

Case Report

Outbreak of Winter Coccidiosis in calves from Northwestern Argentina

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ABSTRACT

Coccidiosis is an intestinal infection caused by protozoans of the genus *Eimeria*. When the disease occurs in winter, it is known as winter coccidiosis. In July 2021, two calves were referred to INTA-Salta SDVE to determine the cause of death. These animals belonged to a herd that had shown clinical signs, such as bloody diarrhea, poor condition and death. Subsequently, a visit was made to the establishment in which fecal samples were taken on the farm for parasitological studies and a necropsy was performed to another dead animal from the same rodeo. In addition, meteorological data (Tmax, Tmin and Tmed) were collected in the period prior to the death of the animals and at the time of the outbreak. The three necropsied calves showed thickening of the cecum mucosa, colon and rectum, with presence of ulcerations in the cecum mucosa and fibrin clots. Average OPG values were 986.6 (± 1693.06), ranging between 0 and 5040 OPG and the most prevalent species was *Eimeria zuernii*. On the other hand, it was observed that the deaths were related to the moment in which the temperatures decreased. Based on the results of the studies, we conclude that the diagnosis is winter coccidiosis.

Keywords: Winter Coccidiosis, Argentina, *Eimeria zuernii*, Cattle.

RESUMEN

La coccidiosis es una infección intestinal causada por protozoos del género *Eimeria*. Cuando la enfermedad ocurre en invierno se conoce como coccidiosis de invierno. En julio de 2021, dos terneros fueron remitidos al INTA-Salta SDVE para determinar la causa de la muerte. Estos animales pertenecían a un rebaño que había presentado signos clínicos, como diarrea sanguinolenta, mal estado y muerte. Posteriormente, se decidió realizar una visita al establecimiento en donde se tomaron muestras de heces en la finca para estudios parasitológicos y se realizó la necropsia de otro animal muerto del mismo rodeo en una visita realizada al establecimiento. Sumado a esto, se recolectaron datos meteorológicos (Tmax, Tmin, Tmed) en el periodo previo a la muerte de los animales y al momento del brote. Los tres terneros a los que se les realizó la necropsia mostraron engrosamiento de la mucosa del ciego, colon y recto, con presencia de ulceraciones en la mucosa del ciego y coágulos de fibrina. Los valores medios de OPG fueron 986,6 ($\pm 1693,06$), oscilando entre 0 y 5040 OPG y la especie de mayor prevalencia fue *Eimeria zuernii*. Por otro lado, se observó que las muertes estuvieron relacionadas al momento en donde las temperaturas disminuyeron. Con base en los resultados de los estudios, concluimos que el diagnóstico es coccidiosis de invierno.

Palabras clave: coccidiosis invernal, Argentina, *Eimeria zuernii*, bovino.

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INTRODUCTION

Coccidiosis of calves is an intestinal infection caused by protozoans of the genus *Eimeria* (Taylor and Catchpole, 1984). The disease affects mainly animals under one year of age and is clinically characterized by diarrhea, anorexia, dehydration and death (Fitzgerald, 1962; Ernst and Benz, 1982).

Outbreaks of coccidiosis may be related to stress-producing factors, such as extreme environmental conditions, transportation, overcrowding, malnutrition, and change of feed (Fitzgerald, 1962; Taylor and Catchpole, 1984), and generally occur in late summer and in winter months. When the disease occurs in winter, it is known as winter coccidiosis. In that season, the disease can be favored by environmental stress imposed by low temperatures and the concentration of parasitized animals due to lack of water and grass (Niilo, 1970; Chakrabarti and Jha, 2016; Hazarica and Das, 2018).

Winter coccidiosis in cattle was first described by Bruce (1921), who observed outbreaks related to low temperatures in British Columbia. Subsequently, this presentation was reproduced experimentally (Niilo, 1970) and observed under field conditions (Arslan *et al.*, 2015; Chakrabarti and Jha, 2016; Hazarica and Das, 2018). In Argentina, the information about this presentation is scarce, with reports of outbreaks in post-weaning calves and related to stress and environmental conditions of late summer and autumn (Rossanigo, 1996; Sanchez and Romero, 2007; Rossanigo, 2009).

This work describes an outbreak of winter coccidiosis in calves in northwestern Argentina, with details of epidemiological, clinical, pathological and etiological aspects.

