Veterinary Parasitology: Regional Studies and Reports 8 (2017) 66–69

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Short Communication

Molecular characterization of zoonotic pathogens *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in calves in Algeria

Djamel Baroudi [a,b], Djamel Khelef [a], Ahcene Hakem [c], Abdelhafidh Abdelaziz [a], Xiaohua Chen [b,d], Colleen Lysen [b], Dawn Roellig [b], Lihua Xiao [b,*]

[a] École Nationale Supérieure Vétérinaire, Rue Issaad Abbes, El Alia, Alger, Algeria
[b] Division of Foodborne, Waterborne and Environmental Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30329, USA
[c] Department of Biology, Université Ziane Achor, Djelfa, Algeria
[d] Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form 3 February 2017
Accepted 11 February 2017
Available online 12 February 2017

Keywords:
Genotyping
Cryptosporidium
Giardia
Enterocytozoon
Calves
Algeria

ABSTRACT

Little is known on the identity and public health potential of *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in farm animals in Algeria. In this study, 102 fecal specimens from pre-weaned dairy calves with or without diarrhea were collected from 19 dairy farms located in 6 provinces. PCR-restriction fragment length polymorphism analysis of the small subunit rRNA gene was used to detect and differentiate *Cryptosporidium* spp., whereas PCR-sequence analysis of the triosephosphate isomerase gene and ribosomal internal transcribed spacer were used to detect and genotype *G. duodenalis* and *E. bieneusi*, respectively. *Cryptosporidium* was found in 14 specimens, among which 7 had *C. parvum*, 4 had *C. bovis*, and 3 had mixed infection of *C. parvum* and *C. bovis* or *C. bovis* and *C. andersoni*. Subtyping of *C. parvum* by PCR-sequence analysis of the 60 kDa glycoprotein gene identified two zoonotic subtypes IIaA16G2R1 and IIaA17G3R1. *G. duodenalis* was found in 28 specimens, with 6 having the host-specific assemblage E, 14 having the zoonotic assemblage A (all belonging to A2 subtype), and 8 having mixed assemblages. Six known genotypes of *E. bieneusi* belonging to Group 2, including I, J, BEB3, BEB4, BEB6 and PtEb XI, were identified in 11 specimens. Diarrhea was mostly associated with the occurrence of *C. parvum*. Data from this study suggest that human-pathogenic *C. parvum* subtypes and *G. duodenalis* and *E. bieneusi* genotypes are common on dairy farms in Algeria.

1. Introduction

Cryptosporidium spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* are common pathogens of a wide range of animals including livestock, companion animals, wildlife and humans, causing diarrhea and other gastrointestinal disorders (Feng and Xiao, 2011; Santín and Fayer, 2011; Checkley et al., 2015). Although infections with these pathogens are often self-limited in immunocompetent individuals, they can become chronic in infants, elderly and immunocompromised persons (Dengjel et al., 2001; Didier and Weiss, 2011). Anthroponotic and zoonotic transmission cycles are known for *Cryptosporidium* spp., with pre-weaned calves as the major reservoir in zoonotic transmission (Xiao, 2010). Although the role of cattle in the zoonotic transmission of giardiasis and microsporidiosis is less clear, identical *G. duodenalis* and *E. bieneusi* genotypes have been found in both humans and cattle (Santín and Fayer, 2009; Feng and Xiao, 2011). Many studies have indicated that *C. parvum*, assemblage A of *G. duodenalis* and Group 1 genotypes of *E. bieneusi* are common zoonotic pathogens (Santín and Fayer, 2009; Feng and Xiao, 2011; Ryan et al., 2014). Some recent studies have demonstrated that a broad range of mammals can be infected with Group 2 *E. bieneusi* genotypes (BEB4, BEB6, I, and J), including humans (Sak et al., 2011; Jiang et al., 2015).

