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Prevalence and risk factors for shedding of *Cryptosporidium* spp. oocysts in dairy calves of Buenos Aires Province, Argentina

Carlos J. Garro [a,*], Gabriel E. Morici [b], Maria E. Utgés [c], Mariela L. Tomazic [d,e], Leonhard Schnittger [d,e]

[a] Grupo de Epidemiología y Medicina Preventiva, Instituto de Patobiología, CICVyA–INTA Castelar, 1585 Hurlingham, Prov. de Buenos Aires, Argentina
[b] Grupo de Parasitología, Instituto de Patobiología, CICVyA–INTA Castelar, 1585 Hurlingham, Prov. de Buenos Aires, Argentina
[c] Centro Nacional de Diagnóstico e Investigación en Endemo-Epidemias, ANLIS "Dr. C. G. Malbrán", Ministerio de Salud de la Nación, Argentina
[d] Grupo de Protozoos Patógenos, Instituto de Patobiología, CICVyA–INTA Castelar, 1585 Hurlingham, Prov. de Buenos Aires, Argentina
[e] CONICET, Av. Rivadavia 1917, C1033AAJ Ciudad Autónoma de Buenos Aires, Argentina

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ABSTRACT

In order to determine the prevalence and risk factors for shedding of *Cryptosporidium* spp. in dairy calves, a cross-sectional study was carried out in the northeastern region of Buenos Aires Province, Argentina. Fecal samples from a total of 552 calves from 27 dairy herds were collected, along with a questionnaire about management factors. *Cryptosporidium* spp. oocysts were detected by light microscopy using Kinyoun staining. Putative risk factors were tested for association using generalized linear mixed models (GLMMs). Oocyst shedding calves were found in 67% ($CI_{95\%}$ = 49–84) of herds (corresponding to a true herd prevalence of 98%) and 16% ($CI_{95\%}$ = 13–19) of calves (corresponding to a true calve prevalence of 8%). Within-herd prevalence ranged from 0 to 60%, with a median of 8%. *Cryptosporidium* spp. excretion was not associated with the type of liquid diet, gender, time the calf stayed with the dam after birth, use of antibiotics, blood presence in feces, and calving season. However, important highly significant risk factors of oocyst shedding of calves was an age of less or equal than 20 days (OR = 7.4; 95% $CI_{95\%}$ = 3–16; $P < 0.0001$) and occurrence of diarrhea (OR = 5.5; 95% $CI_{95\%}$ = 2–11; $P < 0.0001$). The observed association with young age strongly suggests an early exposure of neonatal calves to *Cryptosporidium* spp. oocysts in maternity pens and/or an age-related susceptibility. Association with diarrhea suggests that *Cryptosporidium* spp. is an important enteropathogen primarily responsible for the cause of the observed diarrheal syndrome. Results demonstrate that *Cryptosporidium* spp. infection is widespread in the study region. Monitoring and control of this parasitic protozoan infection in dairy herds is recommended.
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1. Introduction

Cryptosporidiosis is a parasitic protozoan disease caused by several species of the genus *Cryptosporidium*. The parasite infects the gastrointestinal tract in a large number of vertebrate species, including man. A cattle is mostly susceptible to infection by two species of the genus: *Cryptosporidium parvum*, infecting the distal small intestine and *Cryptosporidium andersoni* infecting the abomasum (de Graaf et al., 1999). The overwhelming number of studies report on infection of calves by *C. parvum* while other species, in particular *C. andersoni*, *Cryptosporidium ryanae*, and *Cryptosporidium bovis*, commonly infect adult cattle (Santín et al.,

* Corresponding author at: Institute of Pathobiology, CICVyA–INTA, Los Reseros y Nicolás Repetto, Hurlingham, C.P. 1686, Buenos Aires, Argentina.
E-mail address: garro.carlos@inta.gob.ar (C.J. Garro).

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2008; Delafosse et al., 2015). So far, *C. parvum* has been exclusively identified in dairy calves in Argentina based on two independent molecular studies (Tomazic et al., 2013; Del Coco et al., 2014).

An infected calf may excrete up to 6×10^6 oocysts per gram of feces (Fayer et al., 1998) that are immediately infective, thus heavily contaminating the environment. At favorable conditions of temperature and humidity, oocysts may survive in the environment for months (Fayer et al., 2000). Economic loss associated with *Cryptosporidium* spp. infection are mainly related to diarrhea in calves. Interestingly, diarrhea seems to be a highly variable clinical sign of the infection as it has been observed in 15% (Silverlas et al., 2009) to 100% (Fayer et al., 1998) of oocyst-shedding calves. A conventional method to detect oocysts in stool is Kinyoun's acid-fast stain and microscopical examination. It has been estimated that the sensitivity and specificity of this method is 66.6% and 88.2%, respectively (Elsafi et al., 2014).