Case presentation

In July 2021, two calves were referred to the INTA-Salta Specialized Veterinary Diagnostic Service (SDVE) to determine the cause of death. They belonged to a livestock farm located in Guachipas department, Salta province, Argentina, at an altitude of 1685 masl; the climate is characterized by a summer rainfall regime and a dry period in winter. These animals belonged to a herd of 450 weaned calves around 8 months of age, of which 60 (morbidity: 13,3%) had presented clinical signs, such as bloody diarrhea, poor condition and death (lethality 80%). The animals were necropsied and samples were collected for parasitological and histopathological studies. After calves were necropsied, a visit was made to the farm to observe the health status of the animals, collect fecal samples for parasitological studies, and obtain information about the management and feeding of the herd. In turn, another necropsy was performed during the visit. Therefore, three autopsies were done, two in the laboratory and another one on the ranch.

At necropsy, tissue samples from multiple organs, including small and large intestine, were taken and fixed in neutral buffered (pH 7.2) 10% formalin for 48 h, embedded in paraffin, and stained with hematoxylin and eosin (H&E) for routine histological examination. Fecal samples (n=21) were processed to quantify *Eimeria* oocysts per gram of feces (OPG) using the McMaster method described by Roberts and O'Sullivan (1950). Additionally, the species were identified at x400 magnification following the keys for sporulated oocysts proposed by Levine and Ivens (1967). For this, 3 g of a pool of samples were previously incubated in 2% potassium dichromate in Petri dishes at room temperature for 10 days (Duszynsky and Wilber, 1997).

One hundred oocysts were counted and the relative frequency of each *Eimeria* species was estimated.

RESULTS

At the time of the visit to the livestock farm, 60 calves had died. The herd was grazing in a plot with insufficient pasture for the high stocking rate (50 head of cattle or by hectare / heads per hectare). Upon examination, 20% of the animals in the herd presented poor condition, weakness and hind limbs with fecal matter due to diarrhea, of which 10% had bloody diarrhea (figure 1).

In addition, data on maximum (T max) and minimum (T min) temperatures were obtained, and the thermal amplitude (Tmax-Tmin) of the last two months was calculated in order to correlate these factors with animal death. These variables showed some interesting correlations. First, thermal amplitude observed 25 to 15 days before the first deaths was high and sustained over time. Then, this value decreased due to the occurrence of two snowfall events. The highest thermal amplitude (34°C) was observed about 12 days before the onset of mortality. Finally, during the study period, the mean Tmin was $-3.13 \pm 3.06^\circ\text{C}$, with values close to zero, and mean Tmax was $16.7 \pm 9.7^\circ\text{C}$ (figure 2).

The three necropsied calves showed thickening of the cecum mucosa, colon and rectum, with presence of ulcerations in the cecum mucosa and fibrin clots; a diphtheria mass on the colon mucosa was observed. Histopathological studies showed severe mixed diffuse typhlocolitis with lymphoplasmacytic inflammatory reaction in the lamina propria and surrounding the affected submucosal glands. In the examined section of the colon, clumps of fibrin containing cellular debris and inflammatory cells were adhered to the mucosa. In addition, abundant mature schizonts, oocysts and developing oocysts were also observed deeper in the mucosa, near the muscularis mucosae and crypt epithelial cells, containing different developmental stages (figure 3). Intestinal crypts were distorted or destroyed as a result of coccidial infection. Occurrence of erosion and ulceration of the mucosa was observed. No lesions were noted in other organs.

Parasitological studies of fecal samples from the three calves subjected to necropsy showed the following OPG values: 0, 870 and 17,000. Fecal samples collected on the farm (n= 18) had average OPG values of 986.6 (± 1693.06), ranging between 0 and 5040 OPG. Finally, the relative prevalence of *Eimeria* species showed a high presence of *E. zuernii* (76%), followed by *E. bovis* (18%) and lower proportions of *E. ellipsoidalis* (5%) and *E. auburnensis* (1%). The herd was treated with Toltrazuril 5%. The clinical signs and death of animals remitted after the treatment.

