, 2006; Waitt et al., 2008; Burton et al., 2012; Koehler et al., 2018). One cryptosporidiosis outbreak among alpacas caused by *C. parvum*

involved six alpaca crias from a single farm, and importantly, three people involved in caring for the crias on that farm were subsequently diagnosed with cryptosporidiosis (Starkey et al., 2007). Another study demonstrated that not only can older weaned alpacas succumb to severe clinical cryptosporidiosis attributable to *C. parvum*, but that these animals can act as potential sources of zoonotic infection (Wessels et al., 2013).

Alpacas were originated on the American continent and were imported onto the Chinese mainland from Australia in 2002 (Zhang et al., 2019). As of today, alpacas are raised on farms in Shanxi Province, Xinjiang Uygur Autonomous Region, Shandong Province, Beijing City and some other areas for both meat and wool, and in some zoological gardens for the tourist industry in China. It was reported that there are estimated to be between 5000 and 10,000 alpacas in China in 2018. *Cryptosporidium* infections have been recorded in alpacas in UK, US, Peru, Australia, etc. (Twomey et al., 2008; Burton et al., 2012; Koehler et al., 2018; Gomez-Puerta et al., 2020). However, there has been no study of *Cryptosporidium* infections in alpacas in China. Therefore, the aim of this study was to investigate the prevalence and molecular characteristics of *Cryptosporidium* spp. in alpacas in China.

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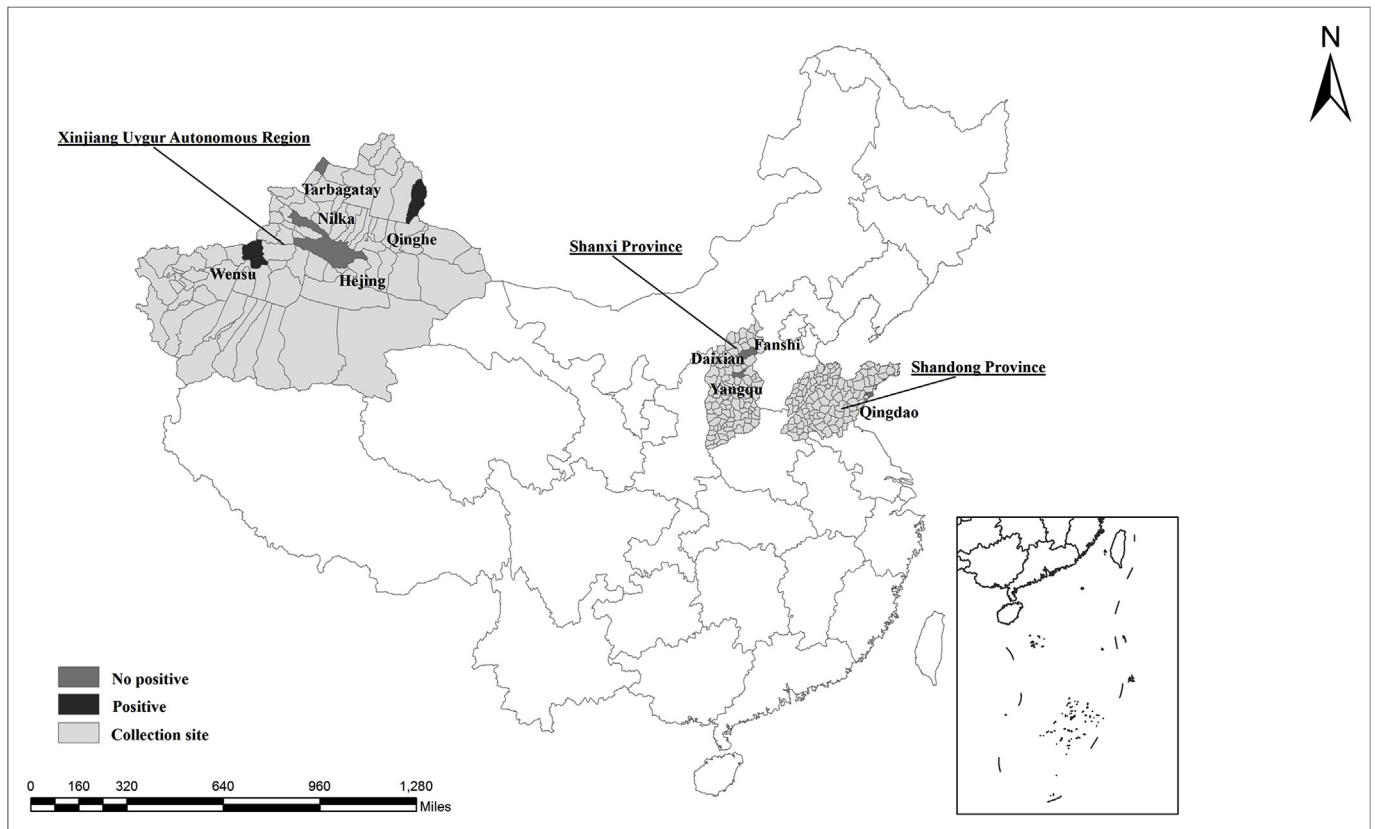


