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Prevalence of Cryptosporidiosis in Calves in Large Scale Dairy Farms in Uasin Gishu County, Kenya

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Abstract
Background: Studies were conducted on prevalence and distribution of *Cryptosporidium parvum* in calves aged below 3 months in University of Eldoret farm and other farms in Uasin Gishu County, Kenya for a period of six months in 2009.During the study period, 241 and 80 dung samples were collected from diarrhoeic and non-diarrhoeic calves respectively and microscopically examined. Stool from the livestock was analysed for the presence of *Cryptosporidium parvum* oocysts by Zinc sulphate floatation technique followed by Ziel-Neelsen staining method. **Results**: Prevalence of *Cryptosporidium parvum* in diarrhoeic calves was 75%, 70.6%, 62.5% and 38.5% for Kobilo, Sirikwa B, University of Eldoret and Sirikwa A farms respectively. Prevalence of *Cryptosporidium parvum* in the non-diarrhoeic calves was 45%, 15%, 10% and 5% for the same farms respectively. **Conclusions:** *Cryptosporidium parvum* was positively related to the diarrhoeic status of the calves ($p< 0.05$). The prevalence of *Cryptosporidium parvum* oocysts was significantly higher in diarrhoeic animals than in non-diarrhoeic ones. It was concluded that the overall prevalence and distribution of *Cryptosporidium parvum* was associated with diarrhoea in calves. However, there is need for a long term study to elucidate the effect of other risk factors for *Cryptosporidium parvum* infection in calves, and the occurrence of diarrhoea in relation to cryptosporidiosis and other infections.
Keywords: *Cryptosporidium parvum*, prevalence, diarrhoea, calves

1.0 BACKGROUND OF THE STUDY

Cryptosporidium spp. (Apicomplexa: *Cryptosporidiidae*) are among the most important coccidian parasites of mammals, birds, reptiles and fish, and are distributed worldwide (Arora and Arora, 2005. Plutzer and Karanis, 2009). These protozoan parasites mainly infect the intestinal tract and rarely the respiratory tract of animals and humans (Arora and Arora, 2005). *C. parvum* and *C. muris* are significant species, causing disease in mammals. *Cryptosporidia* are not host specific and cross infection can occur within and between animal species and people (Upton and Current, 1985). Cryptosporidiosis is a zoonotic disease occurring in a wide range of animals including newborn ruminants and people (Fayer and Ungar, 1986. Chako *et al*., 2010).

The organism is particularly troublesome as an opportunistic infection in immunocompromised individuals in whom the infection fulminates and might be life threatening (Sobieh *et al.*, 1987. Houpt *et al.,* 2005). Death as a direct result of Cryptosporidiosis has been reported among patients with Acquired Immunodeficiency Syndrome (AIDS) (Cook, 1996). In cattle and other hosts cryptosporidiosis appears to parallel the disease in humans, for example, clinical cryptosporidiosis has been observed in calves and infants as young as 4 days (Anderson, 1981). Such zoonotic occurrence of patent infections calls for investigations on the parasite at critical times such as the first three months of infant calves (Xiao and Herd, 1994. Ramirez *et al.,* 2004). Cumulative prevalence rates between 2.3 and 2.5% in industrialized nations and between 7.2 and 8.5% in less developed countries have been reported (O'Donoghue, 1995). Cryptosporidiosis has become a concern for diary producers because of the direct losses due to calves not performing well and the potential for environmental contamination with *C. parvum* (Tzipori and Widmer, 2000).

In calves diarrhoea can be caused by a variety of pathogens including bacteria, viruses, protozoa and intestinal parasites (Moon *et al.,* 1976, Del Coco *et al.,* 2008). In acute neonatal diarrhoea, an important disease of calves, four microorganisms in particular, are of widespread occurrence and proven enteropathogenicity: Rotavirus, Coronavirus, *Cryptosporidia* and enterotoxigenic *E coli* (Morin *et al.*, 1976).

