**Giardia duodenalis** in calves from an isolated farm from northwestern Romania

Diana Onac1, Adriana Jarca2, Zsuzsa Kalmar1, Vasile Cozma1

1 – University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, Department of Parasitology and Parasitic Diseases, Cluj-Napoca 400372, Romania.
2 – 2nd Medical Department, University of Medicine and Pharmacy “Iuliu Hațieganu”, Victor Babeș Street no. 8, Cluj-Napoca, Romania.

Correspondence: Tel. +40264-596384, Fax+40264-593792, E-mail dianaonac@yahoo.com

### Abstract

Giardia is considered one of the most prevalent zoonotic parasite in humans and animals worldwide. Based on high prevalence of Giardia duodenalis, cattle have been considered as potential sources for human infection. This study was conducted in order to identify Giardia assemblages in calves from one isolated farm from north-western Romania. For this purpose, 28 faecal samples from calves with diarrhea were collected. Samples were used for flotation exam and for DNA extraction. Positive samples in nPCR reaction were sequenced, targeting β-giardin gene. 10.7% were positive in coproparasitological examination and 7.1% of the samples tested positive for n-PCR reaction. The sequence analysis of β-giardin locus was obtained for both samples which were identified as assemblage A, respectively one as subassemblage AII and one as subassemblage A3. The results of this study demonstrated the importance of cattle which can be considered as potential sources for human infection.

**Keywords:** Calves; Giardia duodenalis; Assemblages; Romania.

Received 10/08/2015. Accepted 13/09/2015.

### Introduction

*Giardia* sp. is one of the most common intestinal parasites of humans; about 200 million people in Asia, Africa, and Latin America have symptomatic infections (Yason and Rivera, 2007). In children chronic giardiosis can result in long-term growth retardation (Fraser et al., 2000). Is a frequently diagnosed waterborne infection and a major concern to water utilities. Because of the impact on socio-economic development, especially in developing countries, *Giardia* is since 2004 included in the ‘Neglected Disease Initiative’ of the World Health Organization (WHO) (Savioli et al., 2006).

*Giardia* is also a very common enteric parasite of domestic animals, including livestock, dogs, and cats (Thompson, 2004; Thompson and Monis, 2004), and wildlife (Appelbee et al., 2005). To date eight major genetic groups...
Assemblages have been identified, two of which (A and B) are found in both humans and animals, whereas the other six (C to H) are host-specific and do not infect humans (Feng and Xiao, 2011). One species within this genus, *Giardia duodenalis* (syn. *Giardia lamblia* and *Giardia intestinalis*), causes giardiasis in humans and most mammals. Thus, giardiasis is considered a zoonotic disease (Feng and Xiao, 2011). In housed calves, *Giardia* is at present considered as important pathogens in the etiology of diarrhea.

Based on high prevalence of *Giardia duodenalis*, cattle have been considered as potential sources for human infection, although in the last decade molecular tools have provided a more detailed map in the epidemiology of *Giardia*.

**Materials and methods**

Between June – July 2014, 28 faecal samples were collected from calves originated from one farm from North-Western Romania. Faecal samples were collected from all the calves from the farm until 8 month old, animals which suffered of diarrhea. We registered for each calves the age and gender. For all 28 faecal samples it was performed the flotation method for identifying the *Giardia* cysts and then the samples were kept at -20°C until extraction of the DNA. Total DNA extraction was performed with a commercial kit (Isolate Fecal DNA Kit, Bioline, UK), according to manufacturer’s indications using 25 mg of faeces/sample. Total DNA extracts were preserved at -20°C until amplification.

The amplification of β-giardin gene was performed using a nested-PCR protocol (Cacciò et al., 2002; Lalle et al., 2005). In the first PCR reaction it was obtained a 753bp fragment using the primers pair G7-G759. PCR mix consisted in 10µM of each primers, 12.5µl of MasterMix (Rovalab GMBH, Germany) and 4µl of DNA in a total volume of 25µl. PCR conditions were: initial denaturation for 5 min at 96°C, a set of 40 cycles of annealing (30 sec at 95°C, 30 sec at 50°C and 1 min at 72°C) followed by a final extension of 7 min at 72°C.