Fig. 1. Locations of alpaca fecal sampling in China.

2. Materials and methods

2.1. Ethics approval

Appropriate permission was obtained from the herd owners or managers before the alpaca fecal samples were collected, and no specific permit was required for field studies. The protocol was reviewed and approved by the Ethics Committee of Tarim University (Xinjiang, China).

2.2. Sample collection

Between August 2016 and March 2017 and between May and August 2019, a total of 484 fresh fecal samples were collected from individual alpacas at nine sites in the Xinjiang Uygur Autonomous Region (Tacheng, Wensu, Hejing, Qinghe, and Nilka), Shanxi Province (Fanshi, Daixian, and Yangqu), and Shandong Province (Qingdao) (Fig. 1). All the alpacas grazed freely on hay in a fenced pasture during the daytime, and had shelter at night. The alpacas were original imported Australia or Netherlands, and followed by breeding by artificial insemination. The collected samples represented approximately 10%–50% of each alpaca herd. The age group of alpacas involving in present study were available roughly, only for adult or younger, and exact of the days/years were not obtained. The adults alpacas involved in present study all were female, and the sex of younger alpacas were not obtained (Table 1).

Fresh alpaca fecal samples (10–20 g) were collected with sterile gloves and placed in labeled clean plastic bags immediately after defecation. All the collected samples were transported to the testing laboratory in a cooler with ice packs. The samples were stored at 4 °C before DNA extraction, and the DNA was extracted within 1 week of collection. No obvious diarrhea symptoms were observed during sampling.

2.3. DNA extraction and PCR amplification

Total genomic DNA was extracted from approximately 200 mg of each fecal sample with the E.Z.N.A.® Stool DNA Kit (Omega Biotek Inc., Norcross, GA, USA), according to the manufacturer's instructions. Then 200 µL of elution buffer was added to each DNA sample, and the sample was stored at –20 °C until PCR amplification.

Cryptosporidium spp. were screened with nested PCR amplification of the small subunit ribosome RNA (SSU rRNA) locus, as previously described (Cai et al., 2017). *Cryptosporidium parvum* and *C. ubiquitum* were then subgenotyped with an analysis of the 60-kDa glycoprotein locus (*gp60*) sequence (Alves et al., 2003; Li et al., 2014). The 2×EasyTaq® PCR SuperMix (TransGene Biotech Co. Ltd, Beijing, China) reaction system was used for each PCR. To validate the PCR amplification, positive (chicken -derived *C. baileyi* DNA) and negative (distilled water without DNA) controls were included in each PCR batch. The secondary PCR products were examined with electrophoresis in 1.5% agarose gels stained with GelRed™ (Biotium Inc., Hayward, CA, USA).

The intensity of *Cryptosporidium* spp. oocyst shedding was assessed using a SYBR Green-based qPCR of the 18S-LC2 targeting the SSU rRNA locus (Li et al., 2015). The 20 µl qPCR mix consisted of 10 µl of 2×SYBR Green Real-time PCR Master Mix (TransGene Biotech Co. Ltd, Beijing, China), 0.5 µl of 20 µM primers (each), 1 µl of DNA and 8 µl of PCR grade water. The qPCR was conducted on a Mastercycler® ep realplex (Eppendorf, Hamburg, Germany) as previously described (Chen et al., 2019). The number of oocysts per gram of feces (OPG) in *Cryptosporidium*-positive samples was calculated based on the Ct values, using a standard curve generated from spiked with known numbers of oocysts of the *C. parvum* isolate.

Table 1
Prevalence and species/subtypes of *Cryptosporidium* in alpacas in China.