Cryptosporidiosis is also of veterinary impotance since farm animals can be infected with various *Cryptosporidium* species that cause morbidity, with subsequent economic consequences (Smith, 2008). *Cryptosporidium* has a large range of host species and infected hosts can shed large numbers of viable, environmentally robust oocysts that contaminate contaminate wastewater, watersheds, surface and ground waters and foods (Fayer, 2008., Smith, Caccio, Cook, Nichols and Tait, 2007)

No studies on the prevalence of *C. parvum* in diarrhoeic and non diarrhoeic calves below three months have been carried out in the North Rift Kenya, where intensive indoor calf husbandry is common. As a result, information on disease prevalence, distribution and impact on these future replacement stocks are inadequate and not precisely known.

In this study, the prevalence of *C. parvum* in calves below three months in University of Eldoret farm and other farms in Uasin Gishu County was determined. An attempt was made to investigate the potential risk factors for infection in these calves that may point to methods by which infections may be controlled.

Cryptosporidiosis is a major cause of morbidity among calves. However, there is lack of data on the role of this infectious disease in calf morbidity and mortality in dairy farms in Uasin Gishu County. The common conditions affecting calves are merely described as diarrhoea without identification of their aetiology.

There are usually high cases of diarrhoea among calves below three months according to the records kept by the Farm Manager at University of Eldoret farm. It was found out that treatment was usually made for *Salmonella typhi* and other related water borne diseases but less attention was given to *C. parvum.* Similarly, most farmers linked the diarrhoea in the calves to excessive consumption of milk by the calves. Apparently, records indicate that the stool specimens delivered to the Ministry of Livestock and fisheries development, Regional Veterinary Investigation Laboratory in Eldoret was only analysed for bacterial pathogens and no attention was given to protozoans especially *C. parvum.*

The high prevalence of diarrhoea among calves in farms needs to be investigated for other organisms such as *C. parvum* othe*r* than the bacterial and viral causes. Uasin Gishu County was chosen for this study because it has intensive livestock keeping and large scale agricultural activities where both inorganic fertilizers and manure are used. The study population consisted calves below three months in the above-mentioned farms.

The purpose of this study was to establish the prevalence and distribution of *C. parvum* in University of Eldoret farm and other dairy farms among calves below three months and its association with water source for the calves and other potential risk factors associated with its occurrence.

1.1 OBJECTIVES

1. To determine the prevalence of diarrhoea in calves aged less than three months in commercial dairy farms within Uasin Gishu County.
2. To determine the prevalence of Cryptosporidiosis infection in calves aged less than three months in commercial dairy farms in Uasin Gishu County.

2.0 MATERIALS AND METHODS

2.1 The study area

The studies were conducted in Uasin Gishu County, one of the original fourteen districts of the Rift Valley Province of Kenya. The County lies between 34 50'E and 35 37'E and 0 03'S and 0 55'N. It is bordered by Trans Nzoia to the North, Elgeyo Marakwet to the East, Baringo and Nakuru to the south East, Kericho to the South, Nandi to the West and Kakamega to the North West (Figure 1.2). Uasin Gishu County is situated within a highland plateau with altitude varying greatly from 1500 m above sea level to about 2000 m a.s.l. Eldoret Municipality, at 2,085 m above sea level forms the boundary between the highest and lowest altitudes. The County is divided into six administrative divisions. These include central, Ainabkoi, Moiben, Soy, Kesses and Turbo.

Four large scale dairy farms were chosen from some of these divisions namely; University of Eldoret farm, Sirikwa A and B are near Eldoret International Airport, and Kobilo farm is near Moi University main campus. University of Eldoret farm is located on the Eldoret-Iten road about 20km from Eldoret town in Sergoit location, Moiben division, Uasin Gishu County, Rift valley province. It has several breeds of cattle and some sheep on its expansive dairy farm. Across the farm there is permanent swamp water and piped water is used to feed the calves. Sirikwa farm A and B are expansive farms that cover over 6000 acres and are located on Eldoret-Kapsabet road about 10 km from Eldoret town in Kapsaret location, Kapsaret division, Uasin Gishu County, Rift valley province. The two farms are located 2 km apart, separated by River Lolgarini, Sirikwa farm A to the south and Sirikwa farm B to the north. Sirikwa farm A has borehole and river as source of water Sirikwa farm B has a permanent swamp as source of water. Kobilo farm is located 20km from Eldoret town on Eldoret-Nairobi road at Cheplaskei, 3km from Ngeria market within Ngeria location, Kesses division, Uasin Gishu County Rift valley province. It has a permanent swamp as the main source of water.

