

# CONCURRENT INFECTIONS OF *Cryptosporidium* AND *Giardia* IN DAIRY FARMS, MISSISSIPPI STATE, USA

## Infecciones Concurrentes de *Cryptosporidium* and *Giardia* en Fincas Lecheras del Estado de Mississippi, USA

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### ABSTRACT

Fecal samples from 290 bovines randomly selected at 19 dairy farms in ten counties of Mississippi state, USA, were examined during May to August 1995 to investigate the prevalence and association of *Cryptosporidium* and *Giardia* infections. Oocysts of *Cryptosporidium* were identified through the technique of Sheater's sugar flotation, the acid-fast Kinyoun coloration, and Immunofluorescence assay. Age of animals ranged from 4 days to 8 months. Positive animals were found in 18 (94.7%) farms. From total of 290 analyzed samples, 120 (41.3%) were infected: 69 (57.5%) were positive to *Cryptosporidium* spp., 54 (45%) were positive to *Giardia* spp., and 30 (25%) were positive to *Eimeria* spp. Six calves out 120 were positive to both *Cryptosporidium* and *Giardia* infection, four were positive to *Cryptosporidium* and *Eimeria* infection, and seven animals just had all of three types of parasites.

**Key words:** *Cryptosporidium* spp., *Giardia* spp., *Eimeria* spp.

### RESUMEN

Se procesó un total de 290 muestras fecales en el Laboratorio de Protozoología del College of Medicine Veterinary en Mississippi State University, EUA. Las muestras fueron seleccionadas aleatoriamente en 19 fincas lecheras de 10 condados del estado de Mississippi. Dichas muestras se analizaron con la finalidad de conocer la prevalencia y correlación existente entre los parásitos *Cryptosporidium*, *Giardia* y *Eimeria*. Se utilizaron las técnicas siguientes: concentración por flotación con azúcar (Sheater's), coloración Kinyoun (específica para *Cryptosporidium*) e Inmunofluorescencia. La edad de los animales osciló

entre 4 días y 8 meses. Resultaron positivas a la presencia de parásitos *Cryptosporidium* spp., *Giardia* spp., *Eimeria* spp. 18 fincas (94,7%) con 120 muestras (41,3%) del total de muestras analizadas. Para *Cryptosporidium* spp. 69 (57,5%) muestras resultaron positivas, *Giardia* spp. 54 (45%) y para *Eimeria* spp. 30 (25%). Se encontró una correlación entre infestaciones de *Cryptosporidium* y *Giardia* en 06 animales, entre *Cryptosporidium* y *Eimeria* en 04 animales, y solo siete animales positivos a los tres tipos de parásitos (*Cryptosporidium* spp, *Giardia* spp., *Eimeria* spp.).