DISCUSSION

Based on the analyses performed and the data on environmental variables before the outbreak, we conclude that the herd was affected by winter coccidiosis. The OPG results observed in this work were generally low. This finding suggests that the animals sampled for OPG count were probably not at the peak of oocyst clearance. Secondly, the OPG values observed in the necropsied animals were low, considering the observed lesions. Oocyst excretion peak is short (1-3 days) and clinical signs or even death of animals

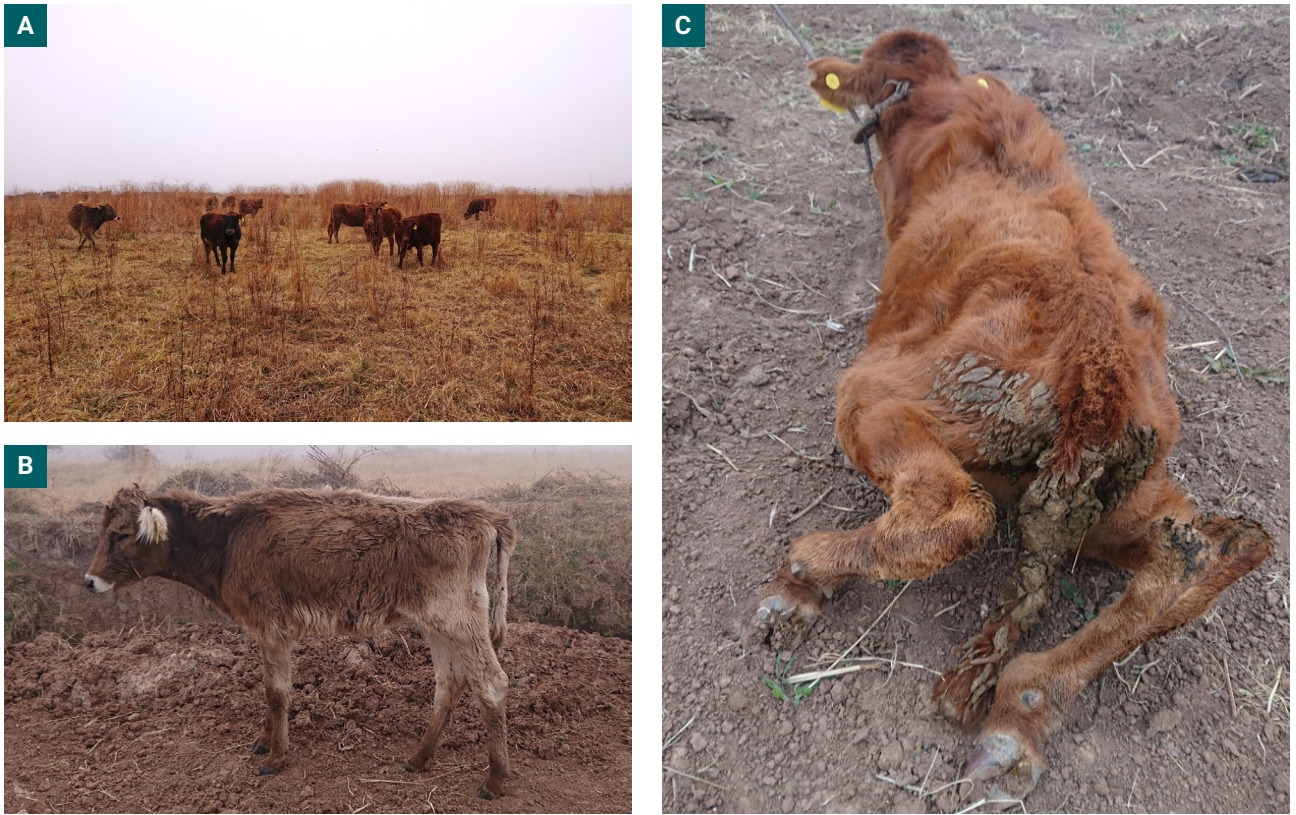


Figure 1. Clinical findings. A. Herd of affected calves. B. and C. Affected calves showing the general poor condition and the dirty perineal area with fecal matter related to diarrhea.