2.2 Study population and subject selection

Calves below the age of three months (Castro-Hermida *et al.,* 2002, Del Coco *et al.,* 2008) from commercial farms formed the target population of 641 calves. The selection of the subjects was based on exclusive criteria by which those under treatment were excluded.

2.3 Study design

Calves were sampled from the four farms in the three administrative divisions; Moiben, Kapsaret and Kesses. The study was cross- sectional and calves were sampled only once with no follow-up to see reinfection. The drainage pattern and source of drinking water was considered and two zones constituted the strata namely the lowland 1500 m above sea level and highland zones 2000 m a.s.l. (Uasin Gishu County Development Plan, 2002 - 2008). University of Eldoret and Kobilo Farms formed the highland stratum while Sirikwa A and Sirikwa B Farms formed the lowland stratum. The selected farms had a level of organisation which allowed gathering of data and collecting samples of reasonable quality for research purposes and are accessible. Similarly, the farms had a past history of diarrhoea in pre weaned calves, and were partly selected based on this history of neonatal diarrhoea

2.4 Management of calves and characteristics of dung samples

Management of calves in the farms studied was in general as follows; Calves were left to suckle for up to 3 days after birth. They were then housed in individual pens and fed with milk and wheat bran. Hay and water were offered adlibitum after this period of suckling. At about two months of age, they were moved to a common pen where they were kept up to the age of 3 to 6 months and milk was gradually replaced by forage and a mixture of cereal by-products. They were later released to grazing fields. The age of the calves on sampling occasion varied from 1 week to 3 months (prepatent period of *C. parvum* oocysts is about 5 days). The breeds of the calves varied from a mixture of Holstein Friesian to local breeds.

Diarrhoea was present if dung was semi-liquid to liquid with or without other abnormal characteristics such as presence of blood or mucous. Any calf with dung without these characteristics was considered non-diarrhoeic. On each sampling occasion, all diarrhoeic calves (excluding those on treatment) and about 20% of the non-diarrhoeic calves were sampled from each farm.

2.5 Methods of data collection

2.5.1 Questionnaire survey

The investigator administered a questionnaire to the four farm managers which required them to respond to various questions relating to the subject under investigation.

A standard questionnaire administered to herd owners captured data on grazing, watering, livestock husbandry practices and worm control practices from the selected herds. This was conducted simultaneously with faecal sampling.

2.5.2 Collection of Dung samples

All samples were collected by the same researcher who also decided whether the calf was diarrhoeic or non-diarrhoeic after stool examination. The dung of diarrhoeic and non-diarrhoeic calves was collected directly from the calf rectum using gloved hands. Each specimen was collected using unused gloves to avoid contamination and infection. Farms were visited every other week throughout the course of the study and a single faecal sample was collected. No calf in the study was sampled more than once. The dung were put in sterile plastic bottles with details of the age labelled and were immediately taken to the laboratory for analysis the same day.

2.5.3 Collection of samples of water for *Cryptosporidium parvum* detection

Water from key drinking sources for the calves acted as the sampling points. Sampling was done at different drinking water troughs containing water from the river, permanent swamp, bore hole and tap. A sample of water for *C. parvum* oocysts testing was collected in sterile bottles of 200 ml capacity. Fifty four samples of this water was collected from the respective farms, labelled and taken to the research laboratory at Chepkoilel University of Eldoret, where analysis to identify *C. parvum* oocysts was done. The water was allowed to settle for 24 hours in the laboratory before analysis.

2.5.4 Detection of *Cryptosporidium parvum* oocysts in the dung specimens

Fresh dung was concentrated to increase the yield of oocysts by sedimentation using zinc sulphate floatation technique.

The modified Ziel-Neelsen Technique staining of dung smears was used as per the procedure described by Henriksen and Pohlenz, (1981), and Clarke and Mcintyre (2001). The procedure was simple, rapid, and inexpensive and allows for distinguishing *C. parvum* oocysts from the yeasts commonly found in dung specimens.

