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Prevalence of *Cryptosporidium* spp. and *Giardia* spp., spatial clustering and patterns of shedding in dairy calves from Córdoba, Argentina

Prevalência de *Cryptosporidium* spp. e *Giardia* spp., distribuição espacial e padrões de eliminação em bezerros de propriedades leiteiras em Córdoba, Argentina

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Abstract

The objectives of this study were to estimate calf and herd prevalence of *Cryptosporidium* spp. and *Giardia* spp., the herd prevalence clustering, spatial distribution according to soil type and shedding patterns in dairy calves from Cordoba, Argentina. Six hundred twenty calves younger than 7 weeks of age from 43 dairy herds were sampled. Samples were processed with the formol-ether and modified Ziehl-Neelsen techniques. Univariate analysis and Kruskall-Wallis tests were used. Factors associated were subjected to multivariate analysis with calf shedding intensity as the response variable. Clustering of herd prevalence was assessed by a scan method, and spatial analysis was applied to explore the overlapping of high prevalence herds and soil type. Overall calf prevalence for *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts were 19.35% (95% CI: 16.14; 22.54) and 34.50% (95% CI: 30.69; 38.34), respectively. Calves younger than two weeks of age were almost four times more likely to be infected with *Cryptosporidium*, in comparison to older ones (RR: 3.78, 95% CI: 2.27; 6.26). *Giardia* spp. shedding showed a similar age pattern (RR: 1.33, 95% CI: 1.02; 1.75). A primary cluster of high *Cryptosporidium* prevalence was found, and high prevalence herds were located in areas with poor drained soil.

Keywords: *Cryptosporidium*, *Giardia*, dairy calves, clustering, soil type.

Resumo

Os objetivos deste estudo foram determinar a prevalência de *Cryptosporidium* spp. e *Giardia* spp., a presença de aglomerados, a distribuição espacial de acordo com o tipo de solo e padrões de eliminação de cistos e oocistos em bezerros de propriedades leiteiras em Córdoba, Argentina. Amostras fecais foram colhidas de 620 bezerros com menos de sete semanas de idade, provenientes de 43 propriedades leiteiras e examinadas pelas técnicas de formol-éter e Ziehl-Neelsen modificada. Foram realizadas uma análise univariada e o teste de Kruskal-Wallis e, em seguida, uma análise multivariada com a intensidade de eliminação de cistos e oocistos, como um evento. A presença de aglomerados foi determinada com o método de varredura e a análise espacial foi realizada para explorar a sobreposição de rebanhos com alta prevalência e tipo de solo. A prevalência de *Cryptosporidium* spp. e *Giardia* spp. foi de 19,35% (IC 95%: 16,14; 22,54) e 34,50% (IC 95%: 30,69; 38,34), respectivamente. A probabilidade de infecção por *Cryptosporidium* spp. foi quase quatro vezes maior para bezerros com menos de 2 semanas em comparação com os bezerros mais velhos (RR: 3,78, IC 95% 2,27; 6,26). O mesmo padrão de infecção relacionada à idade foi observado para *Giardia* spp. (RR: 1,33, IC 95% 1,02; 1,75). Foi encontrado um aglomerado primário com alta prevalência de *Cryptosporidium* spp., e rebanhos com alta prevalência foram localizados em solos mal drenados.

Palavras-chave: *Cryptosporidium*, *Giardia*, bezerros leiteiros, aglomerados, tipo do solo.

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Introduction

Cryptosporidium spp. and *Giardia* spp. are important intestinal pathogens in both people and animals. Cryptosporidiosis is a very common infection in cattle worldwide; calves can become lethargic, anorectic and dehydrated. Calves with severe cryptosporidiosis can take 4 to 6 weeks to fully recover (OLSON et al., 2004), and economic losses due to cryptosporidial infections are related to diarrhea and the extra costs special care of diarrheic calves demand. A mortality rate of 35.5% in calves 0 and 30 days of age has been described (SINGH et al., 2006).

Giardia intestinalis (synomyms *G. duodenalis* and *G. lamblia*) is globally distributed and is a common protozoan parasite in many species of animals and man. The public health implications of giardiasis are well known and its effect on calf performance is not conclusive (HUETINK et al., 2001; OLSON et al., 2004; BARIGYE et al., 2008).

