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Molecular characterization of *Cryptosporidium* isolates from beef calves under one month of age over three successive years in one herd in western France

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ABSTRACT

Cohorts of pre-weaned calves were studied for *Cryptosporidium* infection over three successive years (2010–2012) in one beef cattle herd in western France. Each year 25–34 calves were sampled weekly from 3 days to one month of age in order to characterize oocyst output, *Cryptosporidium* species and clinical features associated with infection. Faecal samples were screened for the presence of oocysts using immunofluorescence analysis. DNA was extracted from positive samples and a PCR SSU rRNA followed by RFLP or sequencing was performed. For the subtyping of *C. parvum*, a gp60 PCR was carried out. Regardless of the year, 92–100% of the animals excreted oocysts on at least one sampling date. Depending on the year of observation, the age of highest prevalence varied. In contrast, the peak of excretion was systematically observed almost at the same age (2nd–3rd week of life) with excretion levels ranging from between 100 and 1.7×10^7 oocysts/g of faeces. Differences concerning clinical signs depending on the year of sampling were observed. Different species patterns were observed, with a predominance of *C. bovis* in the 1st year and a predominance of *C. parvum* in the last year. Moreover, two zoonotic subtypes of *C. parvum*, IIaA15G2R1 and IIaA18G2R1, were recorded in different years. This study shows that, in a given farm, the *Cryptosporidium* species and *C. parvum* subtypes identified as well as the prevalence of infection and level of excretion may vary greatly and show distinct patterns according to the year.

1. Introduction

Cryptosporidiosis is a very common infection in cattle worldwide (Santín et al., 2008; Xiao, 2010). The agent responsible for this intestinal disease is a protozoan of the genus *Cryptosporidium*. This parasite can infect a wide range of hosts including humans (Fayer, 2010; Xiao, 2010). The species *C. parvum* is considered to be one of the most common entero-pathogenic species in humans and ruminants.

In ruminants, which represent a major sector of the agricultural economy in many countries, cryptosporidiosis is a well-recognized cause of neonatal diarrhoea (Noordeen et al., 2000; Fayer and Santín, 2009; Silverlås et al., 2010). The first case reported in cattle was in 1971 (Panciera et al., 1971). Now, bovine cryptosporidiosis is considered as one of the major causes of neonatal calf diarrhoea characterized by emission of yellow watery stool, progressive dehydration, growth retardation and possibly death (de Graaf et al., 1999). In contrast, asymptomatic infection

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commonly occurs in yearling heifers and mature cows (Santín et al., 2008). In cattle, *Cryptosporidium* has become a concern not only because of the direct economic losses associated with the infection, but also from a public health perspective because of the potential for environmental contamination with oocysts and especially contamination of water, an important source of cryptosporidiosis outbreaks as recently reviewed by Baldursson and Karanis (2011). Currently, no drug therapy is available and the high resistance of *Cryptosporidium* oocysts in the environment makes cryptosporidiosis difficult to control (Cacciò and Pozio, 2006).

Among the 26 *Cryptosporidium* species considered valid, cattle are usually infected with four: *C. parvum*, *C. ryanae*, *C. bovis* and *C. andersoni* (Chalmers and Katzer, 2013). A chronological sequence of species from birth to adulthood has been suggested by some authors, with *C. parvum* being predominant in pre-weaned dairy calves (<2 months), *C. ryanae* and *C. bovis* occurring mainly in weaned animals and *C. andersoni* becoming dominant in adult cows (Santín et al., 2004, 2008; Šlapeta, 2006; Fayer, 2010; Xiao, 2010). For other authors the succession according to the age of dairy calves varies according to geographic area and management system adopted (Feng et al., 2007; Geurden et al., 2007; Silverlås and Blanco-Penedo, 2012). In Belgium, Hungary and the USA, the most prevalent species in young dairy calves (<1 month) was *C. parvum* (Geurden et al., 2007; Plutzer and Karanis, 2007; Santín et al., 2008), whereas in other countries including Sweden, India and China, *C. bovis* was shown to be the most prevalent species in young dairy calves (<1 month) (Feng et al., 2007; Silverlås et al., 2010).