2.5.5 Detection of the *Cryptosporidium parvum* in water samples

Zinc sulphate was added to the sample and then centrifuged. Because of the small size of oocysts of *C. parvum,* water samples should be centrifuged at 500 g for at least 10 minutes (Arora and Arora, 2005). The supernatant was then concentrated in tubes in the presence of distilled water after a process of centrifugation. The preparation (deposit smear) on a slide was stained by using basic fuchsine, rinsed using dilute sulphuric acid and then stained again using malachite green. After air drying, the slides were observed microscopically at ×40 and ×100 oil immersions for *C. parvum* oocysts. The slides with *C. parvum* oocysts were recorded positive and those without were recorded as negative.

3.0 RESULTS

3.1 Prevalence of diarrhoea in the calves in the dairy farms within Uasin Gishu County

In this study, a total of 641 calves in four farms were analysed for symptoms of diarrhoea to establish the prevalence of diarrhoea among the four farms in Uasin Gishu County.

Table 3.0 Prevalence of diarrhoea in calves in Commercial farms in Uasin Gishu County.

Division	Farm	Total No. of Calves in the herd	No. of calves with diarrhoea/D (%)	N	T
Moiben	Chepkoilel	177	77(43.50)	20	97
Kapsaret	Sirikwa A	173	73(42.20)	20	93
Kapsaret	Sirikwa B	151	51(33.77)	20	71
Kesses	Kobilo	140	40(28.57)	20	60
Total	4	641	241(37.59)	80	321

The prevalence of diarrhoea in calves ranged between 28.57% - 43.5% giving an average of 37.59% in the four farms studied. Two hundred and forty one (37.6%) of the calves dung were positive for diarrhoea. The highest prevalence of diarrhoea (43.50%) was reported in University of Eldoret farm, while the lowest (28.57%) was in Kobilo farm.

Table 3.1: Prevalence of *Cryptosporidium parvum* in calves

Farm	No. of calves with diarrhoeal (%)	No. of samples N	D	T	No. of samples with *C. parvum* N (%)	D (%)	T (%)
Chepkoilel	77(43.5)	20	77	97	1(5.00)	30(38.50)	31(32.00)
Sirikwa A	73(42.20)	20	73	93	2(10.00)	46(62.50)	48(51.60)
Sirikwa B	51(33.72)	20	51	71	3(15.00)	36(70.59)	39(54.93)
Kobilo	40(28.57)	20	40	60	9(45.00)	30(75.00)	39(65.00)
Total	241(37.59)	80	241	321	15(18.75)	142(59.92)	157(48.90)

N = Non diarrhoeic calves, D = Diarrhoeal calves, T = total

The prevalence of *C. parvum* oocysts in diarrhoeic calves in the farms ranged from 38.5% to 75.00% giving an average of 59.92%. For the non-diarrhoeic calves, the prevalence of Cryptosporidiosis was between 5% - 45% giving a mean of 18.75% in the farms studied. The total prevalence of *C. parvum* oocysts among both non diarrhoeic and diarrhoeic calves ranged between 32.00% - 65.00% giving an average of 48.90%. The prevalence of cryptosporidiosis was higher in diarrhoeic (59.92%) samples compared to non-diarrhoeic (18.75%) as shown in Table 3.1. The values of prevalence in diarrhoeic and non-diarrhoeic calves for cryptosporidiosis differ significantly ($P < 0.05$).

The frequency of diarrhoea in the calves from the population, and their prevalence together with the calculated data on standardized population (for statistical comparison purposes) are shown in Table 3.2. To facilitate statistical comparison, the frequency of diarrhoea data for each of the sampling site was standardized to a uniform population using the formula

$$SF = (\mathrm{F_i} * \frac{SP}{AP}) \text{ where } \mathrm{F_i} = \text{Actual Frequency, SP} = \text{Standardised sampling population,}$$

$$\text{AP} = \text{Actual sampling population}$$

Overall prevalence of diarrhoea among the calves in the farm was 37.6%. Although data indicate that Chepkoilel University College farm had the highest prevalence of diarrhoea among the four farms, the differences were not statistically significant ($\chi^2 = 7.038$, df = 3, P = 0.071).

Table 3.2: Standardised frequency data on prevalence of diarrhoea in calves sampled from four farms in Uasin Gishu County in the year 2009

Farms	UoE	Sirikwa A	Sirikwa B	Kobilo	Total
Population	177	173	151	141	641
Frequency of diarrhoeic calves	77	73	51	40	241
Prevalence of diarrhoeic calves	43.5	42.2	33.8	28.6	37.6
Standardized Frequency of diarrhoeic calves	77	75	60	51	

3.2 Prevalence of *C. parvum* infection in calves in the dairy farms within Uasin Gishu County

During microscopic examination, the oocysts appeared as bright pink spheres on a pale green background as opposed to yeast cells.