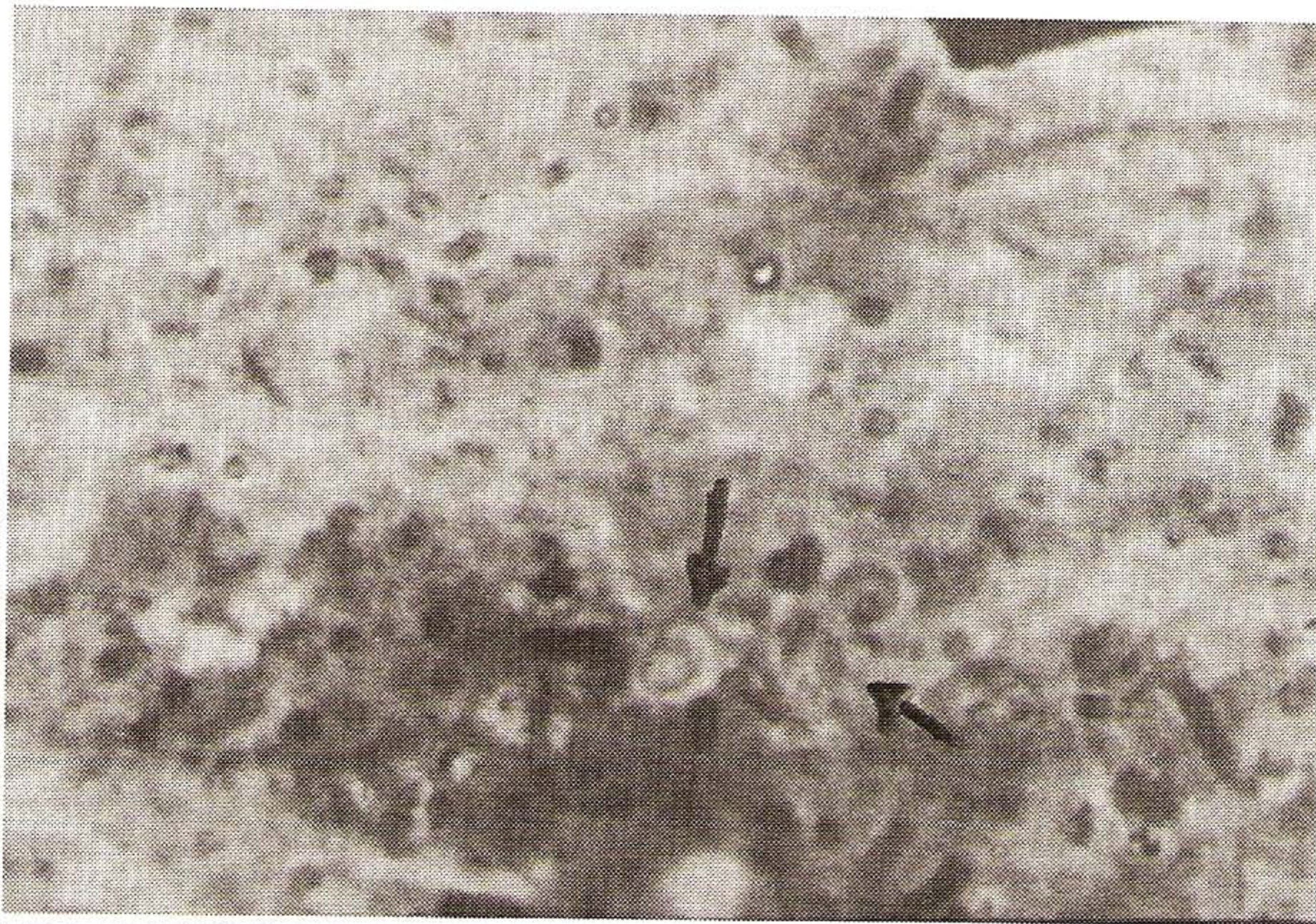
**Palabras clave:** *Cryptosporidium* spp., *Giardia* spp., *Eimeria* spp.

### INTRODUCTION

The most important cause of calf morbidity and mortality is diarrhea [9] and it is primarily caused by infectious agents [24]. *Cryptosporidium* has emerged [18] as a prevalent enteropathogen of neonatal calves and producing bovine cryptosporidiosis. It was first isolated a case of diarrhea in 1971. Subsequently, these organisms have been diagnosed frequently during investigations of calf diarrhea in many countries [2,19]. Two morphologically distinct species of *Cryptosporidium* spp. occur in calves: *C.parvum* and *C.muris* [1].

Clinical neonatal bovine cryptosporidiosis is usually a result of infection with *C. parvum* [1]. The oocyst are 4.5-5.4 µm and contain a residuum and four sporozoite [13], FIG 1. A variety of other enteropathogens like virus, bacteria, are detected. *Cryptosporidium* has direct life cycle which it is completed in 4 days and the transmission is fecal-oral. Cryptosporidiosis is considered to be a zoonosis [14], and the most common route of infection is close contact with diarrheic feces infected. How-





**FIGURE 1. OOCYSTS OF *Cryptosporidium parvum* KINYOUN STAIN. 100X IMMERSION.**

ever, the excretion of oocyst has been found in apparently healthy adult cows [5] and in asymptomatic humans [1]. Studies [20] have indicated that adult cattle are carriers of *C.parvum* and these studies sought to determine more about the role of apparently healthy adults [16]. A number of surveys carried out in recent years show a world distribution of *C.parvum* [25]. In humans and small animals, *Giardia* infection is also common and frequently associated with the occurrence of diarrhea [10, 27]. The earliest reports of *Giardia* were in India [6], Egypt [22]. Outbreaks of diarrhea associated with *Giardia* and *Cryptosporidium* infections occurred in two cattle farms in Ohio [26]. Concurrent infection with two or more agents occur frequently under field conditions [19, 21]. The objective of the study was evaluate the prevalence of the protozoa *Cryptosporidium* and the correlation among the parasites *Cryptosporidium*, *Giardia* and *Eimeria* in calves of Dairy farms of Mississippi state, USA, during the summer time.