In the second PCR reaction it was obtained a 511bp fragment using the primers pair GiarF-GiarR. The PCR mix consisted in 10µM of each primers, 25µl of MasterMix (Rovalab GMBH, Germany), 4µl of DNA in a total volume of 50µl. PCR conditions were: initial denaturation for 5 min at 96°C, a set of 35 cycles of annealing (45 sec at 95°C, 30 sec at 55°C and 45 sec at 72°C) followed by a final extension of 7 min at 72°C (table 1).

PCR products were subsequently sequenced (MACROGEN, Amsterdam, Netherlands).

| Table 1. Primers used for the molecular characterization of *Giardia* isolates |
|---------------------------------|---------------------------------|
| **Forward (5'-3')**               | **Reverse (5'-3')**              |
| G7 - AAGCCGGACGACCTACCCCGGACTGC | G759 - GAGGCCGCCCTGGATCTTCGAGACGAC |
| GiarF - GAACGAACGAGATCGAGGTCCCG | GiarR - GAGCTGAGCTGCCGCTGTT |

**Results**

In coproparasitological examination, 3 (10.7%) out of 28 samples were observed *Giardia* spp. cysts. The amplification of all 28 samples from calves yielded the expected 511bp fragment of β-giardin gene for 2 sample (7.1%). One of the samples was positive also in coproparasitological examination. The comparison with *G. duodenalis* sequences available in GenBankTM revealed that one sample was 100% identical with the isolate KT235953 which corresponds to the assemblage AII and the second one was 95% identical with the isolate FJ472821 correspondent to assemblage A3.

**Discussions**

High prevalence of *Giardia* have been reported in farm animals, such as cattle, sheep and goats (Xiao, 1994; Ryan et al., 2005; Bomfim et al., 2005).
For cattle, the infection rate of *Giardia duodenalis* varied markedly in different studies, being 17.4% to 31.3% in Belgium (Geurden et al., 2004, 2008), 43.6% in Denmark (Langkjaer et al., 2007; Maddox-Hyttel et al., 2006), up to 38.0% in Germany (Jager et al., 2005), 30.0% in Italy (Berrilli et al., 2004), 49.0% in Norway (Hamnes et al., 2006), 2.2 to 14.0% in Poland (O’Handley et al., 2004, 2008), and up to 57.0% in Italy (Caccio et al., 2012). Similar to the present study, a wide distribution of assemblage A among farms was found to be the major subassemblage. In one study conducted in Europe, among 113 samples tested, 70 belonged to subassemblage AI, 39 belonged to subassemblage AII, and 4 belonged to subassemblage AIII (Feng and Xiao, 2011).

In cattle the livestock specific assemblage E is most prevalent, although up to 20% zoonotic assemblage A isolates have been reported, either in pre-weaned calves (O’Handley et al., 2004; Appelbee et al., 2003; Becher et al., 2004; Berrilli et al., 2004; Trout et al., 2004, 2005, 2006, 2007), 58.0% in Australia (O’Handley et al., 2004), and up to 40.6% in New Zealand (Moriarty et al., 2008; Winkworth et al., 2008; Feng and Xiao, 2011).

There are only a few studies of subtypes of *G. duodenalis* in cattle, and subassemblage AI was found to be the major subassemblage. In one study conducted in Europe, among 113 samples tested, 70 belonged to subassemblage AI, 39 belonged to subassemblage AII, and 4 belonged to subassemblage AIII (Feng and Xiao, 2011; Sprog et al., 2009). Similar to the present study, a wide distribution of assemblage A among farms was also reported in the United States (Trout et al., 2004). Contrary to previous reports (Lalle et al., 2005; Van Keulen et al., 2002), assemblage B was not found in the present study. Moreover, the occurrence of assemblage A infections in clinically affected calves seems to suggest that infections with assemblage A are not only transient infections (Caccio et al., 2005), but contribute to the development of clinical giardiasis in calves as in the present research where all the calves take in study were with diarrhea.

The presence only of the zoonotic *Giardia* assemblage A in calves in an isolated farm in Romania might be due to the proximity of intensified livestock industry to human activity, facilitating interaction between the human and livestock transmission cycle.

**Acknowledgments**

This paper was financed under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/136893.

**References**