Results on the prevalence of *C. parvum* between the diarrheic and non-diarrheic calves in the four farms of Uasin Gishu County are shown in Figure 1.3. Generally the prevalence of *C. parvum* was significantly ($P < 0.05$) higher among the diarrheic calves than the non-diarrheic ones at all the farms. Among the diarrheic calves, higher prevalence was recorded in Kobilo farm (75%), followed by Sirikwa B (70.6%) while the least prevalence of *C. parvum* was recorded in UoE farm (38.5%). On the standardized data, there were significant spatial differences in the prevalence of *C. parvum* among the diarrhoeic calves ($\chi^2 = 19.767$, df = 3, P = 0.0012).

Similar trends in prevalence of *C. parvum* prevalence were discerned between the diarrhoeic and non diarrhoeic calves in the sampled farms. Among the non-diarrheic calves, highest prevalence occurred in Kobilo farm (45%) and followed by Sirikwa B farm (15%) while UoE farm had the lowest (5%). However, no statistical comparisons were made in the prevalence data because frequencies of most of the data in the samples were less than 5.

4.0 DISCUSSION

The prevalence of diarrhoea among all calves in this study was 37.59% (Table 4.1). Similar prevalence was found by Olson *et al.,* (1993) and Viring *et al.,* (1993) in Swedish herds. Result from studies in other countries show higher prevalence of diarrhoea (Pohjola *et al.,* 1986 Roy 1990, Mc Donough *et al.,* 1994). In Mozambique, Baule *et al.,* (1995) reported an overall prevalence of diarrhoea calves as high as 39%. In the current study, diarrhoea in calves was observed in all the farms studied. University of Eldoret farm and Sirikwa farm A were the farms with the highest mean prevalence of 43.5% and 42.2% (Table 3.1). This might because more calves were sampled there during that period. Although University of Eldoret farm had the highest prevalence of diarrhoea among the four farms, the differences were not statistically significant. Possibly the relatively big size of these 2 farms with many calves makes them more prone to outbreaks of infectious diseases. The diarrhoeal syndrome has a complex ectopathogenesis, because various infectious agents either alone or in combination may be associated with field outbreak. In addition environmental management and nutritional factors may influence the severity outcome of the disease. The 4 major pathogens associated with neonatal calve diarrhoea are Rotavirus, coronavirus, enterotoxigenic *E. coli* and *C. parvum*. These organisms are responsible for the vast majority (75% to 95%) of enteric infection in neonatal calves worldwide (Tzipori, 1995).

In an analysis of dung samples from 218 diarrhoeal dairy calves by Del Fuente *et al.,* (1998) *C. parvum* and rotavirus were the most commonly detected agents. Since this study was aimed at investigating *C. parvum*, the bacterial pathogens were not investigated. The highest prevalence of diarrhoeal calves came from University of Eldoret farm (43.5%) and Sirikwa farm A (42%) and yet they had the lower prevalence of *C. parvum* of 38.5% and 42.2% respectively. This strengthens the suggestion that other pathogen than the one studied here had caused the diarrhoea.

However, the fact that *C. parvum* investigated was not found in some samples may also have been due to other factors for example shedding of the agent did not coincide with the sampling period, failure to detect the causative agent, some cases of diarrhoea might not be associated with infectious agents but, instead could be due to management or nutritional factors.

Diarrhoea is the most pronounced clinical feature of *Cryptosporidiosis*. Several studies observed a higher prevalence of *C. parvum* infections among diarrhoeic calves as compared with non diarrhoeic calves (Castro-Hermada *et al.,* 2002, Lefay *et al.,* 2000, Naciri *et al.,* 1999, Uga *et al.,* 2000.). Out of the 80 clinically healthy calves, 15(18.75%) were infected by *C. parvum*. This is in agreement with the results of the previous studies showing that healthy calves can be sub clinically infected (Bjorkman, *et al*., 2003, Trotz- Williams, *et al*., 2005, Singh, *et al*., 2006). Another possibility is that the calves had already recovered from diarrhoea caused by the infection. In the present study, *C. parvum* prevalence ranged from 38.5% to 75% for diarrhoeic and 3.8% to 45% for non diarrhoeic calves. This was significantly higher compared to that of 50% and 25.68% for diarrhoeic and non-diarrhoeic calves reported in a study carried out in Punjab by Baarcha (2006). These differences could have been due to contaminated waters used in some farms, hygiene and type of floor of the calf pen.