High *Cryptosporidium* and *Giardia* prevalence in calves has been reported in numerous studies (CASTRO-HERMIDA et al., 2002; STARKEY et al., 2005; TROTZ-WILLIAMS et al., 2005; HAMNES et al., 2006; ALMEIDA et al., 2008).

Management factors affecting the risk of cryptosporidiosis have been identified in calves, for example, method and frequency of cleaning, type of flooring, stocking rate, and length of calving season (CASTRO-HERMIDA et al., 2002; LORINO et al., 2005; HAMNES et al., 2006; TROTZ-WILLIAMS et al., 2008). *Giardia* prevalence varies in cattle, and is reflected by differences in management, climate and study design (OLSON et al., 2004). Also, a decreased intensity of infection has been related with increasing age in calves (HAMNES et al., 2006).

It has been demonstrated that *Cryptosporidium* oocysts are able to move through various soil types (MAWDSLEY et al., 1996), and excretion levels in calves are related with cooler and more humid environment for *Cryptosporidium* and *Giardia* cysts (BARWICK et al., 2003; FAYER, 2004; MADDOX-HYTELL et al., 2006).

Although *Cryptosporidium* spp. has been reported previously in Argentina (BELLINZONI et al., 1990; DEL COCO et al., 2008), there is little knowledge about this protozoa and *Giardia* in calves in the dairy region of central Argentina. Also, no previous research has examined the involvement of herd and calf factors on prevalence as well as the herd prevalence clustering according to soil type.

Therefore, the objectives of this study were to estimate calf and herd prevalence of *Cryptosporidium* spp. and *Giardia* spp., the herd prevalence clustering and spatial distribution according to soil type and shedding patterns in dairy calves from Cordoba, Argentina.

Materials and Methods

1. Study period and area description

A cross sectional study was carried out between April and October of 2008, in 43 dairy farms from San Martín district in the Province of Cordoba, Argentina. Dairy farms with a herd size of 100 to 250 cows were randomly selected from a roster provided by the producer association. This stratum represents 80% of the area dairy operations. Final selection was based on producers' willingness to participate, in this regards, the survey response rate was 70%. All farmers bred Holstein-Fressian cows and their calves were raised on site.

2. Sample and data collection

All calves less than 7 weeks of age were clinically examined and a single fecal sample was obtained directly from the rectum using latex gloves and collected in plastic bags. Samples were stored at 4 °C and transferred to plastic containers with formalin until processed.

A questionnaire of health management was administered to the herdsman. The questions were related to dam and calf management, care, nutrition and hygiene. In addition, calf and dam records were gathered to determine calves age and events of interest around calving. Herd geographic coordinates were recorded using a GPS.

3. Laboratory methods

Samples were processed with formol-ether concentration technique (YOUNG et al., 1979), and the sediment was microscopically examined to determine *Giardi*a spp. cysts. Presence of *Cryptosporidium* oocysts was demonstrated by microscopic examination of faecal smears stained with the modified Ziehl-Neelsen technique (HENRIKSEN; POHLENZ, 1981). A sample was considered positive for *Cryptosporidium* spp. if an oocyst was detected with the correct morphology as internal structure, size and shape (FAYER, 1997).

Cryptosporidium oocyst shedding was scored semi quantitatively according to the average number of oocysts in 20 randomly selected fields at 1000x magnification as follows, 0 (no oocysts), 1 (1-5 oocysts), 2 (6-10 oocysts), 3 (>10 oocysts) (CASTRO-HERMIDA et al., 2002). *Giardia* cysts were scored on a scale ranging from 0 to 3 where: 0 = no parasites present, 1 = few parasites present, 2 = moderate numbers of parasites present and 3 = many parasites present (BARIGYE et al., 2008).

4. Data analysis and mapping

Prevalence of calves with at least one *Cryptosporidium* oocyst or *Giardia* cyst in feces was determined. Univariate associations were used to evaluate the association between calf factors and *Cryptosporidium* or *Giardia* presence in feces (THRUSFIELD, 2007). Calf factors analyzed were dam parity, calving difficulties and age (weeks). Median prevalence of strata within each herd factor was statistically evaluated using Kruskall-Wallis test (DOHOO et al., 2003). All herd and calves variables with $p < 0.20$, were subjected to multivariate analysis. Tests were performed using commercially available software (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL, USA).