As far as clinical infection is concerned, *C. parvum* is frequently recorded as the dominant species in diarrhoeic calves, while other species may occur mainly in subclinical situations (Kváč et al., 2006; Fayer et al., 2008; Santín et al., 2008). Among other common pathogens, *E. coli* is known to cause diarrhoea in calves younger than 1 week, whereas Coronavirus and Rotavirus are mainly involved in 1-to-3-week-old diarrhoeic calves (Foster and Smith, 2009; Silverlås et al., 2010).

Molecular characterization studies of *Cryptosporidium* species are less numerous in pre-weaned beef calves than in dairy cattle and few data are available specifically for non-diarrhoeic beef calves (Geurden et al., 2007; Budu-Amoako et al., 2012; Murakoshi et al., 2012). *Cryptosporidium* infection is usually considered less prevalent in beef calves than in dairy calves (Kváč et al., 2006; Geurden et al., 2007).

Subtyping *C. parvum* at the gp60 gene level gives a high number of subtypes, some of them having zoonotic implications (Plutzer and Karanis, 2009; Xiao, 2010). Previous studies have shown that cattle could be the main animal reservoir for zoonotic subtypes of *C. parvum*, i.e. those belonging to families IIa and IId (Xiao and Fayer, 2008; Chalmers and Giles, 2010). According to Alves et al. (2006), human infections with IIa subtype are especially common in areas where intensive livestock production is found.

In France, three longitudinal studies have recently been conducted. Follet et al. (2011) reported the succession of species previously mentioned and the presence of different subtypes of *C. parvum* in dairy calves. The two other studies reported that the species *C. bovis* can be found early after birth in diarrhoeic and non-diarrhoeic beef calves (Rieux et al., 2013a,b). Our current study was a pluri-annual extension of this previous data and was designed to investigate annual patterns in oocyst excretion and in prevalence of *Cryptosporidium* species and genotypes in pre-weaned beef calves in a single beef herd.

Table 1
Number of beef calves sampled according to the year of sampling in one beef cattle herd.

Cohort	Year	Number of beef calves
1	2010	25
2	2011	34 (18 female and 16 male)
3	2012	32

2. Materials and methods

2.1. Faecal sample collection

This study was carried out in a beef cattle farm located in the Deux-Sèvres region in western France. This herd comprised 52 Parthenais-breed cows. The calving season is from September to December. Calves are usually born in the barn among the other animals. During the winter, from November to February, the young animals are raised indoors together with their mothers. In March and April, they spend sunny days outside, and from May they are always outdoors. Cleaning of premises takes place once a year when animals are outdoors.

This study included 25 calves sampled in 2010, 34 calves (16 males and 18 females) sampled in 2011 and 32 calves sampled in December 2012 (Table 1). Results from calves sampled in 2010 and females sampled in 2011 were published previously, so they will not be presented in detail here (Rieux et al., 2013a,b). Faeces were collected directly from the rectum using sterile plastic gloves once a week from birth to 1 month of age. For each animal, the sampling date, age, animal identification number and the consistency of the faeces (score of 0 or 1, 0: absence of diarrhoea, 1: presence of diarrhoea) were recorded. The samples were transported to the laboratory in a sample pot and then stored at 4 °C for a maximum of 48 h before analysis.

Samples were done in compliance with the animal welfare and did not cause any pain according to the ethics committee for animal experimentation no. 16 (French referential).

2.2. Sample processing (oocyst concentration and immunofluorescence (IFT))

One gram of faeces was used for oocyst concentration using ethyl acetate as previously described (Castro-Hermida et al., 2005). One aliquot of 10 μl of the sediment from each sample was fixed on slides using acetone at 4 °C and processed using an IFT commercial kit (Merifluor® *Cryptosporidium/Giardia*, Meridian Bioscience Europe, Nice, France). The samples were observed by fluorescence microscopy at 400× magnification (Geurden et al., 2007,