According to Kangethe *et al.* (2007), 50 positive households from 285 households in Dagoretti, Kenya where a faecal sample was collected gave an apparent prevalence of 28%. This was lower than one found in the current study even after sampling animals of all age groups.

Sam Thi Nguyen (2006) investigated the prevalence of *Cryptosporidium* infection in relation to age and clinical status in cattle in central region of Vietnam. The percentage of diarrhoeic and non-diarrhoeic calves identified to be shedding *C. parvum* oocysts was 46.57% and 14.0% respectively. In that study the risk of diarrhoea was 1.7 times greater in *C. parvum* infected calves than their non-infected counter parts.

According to Bjorkman (2003), *C. parvum* showed a prevalence of 11% and 5% for diarrhoeic and non-diarrhoeic calves respectively in a study done on Swedish calves. Even though a higher proportion of diarrheic calves shed *C. parvum* the difference between the groups was not statistically significant, possibly due to the low number of positive samples. In the current study carried out in Uasin Gishu County, Kenya, *C. parvum* prevalence in 241 diarrhoeic calves sampled was 38.5% to 75% while in the non-diarrhoeic calves' it was 3.8% to 45%. In this study, the difference in the groups was statistically significant (P<0.05). This may have been due to high number of positive samples probably because of oocysts contaminated water, poor sanitary and hygiene standards in some of the farms studied.

4.1 CONCLUSION

This was the first study with the aim of determining the prevalence of *Cryptosporidium spp.* oosysts in calves in Uasin Gishu County of Rift valley, Kenya. The overall prevalence of diarrhoea was high (37.59%), which was almost distributed evenly among all the four farms studied.

The study partially revealed the aetiology of diarrhoea in calves, which should lead the Livestock Assistants to consider *Cryptosporidium spp*. as a cause of diarrhoea and form a base for more broad and detailed epidemiological studies on *Cryptosporidium* spp. infection in this region. The overall prevalence of *C. parvum* oocysts in diarrhoeic calves was high (59.92%), although University of Eldoret farm had the lowest prevalence of 38.5%. However in non-diarrhoeic calves the prevalence was low (18.75%) although Kobilo farm had the highest occurrence of *C. parvum* oocysts (45%). *C. parvum* in calves from the current study indicates that it is an important cause of diarrhoea and contaminated water may be a key vector for the parasite.

This was the first investigation of *C. parvum* infection in Kenyan dairy calves that involved four large scale dairy farms with a total of 641 young calves. Thus it can be concluded that the overall prevalence and distribution of *C. parvum* was high and that *C. parvum* is associated with diarrhoea in calves.

LIST OF ABBREVIATIONS AND ACRONYMS

AMPATH: Academic Model for Providing Access to Healthcare.
A.s.l: Above sea level
AIDS: Acquired Immunodeficiency Syndrome.
CDC: Centre for Disease Control and prevention
ELISA: Enzyme Linked Immunoassay
DNA: Deoxyribonucleic acid.
dp: decimal place.
Dp: Dual purpose.
HIV: Human Immunodeficiency Virus.
IFN: Interferon.
MN: Moses Ngeiywa
JM: Judith Makwali
OKO: Ombula Kennedy Ombaka
PCR: Polymerase Chain Reaction.
SPSS: Statistical Package for Social Sciences
UOE: University of Eldoret
UG: Uasin Gishu
UK: United Kingdom.
USA: United States of America.
USSR: United Social States of Russia.

DECLARATION OF COMPETING INTERESTS

I Ombula Kennedy Ombaka do declare that there are no financial and non-financial competing interests.

AUTHORS CONTRIBUTIONS

OKO carried out specimen collection, microscopy, design of the study and statistical analysis.
MMN conceived of the study, and participated in its design and co-ordination and guided in drafting the

manuscript.
JM conceived of the study, and participated in its design and co-ordination and guided in drafting the manuscript.

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