A multivariate analysis using Poisson mixed regression model was fitted considering the intensity of shedding (levels 0, 1, 2, 3) as the response and herd as a random effect (SNIJDERS; BOSKER, 1999). Age was categorized in ≤ 2 weeks and > than 2 weeks. The restricted maximum-likelihood method was used to model the covariance structure (random factor), and the maximum likelihood estimator to select the fixed effects. The best predictor subset was selected with the Bayesian information criterion. The GliMMixed procedure (SAS System for Windows V 9.1, SAS Institute, Cary, NC, USA) was used (LITTELL et al., 1996).

Cryptosporidium and *Giardia* herd prevalence were spatially described using a dot map displayed in Arcview 3.2 (ESRI, Redlands, CA, USA). Additional map layers, such soil type (resolution 1:3000), was provided by the National Geography Institute of Argentina.

The spatial scan statistic was used to identify and locate significant spatial clusters of *Cryptosporidium* and *Giardia* herd prevalence within the study area. The size of each cluster detected was set to an upper limit of less than 50% of the total area. The null hypothesis to test was that herds within a particular window have the same prevalence level as herds outside the window. The scan process was also set to detect secondary clusters (KULLDORFF, 1997). All analyses were performed in software SaTScan® v 9.0 (KULLDORFF, 1997; 2010).

Results

1. Overall prevalence and calves shedding pattern

A total of 620 calves from 43 dairies was the study population. Overall prevalence for *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts, were 19.35% (95% C.I.: 16.14, 22.54) and 34.50% (95% C.I.: 30.69, 38.34), respectively.

A decreasing trend of *Cryptosporidium* prevalence (≥ 1 oocyst in feces) was observed, according to calf week of age. For the 1st week of age the value was 36.00%, with prevalence decreasing with age, as 23.01, 16.67, 10.14%, 12.82, 14.71 and 14.04% for 2nd to 7th week of age respectively. With respect to *Giardia* cysts, prevalence was 22.32, 20.18, 15.00, 12.32, 13.93, 6.07 and 10.18% for the 1st to 7th week, respectively.

Regarding shedding intensity of *Cryptosporidium* spp. oocysts, 74 (11.94%) calves were classified as low and 46 (7.42%) was the total of calves with moderate and intense shedding. Shedding decreased with calf week of age. For calves of 1 week of age the values for low and moderate or intense shedding were 17.60% (22/125) and 18.40% (23/125), respectively. Fifteen percent (17/113) of the 2 weeks old calves presented low shedding and 7.96% (9/113) of the same age group had moderate or intense shedding.

For *Giardia* spp. cysts, 147 (23.71%) calves presented low shedding, and 67 (10.81%) moderate or intense shedding. A slight tendency was observed for shedding intensity increasing gradually with calf week of age, reaching a peak at week 5, with 35.90% (28/78) of calves presenting low shedding. Calves of 6 weeks of age had the highest value for moderate/intense shedding 17.65% (6/34), followed by calves of 5 weeks of age with a value of 14.10% (11/78).

Calf age in weeks was the only factor that remained ($p < 0.20$) after the univariate analysis for both *Cryptosporidium* and *Giardia*.

2. Herd prevalence

The average number of calves sampled per herd was 13 (± 8.12). Herd *Cryptosporidium* spp. prevalence was 0% for some herds through 58.33% (Figure 1), the median was 14.29% (Q_1 5.56%,