## MATERIALS AND METHODS

### Sample collection

A total of 290 (181 female, 109 male) Holstein calves from 19 dairy farms of Mississippi, (USA), in ten counties were used for the survey. Fecal samples were collected directly from the rectum and stored at 10°C, until processing. Details of age, breed, sex and calving date were recorded for all the animals in the study.

### Fecal examination

#### • Sheater's sugar solution:

Two g of fresh feces were emulsified with 15 ml of distilled water, strained and pour into 15 ml conical centrifuge tube. The contents were mixed and centrifuged at 1,500 xg for

10 min The supernatant was discarded and the sediment was resuspended in Sheater's sugar solution. A cover slip was placed on the top of the positive meniscus and centrifuged at 1,500 xg for 10 min. The cover slip was placed on a slide and the slides were read immediately using both light and phase-contrast microscopy. Some positive samples were examined by Potassium-Iodine to observe morphology of the *Giardia* cysts, FIG 2.

#### • Kinyoun stain acid-fast:

Thin smears of feces were applied to microscope slides and fixed with gentle heat. Slides were flooded with carbol-fuchsin solution for 3 min. Slides were rinsed with tap water until no more stain appears in the rinse. Decolorized with HCL (95% ethanol, rinsed again with tap water and stained with brilliant green for 30 sec; (Becton Dickinson-Franklyn, Lakes, NJ, TB Kinyoun stain procedures) [12]. Slides were gently rinsed with tap water and dried. Immersion oil and cover slip were applied, and the slides were examined for oocysts at 100x.

#### • Immunofluorescence assay:

Fecal positive samples were examined for *Cryptosporidium* and *Giardia* cysts by direct immunofluorescence staining technique, using a commercial kit (Meriflour-Meridian Diagnostics, Inc., Cincinnati, Ohio). Reagents and samples were brought to room temperature prior to testing. A special transfer loop was used to drop fecal material on to the treated microscope slide. The specimen was spread over the entire well. The slide was allowed to air dry at room temperature for approximately 15-20 min. A drop of detection reagent was placed in each well, and a drop of counterstain was added to each well. The reagents were mixed with an applicator stick and spread over the entire well. The slide was incubated in a humidified chamber for 30 min at room temperature. The slide



was washed and rinsed with a gentle stream of buffer solution, until the excess detection reagents and counterstain were removed. The slide was placed on a clean paper towel to remove the excess of buffer. One to two drop of mounting medium was added to the slide and coverslip was applied. Each well was thoroughly scanned at 100-200x magnification.



FIGURE 2. *Giardia* CYSTS RECOVERED FROM CALVES FAECES SHEATER'S SUGAR SOLUTION. 40X.

### Statistical analysis

All data, collected for each animal were stored in a relational database (The Smart Software System, Innovate Software, Inc.) and including number, age, sex, county, calving date and parasite ova count. Statistical analysis was performed using chi-square test.

### RESULTS

Positive animals were detected in 120 (41.3%) from the total of fecal samples analyzed; 69 (57.5%) were positive for *Cryptosporidium* spp., 54 (45%) were positive for *Eimeria* spp., and 30 (25%) were positive for *Giardia* spp. Six calves out of 120 had combination of *Cryptosporidium* and *Giardia* infection, four were positive for both *Cryptosporidium* and *Eimeria* infection, and just seven calves out 120 had all the three types of parasites *Cryptosporidium-Eimeria-Giardia*, TABLE 1. Infection intensities ranged from light to severe (infection index 1-3). There was correlation demonstrated between infection of *Cryptosporidium* and *Giardia*. Similar with the correlation of *Cryptosporidium* and *Eimeria* although there was not a relationship between presence of *Eimeria* and *Giardia*. There was not correlation among *Cryptosporidium*, *Giardia* and age, but there was relationship between *Eimeria* and the age of three groups

TABLE I  
PREVALENCE OF *Cryptosporidium*, *Giardia*, AND *Eimeria* IN DAIRY FARMS MISSISSIPPI STATE, USA

