Prevalence and Preliminary Genetic Analysis of Giardia Isolated from Adult Sheep in Galicia (Northwest Spain)

JOSÉ A. CASTRO-HERMIDA, ANDRÉ ALMEIDA, MARTA GONZÁLEZ-WARLETA, JOSÉ M. CORREIA DA COSTA and MERCEDES MEZO

*Laboratorio de Parasitología, Centro de Investigaciones Agrarias de Mabeigondo (CIAM), Carretera C-542 de Betanzos a Moeño de Vento, Km 7.5, CP 15318, Abergondo (A Coruña), Galicia, España, and
*Centro de Investigación e Biología Parasitaria-INSIA, Porto, Portugal

GIARDIA duodenalis (synonym G. lambia, G. intestinalis) infects several mammalian species, including man (Adam 2001). Although specimens of G. duodenalis isolated from humans and other animals are morphologically similar, infections are caused by different genotypes of the parasite. The most commonly detected genotypes are: A-I (infects humans, domestic ruminants, dogs, cats, beavers, rats, and other animals); A-II (spread by anthropo-onamic transmission); B (infects humans, beavers, dogs, rats and other animals); C and D (infects canine species); E, F, and G (infects domestic ruminants, cats, and rats) (Fayer et al. 2004). Parasite infection is often asymptomatic but clinical cases have been described in domestic ruminants (Olson et al. 1995; Thompson 2000). The presence of Giardia spp. cysts in sources of drinking water is a serious public health problem as these parasitic forms easily survive standard purification processes (Fayer 2004; Stifko, Smith, and Rose 2000). Information is scarce regarding Giardia epidemiology that include the extent of infection and genotypes involved (Castro-Hermida et al. 2005; Castro-Hermida, de Giacomoni, and Pozio 2006). The main aims of the present study were to determine the prevalence and intensity of infection by G. duodenalis in adult sheep, and to establish the genotypes of the parasite involved in the infections.

MATERIALS AND METHODS

Fecal samples were collected from 575 healthy adult sheep selected at random from 68 herds in Galicia (NW Spain). Giardia duodenalis cysts were detected by direct immunofluorescence with monoclonal antibodies (Aqua-Glo GIC Direct, FL, Comprehensive Kit, Waterborne Inc., New Orleans). The number of parasites/fg feces was determined by the # cysts/ml sample x g feces. In the preliminary genotyping assays, PCR-based procedures were used to amplify and sequence the β giardin gene. A semi-nested PCR protocol was used to amplify the β-giardin gene and for direct sequencing of purified DNA gel bands (Cacció, de Giacomoni, and Pozio et al. 2002; Lulle et al. 2005).

RESULTS AND DISCUSSION

Prevalence. Only few reports are available on the epidemiology of ovine giardiasis in Galicia and there is a lack of information on the importance of adult ovine livestock as a source of infection for humans and animals. In the present survey, we found a high prevalence of Giardia infection (32.7%) among adult sheep. Also, 98.5% of the adult sheep flocks actively shed cysts.

These data demonstrate that infection by G. duodenalis in ovine livestock is widespread in Galicia, Spain. Studies carried out by different workers show that the prevalence of ovine giardiasis ranges between 6.2% and 68.6% (Buret et al. 1990; Olson et al. 1995; Ryan et al. 2005; Taylor et al. 1993), although most of these studies examined only young animals. These data are difficult to compare because of variations in parameters such as age of the animal sampled, the number of samples analyzed, the methods applied, the presence or absence of symptoms, among other parameters.

We believe that the data obtained in the present study are representative of the overall situation in Galicia because the study was not restricted to animals of maximal infection risk ages or those showing disease symptoms. Instead, we analyzed asymptomatic adult sheep selected at random from many different flocks distributed throughout Galicia. The prevalence data we obtained might actually be underestimated because only one fecal sample per animal was collected. Cyst shedding may occur intermittently, usually towards the end of the parasitic patent period. However, in the present study, if an animal tested negative at the time its feces was collected that animal was considered uninfected.

Infection intensity. The intensity of infection by G. duodenalis in adult sheep ranged between 10 and 4,319 cysts/kg of feces. These values are similar to those obtained in previous studies carried out in Spain (Giangaspero et al. 2005). In terms of environmental contamination, the mean number of G. duodenalis cysts that an adult sheep might shed is estimated at 2 × 10^9 to 8638 × 10^3 per day, if it is assumed that the amount of feces produced per animal/day is approximately 2 kg.

Genotyping. Microscopic examination of feces is one of the best methods of determining the prevalence and intensity of Giardia infection. However, molecular techniques that allow characterization of the genes within these cysts provide information on their zoonotic potential and transmission routes (Lulle et al. 2005). The molecular genetics data in our project is at an initial stage and the results obtained so far are preliminary in nature as only a small number of samples were analyzed. The PFGE technique was more sensitive than PCR for detecting G. duodenalis cysts in the feces of adult sheep. Thus, after extraction of DNA from samples with a low parasitic numbers (<100 cysts/fg of feces), we were not able to amplify Giardia DNA by PCR. This could be due to inhibitors in the feces, the small volumes used, or because cysts were only concentrated and not purified. In our opinion, PCR is a highly sensitive and useful technique but so far, we have been able to obtain data on samples with high parasitic load, which more readily allow purification of the cysts. However, the protocol needs modifications in order to amplify DNA of parasites when fecal samples have very low numbers of cysts.

In our initial genetic analyses based on amplification of the β-giardin gene, we detected only genotype E. This genotype is common in bovine livestock and in other domestic ruminants including ovine livestock (Buecher et al. 2004; Hunter and Thompson 2005; Ryan et al. 2003; Trout et al. 2005). There are no
epidemiological or genetic data showing that genotype E infects humans and therefore it is not considered to be zoonotic (Thompson 2003). Studies carried out with bovine livestock in Australia and North America indicated that the risk to public health from calves infected by *G. duodenalis* may be minimal (Hoar et al. 2001; O’Handley et al. 2000). It has been widely demonstrated that domestic ruminants, mainly bovine, ovine, and caprine livestock, are susceptible to infection by zoonotic genotypes of *G. duodenalis*. Genotype A is detected in humans and domestic ruminants over the widest geographical range. However, the results of longitudinal studies carried out in Australia suggest that zoonotic genotypes may be present only as transitory forms and that competitive mechanisms may take place when the frequency of transmission of genotype E is high (Becher et al. 2004). It has even been suggested that the patent periods for genotype A (zoonotic) and genotype E (nonzoonotic) may differ (Trout et al. 2005).

All of the animals examined in the present study were asymptomatic. However, a significant association between the presence of *Giardia* in adult sheep and the occurrence of diarrhea has been reported (Ryan et al. 2005). Further studies are required to analyze larger numbers of samples to confirm whether genotype E predominates in adult sheep in Galicia, which would demonstrate the importance of adult ovine livestock in the transmission of *Giardia* in sheep farms and the possible low risk that these animals represent to public health.

ACKNOWLEDGMENTS

This study was financially supported by Xunta de Galicia through the Consellería de Innovación e Industria (PGIDIT03GAS5035PR) and Ministerio de Educación y Ciencia through the Programa Nacional de Recursos y Tecnologías Agroalimentarias (RTA2006-00007-00-00).

LITERATURE CITED


