

Research paper

Multilocus genotyping of *Giardia duodenalis* isolates from calves in Oromia Special Zone, Central Ethiopia



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ABSTRACT

Giardia duodenalis is a widespread protozoan parasite that infects human and other mammals. Assessing the zoonotic transmission of the infection requires molecular characterization as there is considerable genetic variation within the species. This study was conducted to identify assemblages of *Giardia duodenalis* in dairy calves; and to assess the potential role of cattle isolates in zoonotic transmission in central Ethiopia. A total of 449 fecal samples were collected and screened using microscopy and PCR targeting the small-subunit (*ssu*) rRNA, triose phosphate isomerase (*tpi*), β -giardin (*bg*) and glutamate dehydrogenase (*gdh*) genes. The overall prevalence of *Giardia duodenalis* in dairy calves was found to be 9.6% (43/449). The prevalence of infection based on sex, age and breed difference was statistically not significant ($p > 0.05$). Genotyping results revealed the presence of assemblage E and assemblage A (AI). The genotypic frequency reported was 95.3% (41/43) for assemblage E and 4.7% (2/43) for assemblage A. There was one mixed infection with assemblages AI and E. Sequence analyses showed the existence of 10 genotypes within assemblage E. One genotype that showed novel nucleotide substitution was identified at the *ssu* rRNA locus. The other 9 genotypes, 3 at each locus, were identified at the *tpi*, the *bg* and the *gdh* loci with two of the *gdh* genotypes were novel. Findings of the current study indicate the occurrence of the livestock-specific assemblage E and the potentially zoonotic assemblage A, with the former being more prevalent. Although the zoonotic assemblage was less prevalent, there is a possibility of zoonotic human infection as AI is reported from both animals and humans.

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1. Introduction

Giardiasis is a gastrointestinal disease caused by species of protozoan parasites belonging to the genus *Giardia*. Among the six accepted species of *Giardia*, *G. duodenalis* (*syn. Giardia intestinalis*, *Giardia lamblia*) is the species with great public and veterinary health importance. The public health impact of giardiasis is significant because of its tendency to cause major outbreaks and emergency responses, and because of its adverse effects on growth and cognitive functions in children (Berkman et al., 2002; Halliez and Buret, 2013). Clinical manifestations of the disease in human are quite variable, ranging from asymptomatic to acute or chronic diarrhea, dehydration, abdominal pain, nausea, vomiting, and weight loss (Eckmann, 2003). Although subclinical infection is often reported in animals, infection can result in the onset of diarrhea, ill thrift and decreased weight in young animals (Geurden et al., 2010).

It is well documented that *G. duodenalis* represents a species complex and has the broadest host range (Caccio and Ryan, 2008; Feng and Xiao, 2011; Ryan and Caccio, 2013). Molecular studies have revealed at least eight major genetic groups (assemblages) (A–H) that have different host specificities. Assemblages A and B are known to infect humans and other animals. The remaining six assemblages (C to H) are host-specific (Feng and Xiao, 2011; Ryan and Caccio, 2013; Santin et al., 2012). However, assemblages C, D, E, and F have been reported from human at lower frequency in sporadic cases (Feng and Xiao, 2011; Liu et al., 2014; Strkolcova et al., 2015).

The use of subtyping tools has identified five sub-assemblages within assemblages A and B, named AI–III and BIII–IV, some of which may have zoonotic potential. Sub-assemblages AI and AII are found in both humans and animals but appear to differ in host preference (Sprong et al., 2009; Xiao and Fayer, 2008). Sub-assemblage AI is preferentially found in livestock and pets whereas sub-assemblage AII is predominantly found in humans. Sub-assemblage AIII is almost exclusively found in wild ungulates and is likely to be non-zoonotic. The host distribution of assemblage B is predominantly in human and non-human primates (Feng and Xiao, 2011; Ryan and Caccio, 2013).

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Cattle are commonly infected with assemblages A and E, with E being more prevalent than A (Dixon et al., 2011; Inpankaew et al., 2015; Paz e Silva et al., 2012; Minetti et al., 2014; Santin et al., 2012; Wang et al., 2014). Although assemblage B is less prevalent, it has been reported from many countries (Coklin et al., 2007; Dixon et al., 2011; Lalle et al., 2005; Ng et al., 2011; Ryan and Caccio, 2013; Winkworth et al., 2008). In addition, assemblages C, D and F have been reported from cattle in the United Kingdom (Minetti et al., 2014); and assemblage F from cattle in Spain (Cardona et al., 2015). An age-related distribution of assemblages A and E has also been reported in cattle. Assemblage A was mainly identified in pre-weaned calves and assemblage E was more common in older animals (Mark-Carew et al., 2012; Trout et al., 2007).