Collection sites	Import from (Year)	Collection time	Number of population	Age group	Number of sampled	Number of positive	<i>Cryptosporidium</i> species/subtypes (n)
Tarbagatay	Netherlands (2014)	Aug 2016	46	Adult	18	0	
Wensu	Australia (2014–2015)	Aug 2016	390*	Subtotal	100	3 (3.0%)	<i>C. parvum</i> (2)/IIdA15G1 (2); <i>C. ubiquitum</i> (1)
				Younger	17	0	
				Adult	83	3 (3.6%)	<i>C. parvum</i> (2)/IIdA15G1 (2); <i>C. ubiquitum</i> (1)
Hejing	Australia (2013)	Mar 2017	40	Adult	20	0	
Qinghe	Australia (2004)	Mar 2017	95	Adult	35	11 (31.4%)	<i>C. occultus</i> (11)
Nilka	Australia (2005)	Jul 2017	38	Adult	12	0	
Qingdao	Netherlands (2014–2015)	May 2019	102	Adult	20	0	
Fanshi	Australia (2015–2017)	Aug 2019	210	Subtotal	42	0	
				Younger	7	0	
				Adult	35	0	
Daixian	Australia (2015–2017)	Aug 2019	89	Subtotal	38	0	
				Younger	11	0	
				Adult	27	0	
Yangqu	Australia (2014–2017)	Aug 2019	1200*	Subtotal	199	0	
				Younger	5	0	
				Adult	164	0	
Total			2210*		484	14 (2.9%)	<i>C. occultus</i> (11); <i>C. parvum</i> (2)/IIdA15G1 (2); <i>C. ubiquitum</i> (1)

*approximate.

2.4. Sequencing analysis

The positive PCR amplicons of the target loci were sequenced bidirectionally by a commercial sequencing company (GENEWIZ, Suzhou, China). The nucleotide sequences were compared with reference sequences downloaded from the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/>) using ClustalX 2.1 (<http://www.clustal.org/>) to identify the *Cryptosporidium* species and subgenotypes.

2.5. Statistical analysis and nucleotide sequence accession numbers

A χ^2 test was used to compare the prevalence of the *Cryptosporidium* spp. in the alpacas. Statistical significance was set at $p < 0.05$. The representative nucleotide sequences reported in this study have been deposited in the GenBank database at the National Center for Biotechnology Information under accession numbers MN876846–MN876848 for the SSU rRNA locus and MN879351 for the *gp60* locus.

3. Results

3.1. Prevalence and distributions of *Cryptosporidium* spp. in alpacas

A total of 484 alpaca fecal samples were screened for *Cryptosporidium* spp. with PCR based on the SSU rRNA locus. *Cryptosporidium* spp. were detected in 2.9% (14/484) of the samples. Of the nine collection sites, two were positive for *Cryptosporidium*, Qinghe (31.4%, 11/35) and Wensu (3.0%, 3/100), and all the *Cryptosporidium*-positive samples were identified in adult female alpacas (Table 1).

Sequences analysis identified three *Cryptosporidium* species: *C. parvum* (n = 2), *C. ubiquitum* (n = 1), and *C. occultus* (n = 11). *Cryptosporidium occultus* was the predominant species within the positive alpacas (78.6%, 11/14). The number of OPG of one *C. ubiquitum*-positive samples was 13,121, and the average number of OPG of two *C. parvum*-positive samples was $16,011 \pm 5803$, 11 *C. occultus*-positive samples was 472 ± 526 , respectively.

3.2. Sequences analysis and phylogenetic analysis of SSU rRNA

Two SSU *C. parvum* sequences were identical to isolates from a human (MK990043), dairy cattle (MF074692), sika deer (KX259139), and donkey (KU200953) in China. One *C. ubiquitum* sequence shared 99.9% homology with that of an isolate from a bactrian camel (MH442993) in China, with one substitution at nucleotide position 83 (T→C). The remaining 11 *C. occultus* sequences were identical to isolates from a human (HQ822146) in the UK, bamboo rat (MK731963) in China, and rats (MG699176, MG699177, MG699178, and MG699179) in the Czech Republic.