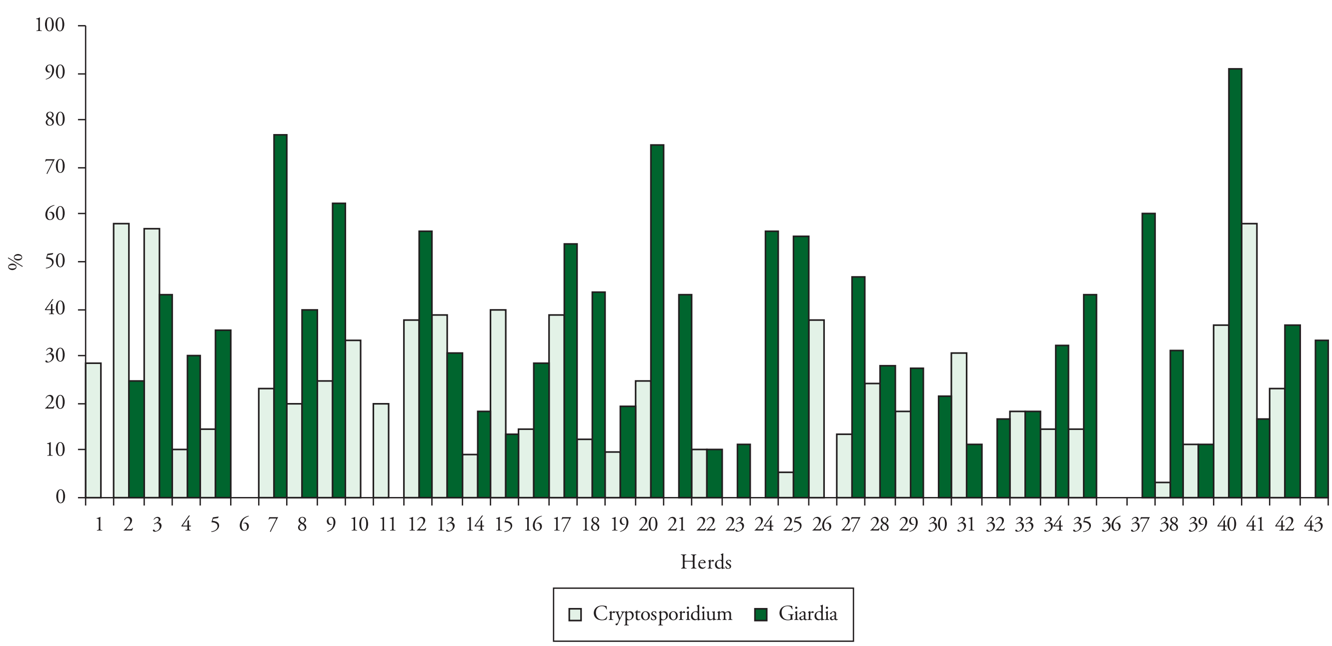

Figure 1. Herd prevalence of *Cryptosporidium* and *Giardia* spp. in dairy calves from San Martín district, Province of Córdoba, Argentina.

Q_3 28.57%). *Giardia* spp. prevalence ranged from 0 to 90.91% (Figure 1), with a median of 30.77% (Q_1 16.67% and Q_3 43.75%). Results from Kruskall Wallis test showed that none of the herd variables were significantly associated with *Cryptosporidium* and *Giardia* median prevalence (Table 1).

3. Multivariate analysis

After testing all the herd and calf variables available, only calf age remained associated with *Cryptosporidium* or *Giardia* shedding in the regression model. Calves younger than two weeks were almost four times more likely to be *Cryptosporidium* positive, in comparison to older ones (RR: 3.78, 95% CI: 2.27; 6.26). The risk of shedding *Giardia* showed a similar age pattern, although the strength of the association was lower (RR: 1.33, 95% CI: 1.02; 1.75). Both models showed herd as significant random effect, so that the calf shedding was highly clustered within farm. The size of the variance component for herds was larger for *Cryptosporidium* (σ_{herd} 0.1499/σ_{calves} 0.1976) than for *Giardia* (σ_{herd} 0.1253/σ_{calves} 0.3253).

4. Spatial analysis

Cryptosporidium spp. prevalence varied among herds, however a group of herds located in the Northern part of the district showed the highest values (Figure 2). Another spatial feature was that most of the high prevalence herds were located in areas with poor drained soil (Figure 2). A primary cluster (P-value = 0.003) of farms was detected in the same area. The risk of having high prevalence herd, within the cluster area, was almost three times higher (RR = 2.85, P-value = 0.001) in comparison with herds outside the cluster area. *Giardia* spp. herd prevalence showed no significant cluster. Similarly to *Cryptosporidium*, high prevalence herds were mostly located within poor drained soil areas (Figure 3).