Molecular studies conducted in Ethiopia have shown the occurrence of assemblages A and B in humans (Gelanew et al., 2007; Flecha et al., 2015; Wegayehu et al., 2016). Mixed infections of assemblages A and F

have also been reported (Gelanew et al., 2007). However, no investigation has been conducted in describing the assemblages of *G. duodenalis* in cattle from Ethiopia. The objective of this study was to characterize the assemblage and sub-assemblage of *G. duodenalis* in calves; and to assess the potential role of cattle isolates in zoonotic transmission.

2. Materials and methods

2.1. Study area

This cross-sectional study was conducted in Holetta, Sendafa and Chancho and their surroundings of Oromia Special Zone, central Ethiopia between January and June 2014 (Fig. 1). The three study areas are located at a distance of ~40 km west, northeast and north of the capital city, Addis Ababa. Based on the available climatological data, the mean

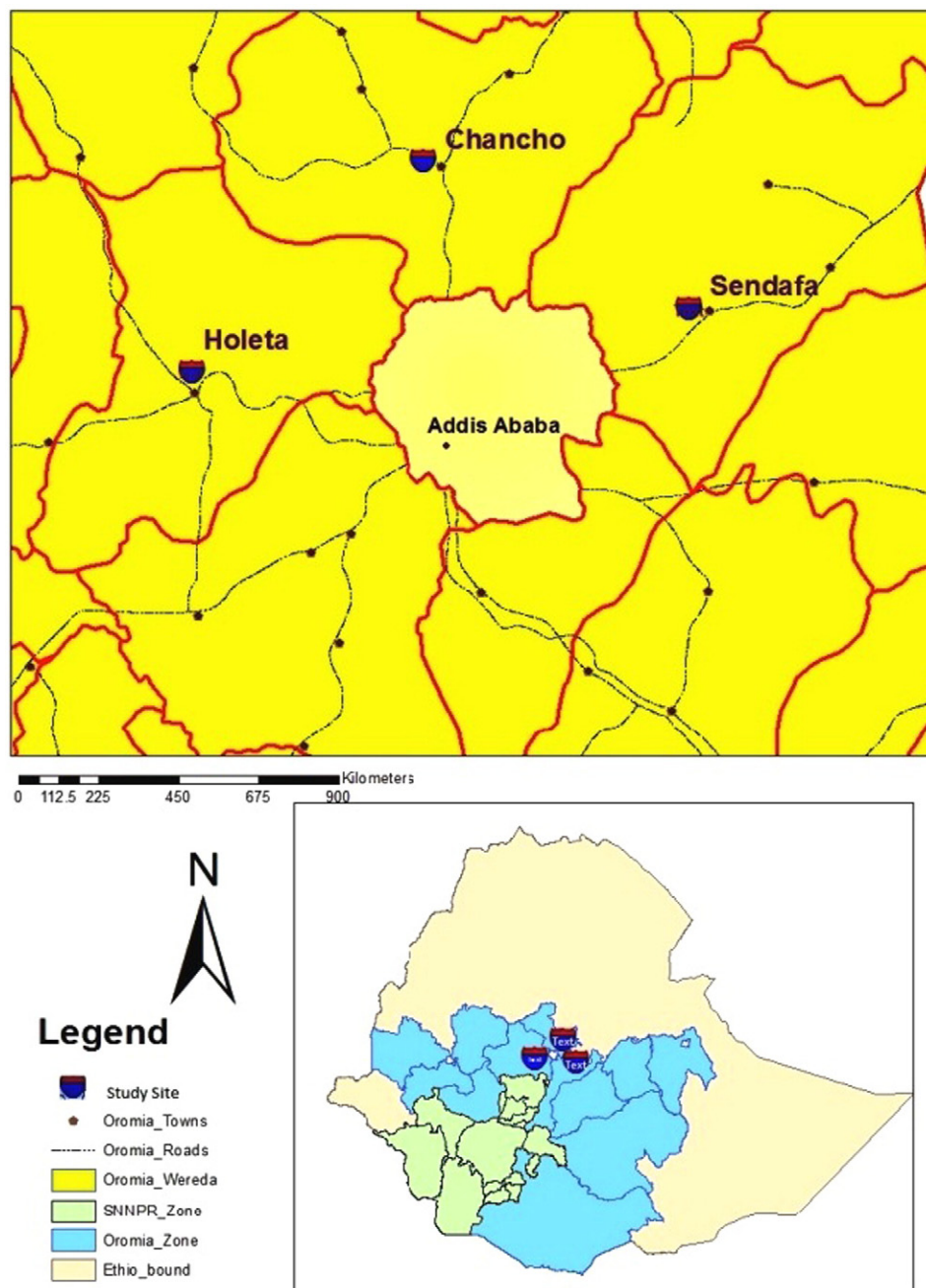


Fig. 1. Map of Ethiopia showing the location of study sites, Holetta, Sendafa and Chancho.

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