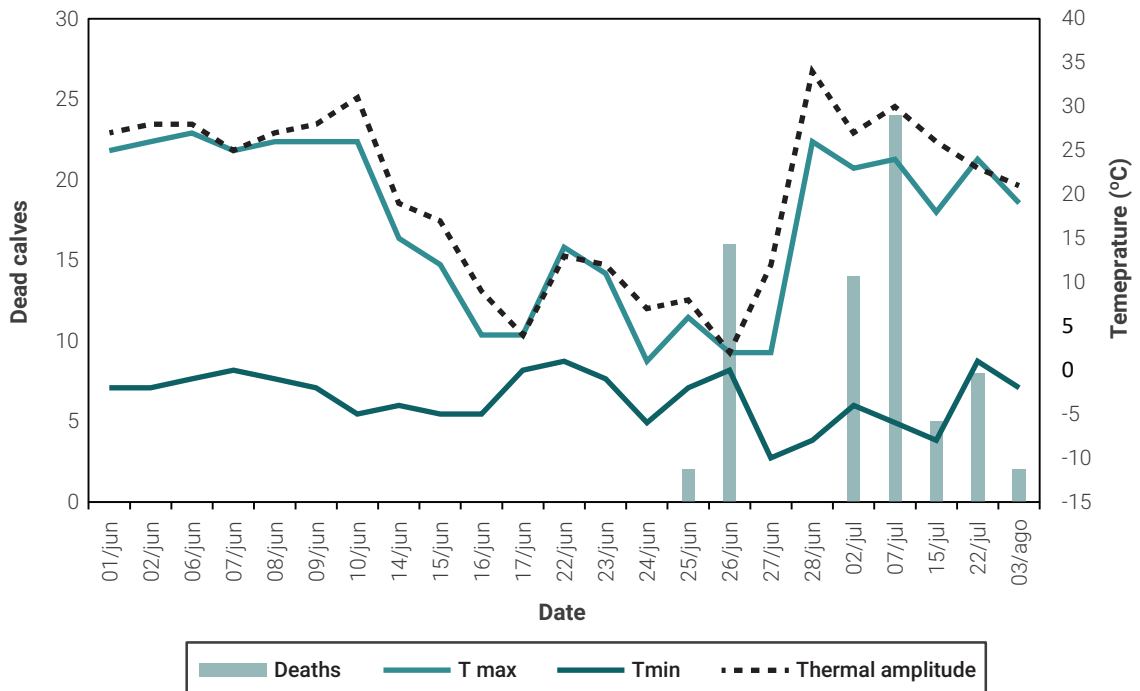


Figure 2. Relationship between thermal amplitude and the distribution of deaths during the months of June/July 2021.