Discussion

The widespread occurrence of *Cryptosporidium* and *Giardia* found in the herds and calves studied, agrees with previous studies (CASTRO-HERMIDA et al., 2002; TROTZ-WILLIAMS et al., 2005; HAMNES et al., 2006; TROTZ-WILLIAMS et al., 2007).

In Latin America, *Cryptosporidium* prevalence varied from 21.2 to 25% in calves younger than 30 days old (MALDONADO-CAMARGO et al., 1998; LANGONI et al., 2004; ALMEIDA et al., 2008; CARDOSO et al., 2008). Similar values have been reported in Argentina (BELLINZONI et al., 1990; DEL COCO et al., 2008) and are in accordance with the current study. Bellinzoni et al. (1990) studied 452 fecal samples of calves with diarrhea from 33 dairy farms and 36 beef herds from different Argentina provinces, while the later study, involved 280 calves from one dairy farm. In contrast, the present research involved a greater number of dairy farms and calves and focused to understand the prevalence variation among dairy herds from Córdoba. In addition, the prevalence estimated may be less prompt to bias because the sampling was regardless calves health status. To our knowledge, no previous study had reported the presence of *Giardia* spp. in calves in Argentina.

Table 1. *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts median prevalence (%) and herd management factors (n = 43).

Herd median prevalence (%)					
Herd management factors	**Exposure**	***Cryptosporidium***	**P-value**	***Giardia***	**P-value**
Individual system	Yes	14.29	0.674	31.80	0.799
	No	18.18		21.74	
Shade availability	Yes	16.44	0.142	29.67	0.289
	No	10.00		43.75	
Milk bucket cleaning	Never	13.33	0.318	27.27	0.301
	At least once a week	23.33		32.35	
Starter bucket cleaning	Never	14.29	0.820	31.01	0.581
	At least once a week	14.71		32.35	
Weaning age	More than a week	16.23	0.515	29.29	0.741
	Within a week	13.81		31.80	
Treatment at birth	Yes	16.44	0.803	31.01	0.527
	No	11.69		19.96	
Navel disinfections	Yes	14.02	0.630	31.56	0.714
	No	14.29		29.29	
Bucket drinking training	No	20.00	0.974	25.00	0.610
	Yes	14.29		31.01	
Liquid diet duration	≥ 9 weeks	7.96	0.077	30.67	0.410
	< 9 weeks	14.29		26.79	
Herdsman assistant	No	14.50	0.530	27.92	0.673
	Yes	13.33		31.25	
Working only with calves	Yes	14.29	0.892	26.79	0.952
	No	13.40		32.05	

