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A multicentre prevalence study in Europe on *Giardia duodenalis* in calves, with molecular identification and risk factor analysis

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ABSTRACT

The present study aimed to obtain data on the occurrence of *Giardia duodenalis* in calves in four major cattle rearing countries in Europe (Germany, UK, France and Italy), along with genotyping data and risk factors associated with these infections. A total of 2072 calves were sampled on 207 farms. The majority of the animals were Holstein dairy or mixed Holstein calves ($n = 1565$ or 75.5%), and were female ($n = 1640$ or 79.1%). The average age was 7.8 weeks (SD = 4.1; median = 7; range = 2–16 weeks). All fecal samples were tested using a commercially available monoclonal antibody-based ELISA. The overall apparent prevalence of *G. duodenalis* for the four countries was 45.4% ($n = 942/2072$) and the overall farm prevalence was 89.9% (186/207), with differences in both animal and farm prevalence between the four countries. The prevalence was significantly higher in animals up to 8 weeks (OR = 1.88; $P < 0.001$) compared to older calves, and several management factors including contact with the Dam, Frequency of cleaning of the Maternity Pens, and Disinfection of the Calf Housing were found to be associated with infection. Positive samples were withheld for genotyping using the β-giardin and triose phosphate isomerase gene: *G. duodenalis* assemblage E was most prevalent, although 43% of the isolates were typed as assemblage A, with differences in between countries. Furthermore, 32% of the examined samples was found to be a mixed assemblage A and E infection, which is consistent with previous reports. The results of the present study confirm previous findings in other European countries that *G. duodenalis* infections are common in calves. The infection especially occurs in animals younger than 2 months, and the proportion of positive animals gradually decreased with increasing age.

1. Introduction

Giardia duodenalis has been reported worldwide in production animals, mainly cattle. Although infections in cattle are often subclinical, *Giardia* is able to induce diarrhea and a reduction in weight gain in calves (Geurden et al., 2009). Most of the epidemiological research into *G. duodenalis*

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has been driven by public health concerns, as the parasite has been implicated in a number of waterborne outbreaks with cattle as a potential source for the contamination (Thompson, 2004). *G. duodenalis* is a species complex comprising of eight assemblages (Thompson, 2004; Lasek-Nesselquist et al., 2010), which have a distinct host range. In cattle, both the potentially zoonotic assemblage A and the livestock-specific assemblage E have been identified (Geurden et al., 2006). Assemblage E is the most prevalent in calves (Thompson, 2004), yet reports of mixed infections in calves add a new perspective to the zoonotic potential of bovine infections (Geurden et al., 2008). Furthermore, assemblage A seems to occur more frequently in European cattle, compared to studies in Northern America or Australia (Geurden et al., 2008; Lalle et al., 2005), though this is based on a limited number of studies.

Although prevalence estimates in cattle are available for several European countries (Geurden et al., 2009), data are lacking for other important cattle-rearing countries and major European markets. Moreover, the animal and the farm prevalence vary considerably between studies and countries, and differences in management, geography and climate are considered to partially account for this variation, along with differences in study design (Geurden et al., 2009). A few studies identified risk factors associated with infection with *G. duodenalis* in calves. The type of flooring (Maddox-Hyttel et al., 2006; Muhid et al., 2011), type of calf housing (Ruest et al., 1998), maternity management practices (Wade et al., 2000) and direct contact with infected animals (Gow and Waldner, 2006) were considered to be significantly associated with infection.

The objective of the present study was to obtain data on the occurrence of *G. duodenalis* in calves in four major cattle rearing countries in Europe (Germany, UK, France and Italy), along with data on the *Giardia* genotypes. A risk factor analysis based on questionnaire data from these 4 countries and from a previous study in Belgium (Geurden et al., 2004) was performed in order to evaluate the management factors associated with infection.

2. Materials and methods

2.1. Study design

In each participating country (France, Germany, Italy and UK), one or more regions were selected, considered to be representative of the dairy cattle industry in the respective country. Within each region, at least 30 dairy farms were randomly selected and were visited on one occasion between January and June 2010. On each farm, fecal samples (minimum 2 g) were collected per rectum from all animals within the predefined age range (2–16 weeks). After collection, the fecal samples were labeled with pre-designed labels containing the farm number, animal number and date of collection. Additionally, fecal consistency scores [liquid (L), semi-liquid (SL), semi-firm (SF) and firm (F)] were recorded. Within a country, the fecal scoring was performed by the same investigator. The investigators in the different countries received the same training (with photos) on fecal scoring in order to standardize the procedure. Next to fecal consistency, age, breed and sex of the animals were recorded along with a questionnaire pertaining to farm size, farm management, housing and disinfection procedures, and calf-feeding management on each farm.

The samples were stored at −20 °C until examination. All samples were examined using a commercially available monoclonal antibody-based ELISA (*Giardia* II, catalog No. 30405, Techlab, Blacksburg, VA24060), following the manufacturer's instructions. This assay was previously found to be a sensitive (89%) and specific (90%) diagnostic tool for the diagnosis of *G. duodenalis* in calves for epidemiological studies (Geurden et al., 2004).

2.2. Molecular characterization

At least one positive sample was randomly selected per two positive farms in each country for subsequent genotyping. DNA was extracted from *Giardia* positive fecal samples, using the QIAamp® Stool Mini Kit (Qiagen) according to the manufacturer's instructions, incorporating an initial step of 3 freeze-thaw cycles (freezing in liquid nitrogen for 5 min and heating at 95 °C for 5 min) in the protocol to maximize cyst lysis. The eluted DNA was dissolved in 15 μl ultrapure water. *Giardia* positive samples were characterized using the β-giardin gene (Lalle et al., 2005), and the triose phosphate isomerase (tpi) gene (Geurden et al., 2008). In all of these PCR protocols, bovine serum albumin (BSA) was added to a final concentration of 0.1 μg BSA/μl reaction mixture. Amplification products were visualized on 1.5% agarose gels with ethidium bromide. A positive (DNA from cultured assemblage A and E trophozoites) and negative (PCR water) control sample were included in each PCR reaction. PCR products were purified using the Qiaquick® purification kit (Qiagen) and fully sequenced using the Big Dye Terminator V3.1 Cycle sequencing Kit (Applied Biosystems). Sequencing reactions were analyzed on a 3100 Genetic Analyzer (Applied Biosystems) and assembled with Seqman II (DNASTAR, Madison WI, USA). Sequences were compared with known sequences by BLAST-analysis against the NCBI database.

2.3. Risk factor analysis

On each farm a questionnaire was completed containing information on farm management (Table 1), with a specific emphasis on management practices. Next to the data collected in the 4 countries investigated in the present study, the data from a previous prevalence study in Belgium (Geurden et al., 2004) collected using the same questionnaire and the same study design, including diagnostic technique, were included in the risk factor analysis. Although the Belgian study encompassed several years and included sampling year round, season was not found to be a determining factor in this study.

Multilevel logistic regression models, with maximum likelihood estimates for countries and herds as random variables, were fit to explain the effects of management practices on the risk of *G. duodenalis* infections in calves. Model diagnostics, for the multilevel logistic regressions, included the assessment of residuals at the country and herd levels. The intraclass correlation coefficient (ICC) was