Compared to industrialized nations, molecular epidemiologic studies of *Cryptosporidium* spp., *G. duodenalis* and *E. bieneusi* in cattle are scarce in developing countries. In Algeria, efforts have been made to characterize *Cryptosporidium* spp. in birds (Baroudi et al., 2013) and *Cryptosporidium* spp. and *E. bieneusi* in horses (Laatamna et al., 2015). The present study was conducted to determine the identity of *Cryptosporidium* spp., *G. duodenalis* and *E. bieneusi* in pre-weaned dairy calves in Algeria, which is needed in understanding of the epidemiology of these important parasites.

2. Materials and methods

2.1. Study area and specimen collection

This study was undertaken from January 2013 to January 2014 on 19 dairy farms located in 6 provinces east of Algiers, Algeria (Table 1). A

* Corresponding author at: Division of Foodborne, Waterborne and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Mail Stop D66, 1600 Clifton Road, Atlanta, GA 30329-4018, USA.
E-mail address: lxiao@cdc.gov (L. Xiao).

http://dx.doi.org/10.1016/j.vprsr.2017.02.005
2405-9390/

total of 102 fecal specimens were collected directly from the rectum of pre-weaned calves (younger than 90 days) into plastic specimen cups. Fecal consistence was recorded for each specimen. The specimens were transported in an isothermal box to the laboratory and stored at 4 °C in 2.5% potassium dichromate. The field study protocol was approved by École Nationale Supérieure Vétérinaire, Algiers, Algeria.

2.2. DNA extraction

After washing the specimens twice with distilled water by centrifugation, genomic DNA was extracted from 0.2 ml of fecal slurry using the FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, CA) and manufacturer-recommended procedures. The extracted DNA was stored at −80 °C before PCR analysis.

2.3. Detection and typing of Cryptosporidium spp. G. duodenalis and E. bieneusi

All DNA preparations were analyzed for *Cryptosporidium* spp. by nested PCR amplification of the small subunit (SSU) rRNA gene. *Cryptosporidium* species in positive PCR products were determined by restriction fragment length polymorphism (RFLP) analysis using restriction enzymes *Ssp*I and *Mbo*II (Feng et al., 2007). Specimens identified as *C. parvum* were further subtyped by PCR-sequence analysis of ~850-bp fragment of the 60 kDa glycoprotein (gp60) gene (Alves et al., 2003). The recommended subtype nomenclature was used in naming *C. parvum* subtypes (Xiao, 2010). Similarly, *G. duodenalis* and *E. bieneusi* were detected by nested PCR targeting a 530-bp fragment of the triosephosphate isomerase (tpi) gene and 392 bp of the ribosomal internal transcribed spacer (ITS), respectively (Sulaiman et al., 2003, 2004). DNA sequence analysis of PCR products was used in identification of *G. duodenalis* and *E. bieneusi* genotypes.

2.4. DNA sequence analysis

Secondary PCR products of the gp60, tpi and ITS genes were purified using Montage PCR Cleanup Filters (Millipore, Bedford, MA) and sequenced in both directions using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and ABI 3130 Genetic Analyzer (Applied Biosystems). The obtained nucleotide sequences were read and assembled using ChromasPro (http://technelysium.com.au/ChromasPro.html), edited using BioEdit 7.0.4 (http://www.mbio.ncsu.edu/BioEd-it/bioedit.html), and aligned with reference sequences of each target using ClustalX 2.1 (http://www.clustal.org/).

2.5. Statistical analysis

The Fisher's exact test implemented in the R package 3.3.2 (https://www.r-project.org/) was used to compare differences in occurrence rates of pathogens or diarrhea between age groups. Differences were considered significant at $P \leq 0.05$.