3.3. Subtypes of *C. parvum*

The *C. parvum* and *C. ubiquitum* isolates were subtyped based on a *gp60* locus sequence analysis. The two *C. parvum* isolates were identified as IIdA15G1, and the one *C. ubiquitum* was not subtyped successfully. The two *C. parvum* isolates shared 100% nucleotide sequence identity at the *gp60* locus with the corresponding regions of *C. parvum* isolates from humans (JF268648) in India and various animals in China, including dairy cattle (KT964798), macaque (KJ917586), sheep (MH794167), bamboo rat (MK731965), horse (MK770629), and rodents (GQ121027).

4. Discussion

Cryptosporidium spp. are important pathogens causing diarrhea in preweaned alpacas (Cebra et al., 2003; Rojas et al., 2016). The observed *Cryptosporidium* spp. infection rate in alpacas in China was 2.9% (14/484) in the present study. Slightly higher prevalence rates have been reported in alpacas in Australia (3.7%, 3/81) (Koehler et al., 2018), Peru (4.4%, 12/274) (Gómez-Couso et al., 2012), and America (7.7%, 17/220) (Burton et al., 2012). However, much higher prevalence rates were documented in newborn alpacas in a report from Peru, in which 12.4% of neonatal alpacas were infected (159/1312) (Gomez-Puerta et al., 2020). The different prevalence rates reported in these studies may be attributable to differences in the feeding sites, age distributions, sample sizes, host health status, management systems, and population densities of the animals tested, as well as some other identified factors.

However, the *Cryptosporidium*-positive were not observed in younger alpacas in present study, a further study is required to investigate the relationships between *Cryptosporidium* infections and the age of alpacas in China.

Three zoonotic *Cryptosporidium* species (*C. parvum*, *C. cuniculus*, and *C. ubiquitum*) have been reported in alpacas in previous studies (Koehler et al., 2018; Gomez-Puerta et al., 2020). In the present study, three *Cryptosporidium* species, *C. parvum*, *C. ubiquitum*, and *C. occultus*, were identified in alpacas (Table 1). To our knowledge, this is the first report of *C. occultus* infection in alpacas. *Cryptosporidium parvum*, *C. ubiquitum*, and *C. occultus* have extensive host ranges, which include humans, ruminants, and rodents, in various countries (Kváč et al., 2018; Huang et al., 2018; Zhao et al., 2018). *Cryptosporidium parvum* has been predominantly reported in alpacas, and was the only species detected in 153 *Cryptosporidium*-infected newborn alpaca as in a study in Peru (Gomez-Puerta et al., 2020). *Cryptosporidium ubiquitum* appears to be the most common *Cryptosporidium* species infecting humans and ruminants in China, especially sheep (Mi et al., 2018). *Cryptosporidium occultus*, previously known as the *C. suis*-like genotype, has been identified in humans, cattle, yaks, and rats, which suggests that it has a wide host range (Kváč et al., 2018; Zhao et al., 2018).

In this study, we detected *C. parvum* subtype IIdA15G1, which is commonly found in humans and animals (yaks, buffalos, rodents, and calves) in China (Feng and Xiao, 2017). Subtype IIdA15G1 was responsible for an outbreak of lethal cryptosporidiosis in the Ningxia Hui Autonomous Region of China that resulted in the deaths of hundreds of preweaned calves following severe diarrhea (Cui et al., 2014). However, an alpaca-derived *C. parvum* isolated in Canada was subtype IIA (n = 1) (O'Brien et al., 2008), and one isolated in Australia was subtype IIA20G3R1 (n = 1) (Koehler et al., 2018). These results indicate that there are genetic differences in the subtypes of *C. parvum* infecting alpacas in different regions.

In conclusion, to our knowledge, this is the first report of *Cryptosporidium* spp. in alpaca in China. Three *Cryptosporidium* species (*C. parvum*, *C. ubiquitum*, and *C. occultus*) were identified, and this is also the first report of *C. occultus* infections in alpacas. It provides the basic data of molecular characteristic for the study of *Cryptosporidium* in animals in China.

Declaration of competing interest

The authors declare that they have no conflict of interests.

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References

Alves, M., Xiao, L., Sulaiman, I., Lal, A.A., Matos, O., Antunes, F., 2003. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. *J. Clin. Microbiol.* 41, 2744–2747.

Bidewell, C.A., Cattell, J.H., 1998. Cryptosporidiosis in young alpacas. *Vet. Rec.* 142 (11), 287.

Burton, A.J., Nydam, D.V., Mitchell, K.J., Bowman, D.D., 2012. Fecal shedding of *Cryptosporidium* oocysts in healthy alpaca crias and their dams. *J. Am. Vet. Med. Assoc.* 241 (4), 496–498.