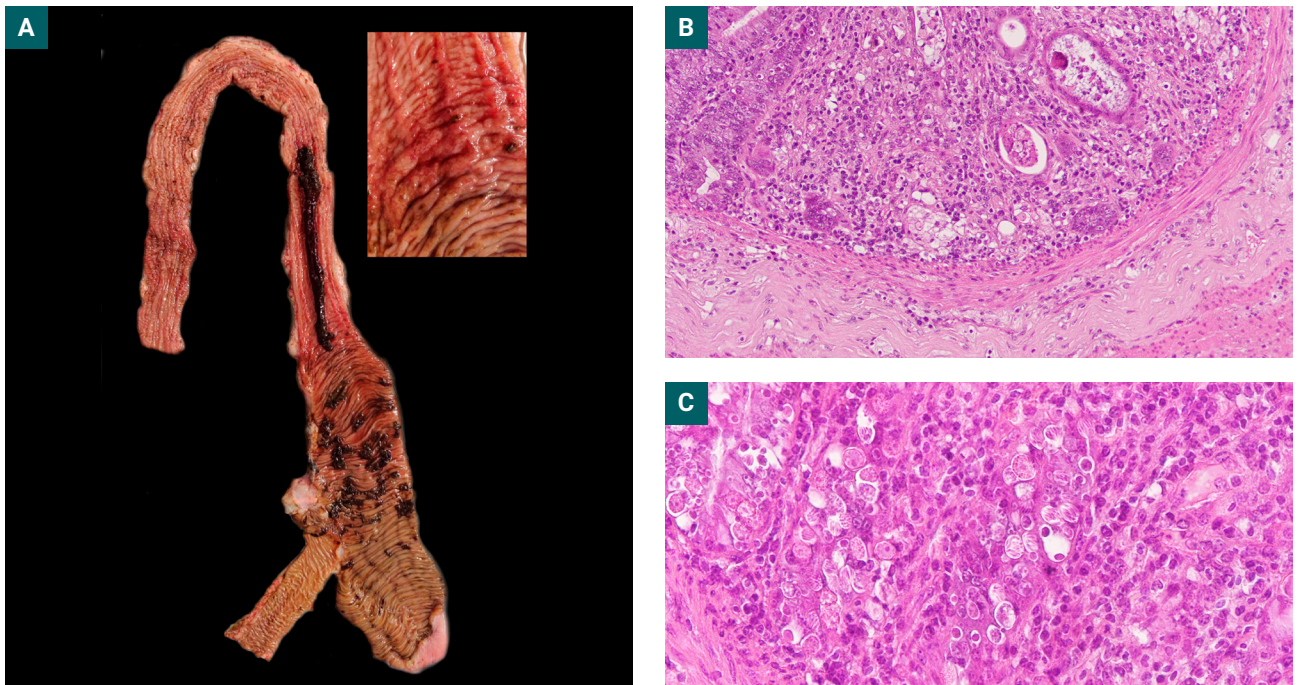


Figure 3. Pathological findings. **A.** Gross lesion. Diffuse necrohemorrhagic typhlocolitis with pseudomembrane formation. Note the proliferative response of the mucosa (colon). **B.** Degenerating mature first generation schizont infiltrated by inflammatory cells in a villus. Note the merozoites within the schizont. **C.** Epithelial cells at the base of a gland infected with *Eimeria* spp. with cellular dissociation and variation in size and shape.

can occur with no occurrence of the prepatent period, and the severity of the clinical signs depends on the number of ingested oocysts (Boughton, 1945; Taylor and Catchpole, 1984; Sanchez and Romero, 2007). Therefore, when coccidiosis is suspected, animals with or without clinical signs must be immediately sampled.

In this study, the pathological findings were crucial to reach the diagnosis of coccidiosis. Microscopically, a diffuse necrotic fibrinous typhlocolitis was observed, with abundant presence of different evolutionary forms of intralesional coccidia. These findings agree with those reported by Stockdale (1977) for calves experimentally infected with *E. zuernii*. Our observations may be correlated to the stage of infection, which is presumed to be between 15 to 18 days before the death events. Stockdale (1977) described that between days 18 and 21, the submucosa and external muscle layers were also involved in the colitis and typhlitis. Later, from day 22, the lesions started to resolve in surviving animals. By contrast, *E. bovis* infection causes severe lesions in the ileum, without spreading as far into the large intestine (Friend and Stockdale, 1980).

Previous works reported outbreaks in late summer-autumn seasons and therefore, temperatures were higher than those recorded in the present study (Rossanigo, 1996; Sánchez and Romero, 2007; Rossanigo, 2009). In this work, the occurrence of the outbreak has an epidemiological peculiarity that would be related to high thermal amplitude and low T_{min} recorded in the study period as well as the overcrowding of animals in the grazing plot. However, the findings obtained at T_{max} agree with the previously mentioned records and it can be assumed that the observed values would allow maturation of oocysts in

the environment. On the other hand, these T_{max} values may be similar to those observed in late summer-autumn period in other regions (Humid Pampa-Semiárid Pampa), where other outbreaks were recorded in Argentina.

The analysis to identify *Eimeria* species showed a high prevalence of *E. zuernii* (76%). This result agrees with findings of Geuden *et al.* (2005), who determined that *E. zuernii* is the most prevalent *Eimeria* species in winter coccidiosis outbreaks, whereas *E. bovis* is abundant in summer outbreaks. This species was also reported as causing this disease in other parts of the world (Arslan *et al.*, 2015; Chakrabarti and Jha, 2016; Hazarica and Das, 2018) and Argentina (Rossanigo, 1996; Sánchez and Romero, 2007; Rossanigo, 2009).

CONCLUSIONS

This work describes winter coccidiosis in northwestern Argentina for the first time. Our findings indicate that in regions with great thermal amplitude, young calves should be monitored and adequate animal load should be maintained to avoid the occurrence of this kind of outbreaks, especially during the periods of greatest differences between daily maximum and minimum temperatures. On the other hand, since oocyst excretion peak period is short, OPG numbers may be low during coccidiosis outbreaks. Thus, if the clinical, pathological and epidemiological findings suggest the occurrence of this disease, complementary diagnostic methods such as histopathology should be used or the analysis of a greater number of samples should be considered. Finally, the favorable response to treatment with Toltrazuril 5%, allows to conclude the diagnosis of coccidiosis.

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