3. Results

3.1. Occurrence of Cryptosporidium spp., G. duodenalis and E. bieneusi

Cryptosporidium spp., *G. duodenalis* and *E. bieneusi* were detected in 14 (13.7%), 28 (27.5%) and 11 (10.8%) of the 102 specimens, respectively (Table 1). The occurrence rate of *Cryptosporidium* spp. in one month or younger calves was higher (20.7% or 6/29) than that in 2-month-old calves (11.4% or 4/35) and 3-month-old calves (10.5% or 4/38) ($P > 0.05$). In contrast, higher occurrence rates of *G. duodenalis* were seen in older calves: 42.9% (15/35) in 2-month-old calves and 28.9% (11/38) in 3-month-old calves, compared to 6.9% (2/29) in 1-month-old calves ($P < 0.05$). Similarly, higher occurrence rates of *E. bieneusi* were also seen in older calves: 17.1% (6/35) in 2-month-old calves and 10.5% (4/38) in 3-month-old calves, compared to 3.4% (1/29) in 1-month-old calves ($P > 0.05$; Table 2).

3.2. Cryptosporidium species and subtypes

RFLP analysis of the SSU rRNA PCR products identified the presence of 3 *Cryptosporidium* species, including *C. parvum*, *C. bovis* and *C. andersoni* in 9, 7 and 1 specimens, respectively. Among them, 2 specimens had concurrence of *C. parvum* and *C. bovis* and one had *C. bovis* and *C. andersoni*. *C. parvum* occurred in calves up to 2 months of age, whereas *C. bovis* and *C. andersoni* were seen in 2- and 3-month-old calves (Table 2). Sequence analysis of the gp60 PCR products from the 9 *C. parvum*-positive specimens showed that 6 belonged to the zoonotic IIa subtype family, including IIaA16G2R1 in 5 specimens and IIaA17G3R1 in one specimen. The subtype identity could not be determined for the remaining three *C. parvum*–positive specimens (Table 1). The concurrence of *C. parvum* and *C. bovis* in one calf was confirmed by gp60 PCR, which identified the presence of the *C. parvum* IIaA16G2R1 subtype.

3.3. G. duodenalis genotypes and subtypes

Among the 28 *G. duodenalis*–positive specimens, 2 genotype assemblages were identified by DNA sequencing of the tpi PCR products, including assemblage A in 14 specimens, assemblage E in 6 specimens, and mixed assemblages (A and E) in 8 specimens. All assemblage A

Table 1
Distribution of genotypes/subtypes of *Cryptosporidium* spp., *G. duodenalis* and *E. bieneusi* in pre-weaned calves on dairy farms in east Algeria.

Location/no. of farms	No. of specimens	Pathogen						
		Cryptosporidium			*G. duodenalis*		*E. bieneusi*	
		No. positive	Species	Subtype	No. positive	Assemblage/subtype	No. positive	Genotype
Rouiba (3)	19	3	*C. parvum* (2) *C. parvum* + *C. bovis* (1)	IIaA16G2R1 (1) IIaA17G3R1 (1)	6	A(A2) (3) (A + E) (3)	3	I (1), BEB6 (1), PtEb XI (1)
Zemmouri (5)	24	4	*C. parvum* (3) *C. bovis* (1)	IIaA16G2R1 (2)	8	A(A2) (3) E (2) (A + E) (3)	2	J (1), BEB6 (1)
Boumerdes (4)	22	3	*C. parvum* (1) *C. bovis* (1) *C. bovis* + *C. andersoni* (1)	IIaA16G2R1 (1)	6	A(A2) (2) E (2) (A + E) (2)	2	BEB4 (1), BEB3 (1)
Legata (2)	8	1	*C. parvum* (1)	IIaA16G2R1 (1)	3	A(A2) (3)	1	BEB4 (1)
Djenet (3)	18	2	*C. bovis* (1) *C. parvum* + *C. bovis* (1)		3	A(A2) (3)	2	BEB4 (2)
Si-Mustapha (2)	11	1	*C. bovis* (1)	0	2	E (2)	1	Mixed (1)

Numbers in parentheses are numbers of farms or positive specimens.