Cai, M., Guo, Y., Pan, B., Li, N., Wang, X., Tang, C., Feng, Y., Xiao, L., 2017. Longitudinal monitoring of *Cryptosporidium* species in pre-weaned dairy calves on five farms in Shanghai, China. *Vet. Parasitol.* 241, 14–19.

Cebra, C.K., Mattson, D.E., Baker, R.J., Sonn, R.J., Dearing, P.L., 2003. Potential

pathogens in feces from unweaned llamas and alpacas with diarrhea. *J. Am. Vet. Med. Assoc.* 223 (12), 1806–1808.

Checkley, W., White Jr., A.C., Jaganath, D., Arrowood, M.J., Chalmers, R.M., Chen, X.M., Fayer, R., Griffiths, J.K., Guerrant, R.L., Hedstrom, L., Huston, C.D., Kotloff, K.L., Kang, G., Mead, J.R., Miller, M., Petri Jr., W.A., Priest, J.W., Roos, D.S., Striepen, B., Thompson, R.C., Ward, H.D., Van Voorhis, W.A., Xiao, L., Zhu, G., Houpt, E.R., 2015. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. *Lancet Infect. Dis.* 15 (1), 85–94.

Chen, L., Hu, S., Jiang, W., Zhao, J., Li, N., Guo, Y., Liao, C., Han, Q., Feng, Y., Xiao, L., 2019. *Cryptosporidium parvum* and *Cryptosporidium hominis* subtypes in crab-eating macaques. *Parasites Vectors* 12 (1), 350–2019.

Cui, Z., Wang, R., Huang, J., Wang, H., Zhao, J., Luo, N., Li, J., Zhang, Z., Zhang, L., 2014. Cryptosporidiosis caused by *Cryptosporidium parvum* subtype IIdA15G1 at a dairy farm in Northwestern China. *Parasites Vectors* 7, 529.

Feng, Y., Xiao, L., 2017. Molecular epidemiology of cryptosporidiosis in China. *Front. Microbiol.* 8, 1701.

Feng, Y., Ryan, U.M., Xiao, L., 2018. Genetic diversity and population structure of *Cryptosporidium*. *Trends Parasitol.* 34 (11), 997–1011.

Gómez-Couso, H., Ortega-Mora, L.M., Aguado-Martínez, A., Rosadio-Alcántara, R., Maturrano-Hernández, L., Luna-Espinoza, L., Zanabria-Huisa, V., Pedraza-Díaz, S., 2012. Presence and molecular characterisation of *Giardia* and *Cryptosporidium* in alpacas (*Vicugna pacos*) from Peru. *Vet. Parasitol.* 187 (3–4), 414–420.

Gomez-Puerta, L.A., Gonzalez, A.E., Vargas-Calla, A., Lopez-Urbina, M.T., Cama, V., Xiao, L., 2020. *Cryptosporidium parvum* as a risk factor of diarrhea occurrence in neonatal alpacas in Peru. *Parasitol. Res.* 119 (1), 243–248.

Huang, J., Zhang, Z., Zhang, Y., Yang, Y., Zhao, J., Wang, R., Jian, F., Ning, C., Zhang, W., Zhang, L., 2018. Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in deer in Henan and Jilin, China. *Parasites Vectors* 11 (1), 239.

Koehler, A.V., Rashid, M.H., Zhang, Y., Vaughan, J.L., Gasser, R.B., Jabbar, A., 2018. First cross-sectional, molecular epidemiological survey of *Cryptosporidium*, *Giardia* and *Enterocytozoon* in alpaca (*Vicugna pacos*) in Australia. *Parasites Vectors* 11 (1), 498.

Kváč, M., Vlnatá, G., Ježková, J., Horčíčková, M., Konečný, R., Hlásková, L., McEvoy, J., Sak, B., 2018. *Cryptosporidium occultus* sp. n. (Apicomplexa: Cryptosporidiidae) in rats. *Eur. J. Protistol.* 63, 96–104.

Li, N., Xiao, L., Alderisio, K., Elwin, K., Cebelski, E., Chalmers, R., Santin, M., Fayer, R., Kvac, M., Ryan, U., Sak, B., Stanko, M., Guo, Y., Wang, L., Zhang, L., Cai, J., Roellig, D., Feng, Y., 2014. Subtyping *Cryptosporidium ubiquitum*, a zoonotic pathogen emerging in humans. *Emerg. Infect. Dis.* 20 (2), 217–224.