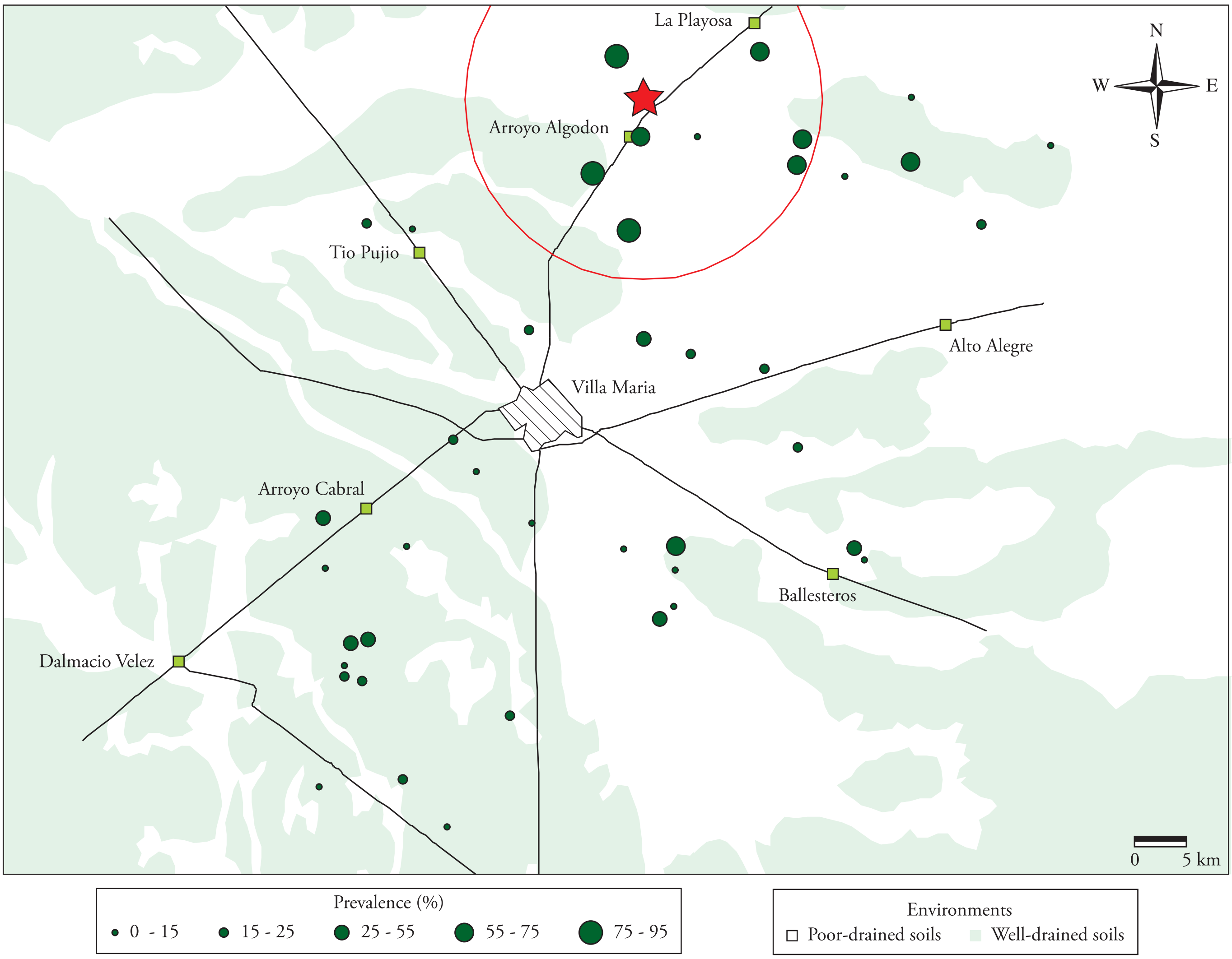

Figure 2. *Cryptosporidium* spp. herd prevalence, spatial distribution and clustering. Province of Córdoba, Argentina. Primary cluster in circle (RR = 2.85, P = 0.001).

Regarding the test used, if quick and relatively quantitative, fecal smears for *Cryptosporidium* oocysts diagnosis have limited sensitivity (KUCZYNSKA; SHELTON, 1999). However, moderate agreement has been reported for the acid-fast staining technique and the enzyme immunoassay. In most cases, herd level determination of cryptosporidiosis requires a smaller sample size, is more economic and easily accomplished with the detection method used (McCLUSKEY et al., 1995). Still, the prevalence may be underestimated because of the examination of a single sample from each calf.

With respect of the infestation intensity, in dairy calves, shedding of *Cryptosporidium* spp. oocysts has been reported to be highest among animals 7-21 days of age (BROOK et al., 2008). This finding agrees with the current study where calves of 1 week of age were the group with increased oocysts' shedding, followed by the 2 weeks of age group.

Infection may have occurred immediately after birth. Dairy calves become infected with *Cryptosporidium* spp. either from the postparturient dam or from hutches contaminated with oocysts from a previous calf (MALDONADO-CAMARGO et al., 1998). Under confinement, the initial exposition to infective oocysts seems to take place in the maternity pen, or after being transferred to the cowshed, indicating a heavy environmental contamination in the calving area (DEL COCO et al., 2008). Under Argentina conditions, the soil parasitic burden may be critical as exposure because the calf paddock area in all herds studied was not subjected to rotation as newborn calves were introduced to the system. In this sense, continual introduction of dairy calves provides the opportunity for year-round transmission to a new susceptible population (OLSON et al., 2004).