In Argentina, depending on the methodology used and the area of study, the prevalence in dairy calves has been reported between 17 and 29% (Bellinzoni et al., 1990; Del Coco et al., 2008; Modini et al., 2011; Tiranti et al., 2011). Although these results seem to agree, they cannot be extrapolated to other dairy calves' populations. An important quantity of dairy activity is located in the northeastern region of the Buenos Aires Province but knowledge of the cryptosporidiosis prevalence in this region is lacking. On the other hand, several studies have identified risk factors for shedding *Cryptosporidium* spp. oocysts, yet the results have been discordant (Silverlas et al., 2009; Trotz-Williams et al., 2007; Maldonado-Camargo et al., 1998). Risk factors provide important information to establish control strategies allowing to diminish and to prevent the spread of calf infection thus minimizing environmental contamination with parasite oocysts. As efficient drugs and vaccines against cryptosporidiosis are not currently available, knowledge of risk factors is paramount to confine the infection.

Based on this rational, the objective of this study was to estimate the prevalence and determine the relevant risk factors for shedding of *Cryptosporidium* spp. in calves of dairy farms in the northeast region of Buenos Aires Province, Argentine.

2. Materials and methods

2.1. Sampling frame

There are about 874 dairy herds in the northeast region of Buenos Aires Province, representing about 33% of all dairy farms of this province (M.A.A., 2010). Dairy herds included in this study are situated in the district of Exaltación de la Cruz, General Belgrano, Lobos, Luján, Marcos Paz, Monte, Navarro, and San Miguel del Monte. The common practice in this area is that cows enter maternity pens approximately four weeks before parturition. Between 6 and 36 h after birth, calves are separated from their mother and are then individually tied to stakes fixed in the ground and raised with the use of feed buckets.

2.2. Study design and data collection

A cross-sectional study was carried out between August 2013 and December 2014. Based on data from a previous report (Bellinzoni et al., 1990), the minimum number of dairy farms to be included in this study should be 27 to accept an error of 15% in the estimated prevalence with a confidence level of 95%. Furthermore, it was estimated that a minimum number of 12 calves should be examined per dairy herd (de Blas, 2016); this calculation was based on the assumption of a within-herd prevalence of 20% (Del Coco et al., 2008) and an expected population of 40 dairy calves. Within each farm, calves were randomly selected. Due to limitations in operational capacity, a maximum number of 30 calves were examined per herd.

A questionnaire was used to collect information about management factors filled by a single member of the study team on all farms. The questions were designed in order to gather information about potential factors associated with *Cryptosporidium* spp. shedding. Physical appearance of feces was evaluated at the time of collection using the scheme to categorize the fluidity of stools proposed by Larson et al. (1977). The occurrence of diarrhea was assigned to feces of score 3 and 4 (liquid or semi-liquid stool) while no diarrhea was assigned to feces that scored 1 and 2 (firm or slightly deformed stool).

2.3. Sampling and detection of oocysts

A single fresh feces sample per calf was collected in a clean polyethylene bag directly from the rectum after anal massage or immediately after deposition. Samples were refrigerated at 4 °C until they were further processed in the laboratory within 48 h. Fecal smears on slides were allowed to air dry and fixed with methanol. Subsequently, Kinyoun staining was performed as previously described (Elsafi et al., 2014; Henriksen and Pohlenz, 1981) and preparations examined with the aid of an optical microscope using oil immersion at 1000× magnification. Oocysts of *Cryptosporidium* spp. were considered those whose morphology, optical properties, internal structure, and size matched those described by Trotz-Williams et al. (2005) and Fayer et al. (2008). At least 40 randomly selected fields were observed until the result was determined as positive or negative.

2.4. Data analysis

The analysis was based on a dichotomous outcome (calf positive or negative for shedding of oocysts). An animal was considered positive when a single oocyst was detected after microscopical examination. The studied factors represent categorical variables of the individual animal: age of calf, type of liquid diet, occurrence of diarrhea, length of time the calf stayed with the dam after birth, gender, blood in feces, and calving seasons (spring–summer months: October to April; autumn–winter months:

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