Li, N., Neumann, N.F., Ruecker, N., Alderisio, K.A., Sturbaum, G.D., Villegas, E.N., Chalmers, R., Monis, P., Feng, Y., Xiao, L., 2015. Development and evaluation of three real-time PCR assays for genotyping and source tracking *Cryptosporidium* spp. in water. *Appl. Environ. Microbiol.* 81 (17), 5845–5854.

Mi, R., Wang, X., Huang, Y., Mu, G., Zhang, Y., Jia, H., Zhang, X., Yang, H., Wang, X., Han, X., Chen, Z., 2018. Sheep as a potential source of zoonotic cryptosporidiosis in China. *Appl. Environ. Microbiol.* 84 (18) pii:e00868-18.

O'Brien, E., McInnes, L., Ryan, U., 2008. *Cryptosporidium* GP60 genotypes from humans and domesticated animals in Australia, North America and Europe. *Exp. Parasitol.* 118 (1), 118–121.

Rojas, M., Manchego, A., Rocha, C.B., Fornells, L.A., Silva, R.C., Mendes, G.S., Dias, H.G., Sandoval, N., Pezo, D., Santos, N., 2016. Outbreak of diarrhea among preweaning alpacas (*Vicugna pacos*) in the southern Peruvian highland. *J. Infect. Dev. Ctries* 10 (3), 269–274.

Rulofson, F.C., Atwill, E.R., Holmberg, C.A., 2001. Fecal shedding of *Giardia duodenalis*, *Cryptosporidium parvum*, *Salmonella* organisms, and *Escherichia coli* O157:H7 from llamas in California. *Am. J. Vet. Res.* 62 (4), 637–642.

Ryan, U., Fayer, R., Xiao, L., 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology* 141 (13), 1667–1685.

Ryan, U., Hijjawi, N., Xiao, L., 2018. Foodborne cryptosporidiosis. *Int. J. Parasitol.* 48 (1), 1–12.

Starkey, S.R., Johnson, A.L., Ziegler, P.E., Mohammed, H.O., 2007. An outbreak of cryptosporidiosis among alpaca crias and their human caregivers. *J. Am. Vet. Med. Assoc.* 231 (10), 1562–1567.

Thomson, S., Hamilton, C.A., Hope, J.C., Katzer, F., Mabbott, N.A., Morrison, L.J., Innes, E.A., 2017. Bovine cryptosporidiosis: impact, host-parasite interaction and control strategies. *Vet. Res.* 48 (1), 42.

Twomey, D.F., Barlow, A.M., Bell, S., Chalmers, R.M., Elwin, K., Giles, M., Higgins, R.J., Robinson, G., Stringer, R.M., 2008. Cryptosporidiosis in two alpaca (*Lama pacos*) holdings in the South-West of England. *Vet. J.* 175 (3), 419–422.

Waitt, L.H., Cebra, C.K., Firshman, A.M., McKenzie, E.C., Schlipf Jr., J.W., 2008. Cryptosporidiosis in 20 alpaca crias. *J. Am. Vet. Med. Assoc.* 233 (2), 294–298.

Wessels, J., Wessels, M., Featherstone, C., Pike, R., 2013. Cryptosporidiosis in eight-month-old weaned alpacas. *Vet. Rec.* 173 (17), 426–427.

Whitehead, C.E., Anderson, D.E., 2006. Neonatal diarrhea in llamas and alpacas. *Small Rumin. Res.* 61, 207–215.

Xiao, L., 2010. Molecular epidemiology of cryptosporidiosis: an update. *Exp. Parasitol.* 124 (1), 80–89.

Zhang, Q., Wang, H., Zhao, A., Zhao, W., Wei, Z., Li, Z., Qi, M., 2019. Molecular detection of *Enterocytozoon bienersi* in alpacas (*Vicugna pacos*) in Xinjiang, China. *Parasite* 26, 31.

Zhao, W., Wang, J., Ren, G., Yang, Z., Yang, F., Zhang, W., Xu, Y., Liu, A., Ling, H., 2018. Molecular characterizations of *Cryptosporidium* spp. and *Enterocytozoon bienersi* in brown rats (*Rattus norvegicus*) from Heilongjiang Province, China. *Parasites Vectors* 11 (1), 313.