Regarding calf factors, of all the variables analyzed, age was the only one associated with *Cryptosporidium* and *Giardia* shedding. Calves younger than two weeks of age were almost four times more likely to be infected with *Cryptosporidium* than those older than two weeks of age. The effect of age on risk of shedding *Cryptosporidium* spp. has been described as highly significant, with calves between 8 and 21 days being most at risk (MADDOX-HYTELL et al., 2006; BROOK et al., 2008; CARDOSO et al.,

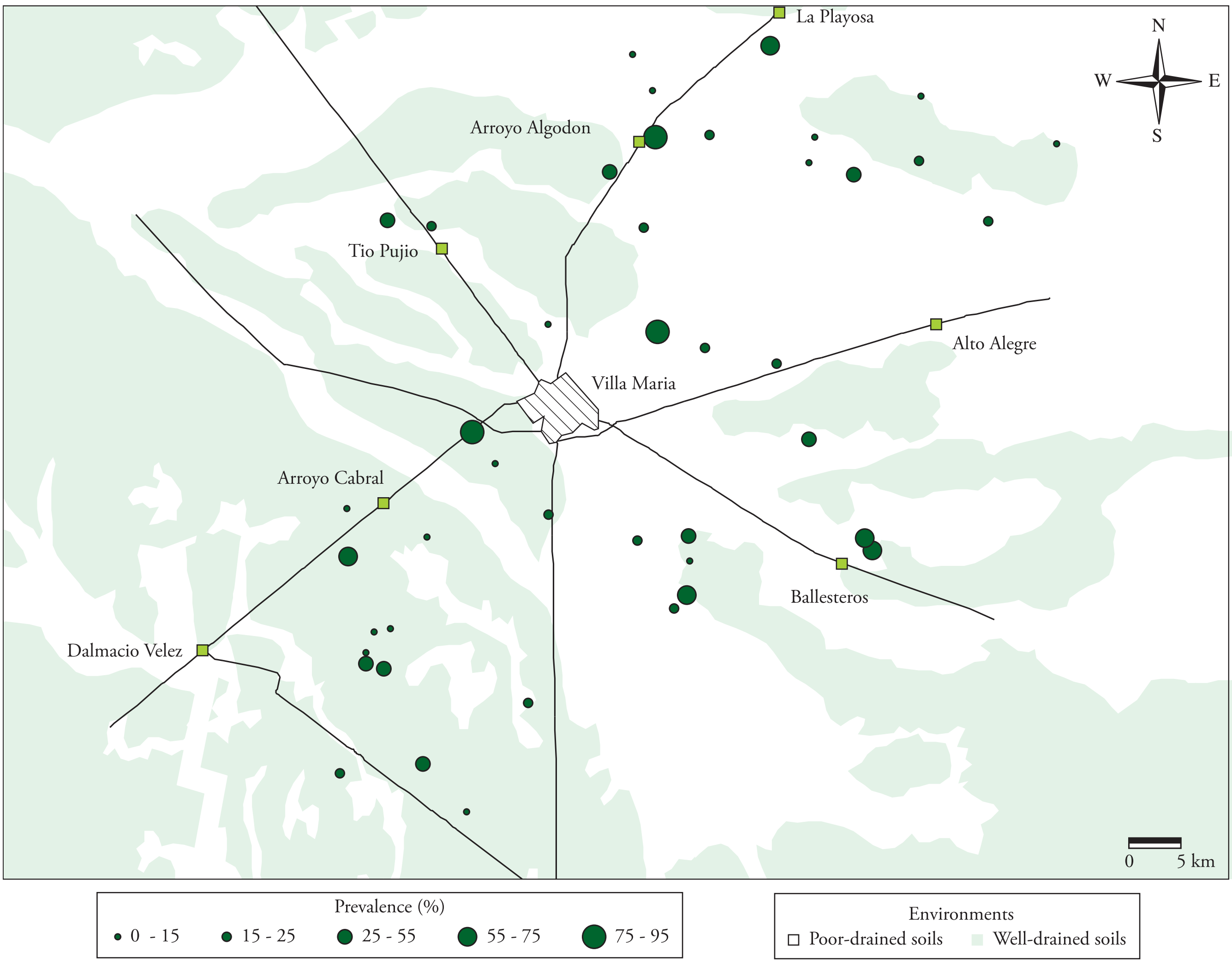

Figure 3. *Giardia* spp. herd prevalence and spatial distribution. Province of Córdoba, Argentina.