Farms	County	Total animals	Infested animals	%	<i>Crypto.</i>	%	<i>Giardia</i>	%	<i>Eimeria</i>	%
1	1	10	07	70	02	28.5	05	71.4	0	0
2	1	27	14	51.8	05	35.7	11	78.5	0	0
3	2	21	10	47.6	05	50.0	07	50.0	03	30.0
4	3	14	02	14.2	01	50.0	01	50.0	0	0
5	2	08	01	12.5	0	0	01	50.0	0	0
6	2	14	05	35.7	04	80.0	0	0	01	20.0
7	2	26	05	19.2	04	80.0	0	0	02	40.0
8	2	21	10	47.6	04	40.0	02	20.0	05	50.0
9	3	08	05	62.5	03	60.0	03	60.0	02	40.0
10	4	02	02	100	01	50.0	01	50.0	0	0
11	2	08	05	62.5	01	20.0	02	40.0	04	80.0
12	9	07	04	57.1	04	100	02	50.0	01	25.0
13	6	09	02	22.2	01	50.0	0	0	01	50.0
14	7	23	09	39.1	08	88.8	03	33.3	01	11.1
15	8	12	04	33.3	04	100	0	0	0	0
16	10	07	05	71.4	02	40.0	04	80.0	0	0
17	1	11	0	0	0	0	0	0	0	0
18	8	52	22	42.3	12	54.5	12	54.5	02	9.0
19	5	10	08	80.0	08	100	0	0	08	100
Total		290	120	41.3	69	57.5	54	45.0	30	25.0





**FIGURE 3. PREVALENCE OF *Cryptosporidium*, *Giardia* AND *Eimeria* INFECTIONS IN CALVES IN MISSISSIPPI STATE, USA. COUNTIES: 1- 43.7%, 2- 40.1%, 3- 31.8%, 4- 100%, 5- 90%, 6- 22.2%, 7- 7.8%, 8- 71.8%, 9- 57.1%, 10- 71.4%.**

>10 weeks old. There was a significant correlation among animal's infection and the county in which the farm was located. The county number 4 had a higher incidence of infestation (100%), followed by the number 5 (90%) and number 8 (71.87%), FIG. 3.

## DISCUSSION

First survey of *Cryptosporidium* in calves in Mississippi state, USA. The prevalence rate of infection of *Cryptosporidium* was 57.5% in the animals aged 1-4 weeks which was comparable with the 51% of prevalence found among 1-3 week old calves surveyed in Washington state, USA [17]. Although one study suggested that there is not difference in the frequency of infection among various age groups of calves [23], others have indicated that infection is more prevalent among younger animals [15]. *Giardia* was more prevalent (45%) than previously reported in others surveys [26]. A more sensitive test, immunofluorescence assay, was used which explain the higher inci-

dence of *Giardia*. There has been much debate about the zoonotic potential of Giardiasis [3, 7]. There was a high correlation between infection current of *Cryptosporidium* and *Giardia* (13.72%) in those animals sampled. These results were similar to a study that reported this correlation in Czechoslovakia [11,26]. *Cryptosporidium* and *Giardia* are probable more common than reported. Giardiasis and Cryptosporidiosis are considered to be significant zoonotic diseases and domestic animals are believed to be important reservoirs [4, 8].

## CONCLUSION

This study reveals that *Cryptosporidium* spp., *Giardia* spp., and *Eimeria* spp., are the most frequent parasites in calves in Mississippi state dairy farms. Results of the current study demonstrate that *Cryptosporidium* spp., is frequently involved in the aetiology of calf neonatal diarrhea in this region and adult carries seem to play an important role as a source of infection. *Giardia* infections have also been found to be unexpectedly frequent in cattle, although the role of this protozoan in the aetiology of diarrhea in calves remains unclear. The dairy farms of the counties studied use multiple cow calving facilities and these increase the pathogen load in the susceptible calf's environment. These infections play an important role in animal production as it has been demonstrate that these parasites are able to cause diarrhea, which negatively influence growth, impair feed conversion and reduce milk production in this region, because small herds were more likely to be positive for these infections. *Giardia* and *Cryptosporidium* may also act as an infection source for humans by direct contact with contaminated feces or water. The neonatal period is critical time for exposure. Hygiene measures to control and prevent *Giardia* and *Cryptosporidium* infections must be considered in dairy calves farms of Mississippi State. Also, put the young animals in individual pens in order to prevent cross-contamination.

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