2008). Infection dynamics peaked at 1-2 weeks of age, with a shedding duration of 1-2 weeks.

Previous studies in beef calves showed that dam and calf age were predictors for *Giardia* spp. presence. Calves born to 2-year-old heifers were 2.3 times more likely to be shedding *Giardia* spp. than calves born to cows that were 4-10 years of age, while calves 9 days of age and older were at a higher risk of shedding *Giardia* spp. than calves ≤ 4 days of age (GOW; WALDNER, 2006). Age of peak shedding has been described to occur at 5 weeks, with duration of more than 30 weeks for *Giardia* (OLSON et al., 2004).

However, according to other studies, intensity of infection appeared to decline with age (HAMNES et al., 2006), in agreement with the current results where calves younger than two weeks of age were 1.3 times more likely to be infested with *Giardia* than those older than two weeks of age.

In our study calving difficulties was not associated with *Cryptosporidium* spp. oocysts' shedding. Difficult calving causes stress for the newborn calf, which decreases resistance to pathogens due to a combination of reduced calf vigor and delayed ingestion of colostrum (LORINO et al., 2005); it has been also found associated to increased mortality and morbidity in calves (LOMBARD et al., 2007). Therefore calving difficulties should not be disregarded as a risk factor.

Several herd factors were hypothesized as presenting an increased risk on *Cryptosporidium* and *Giardia* prevalence levels, however none of the variables analyzed had a significant effect. Feeding of starter grain to newborn dairy calves was associated with increased *Cryptosporidium* shedding (MALDONADO-CAMARGO et al., 1998), while feeding milk replacer was associated with a decreased risk of shedding (MOHAMMED et al., 1999) but also was found to be associated with higher prevalence (TROTZ-WILLIAMS et al., 2008). At the same time, lower *Cryptosporidium* prevalence has been reported on farms using soap or detergent when washing feeding utensils, and may be related to a better overall calf management on farms (TROTZ-WILLIAMS et al., 2008). The lack of power due to sample size (n = 43) could explained the current results. Besides that, some numerical trends in consistency with previous hypothesis were observed for collective system, shade availability and milk bucket cleaning.

The herd effect on calf shedding was significant and large and means that the magnitude of calf shedding was not independent from the herd they belonged. The herd effect detected in this study may be due and seemed to be explained mostly for the geographic location of the farm. The high prevalence herds, for *Cryptosporidium* and *Giardia*, were mostly located within areas of poor drained soil. Oocysts excretion levels in calves with cooler and more humid environment can be attributed to a season effect. This conditions support the survival of oocysts for extended periods of time (FAYER, 2004; MADDOX-HYTELL et al., 2006). In addition, a significant association between the detection of *Giardia* cysts and the percent moisture of the soil has been described (BARWICK et al., 2003). As the percent moisture increased, the likelihood of detecting cysts also increases.

A limitation of this study was that the molecular characterization of the isolates of *Cryptosporidium* and *Giardia* was not performed therefore further research is needed to assess the zoonotic implications of these results. However, infections in cattle are age related, with *C. parvum* found in 85% of the *Cryptosporidium* infections in pre-weaned calves. In post-weaned calves and older animals *C. parvum* is almost completely replaced with *C. andersoni*, *C. bovis*, and the deer-like genotype (FAYER et al., 2007; THOMPSON et al., 2007). Based on the high prevalence of *C. parvum* in young animals, it has been reported that only neonatal calves are an important source of zoonotic transmission (XIAO et al., 2007).

Conclusions

The current study revealed that *Cryptosporidium* spp. and *Giardia* ssp. are widely distributed among dairy calf operations in the area studied. The infection intensity varied according to calf week of age. A primary cluster of *Cryptosporidium* herd prevalence was detected, while for *Giardia* spp. no significant cluster was identified. High prevalence herds were mostly located within areas of poor drained soil for both parasites.

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