

Prevalence of some *Cryptosporidium* species in cattle in Baghdad, Iraq.

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Abstract

A total of 268 fecal samples were collected from calves between 1 week to 2 years old from Al-Nasr station for dairy cattle and three regions in Baghdad (AL-Taji, AL-Shula, and AL-Gazaliya). Modified Zehil Neelson stain was used to detect *Cryptosporidium* oocysts in these samples. Oocyst shape and size were used as criteria for species identification. The overall prevalence of *Cryptosporidium* infection was 35.44%. No sex preponderance was found, but there was decreasing in the prevalence versus age, with *C. parvum* was the dominated species before six month age, and *C. andersoni* in calves older than that.

Introduction

Cryptosporidiosis is considered as one of the leading cause of diarrhea in neonate calves (1). Adult cattle are also infected but usually without obvious clinical signs. Initially, based on microscopic identification of oocysts, cattle were reported to harbor two species: *Cryptosporidium parvum* and *Cryptosporidium muris*, infecting intestine and abomasums, respectively(2). After application of molecular techniques and cross species transmission studies, *C. muris* from the cattle properly identified as

a new species, *C. andersoni* (3). *C. andersoni* infect the gastric gland of abomasums (4) and have been reported in calves older than 4 weeks of age (5). Infections produce no diarrhea and followed a more chronic course than *C. parvum*; oocyst production is less than *C. parvum* (6). This study aimed to determine the prevalence of the two main *Cryptosporidium* species: *C. parvum* and *C. andersoni* in cattle. For our knowledge, this is the first study which involved the prevalence of *C. andersoni* in cattle in Iraq.

Material and Methods

1- Source and collection of specimens:

Fecal samples (~5 grams each) were collected from 268 calves and heifers between 1 week to 2 years old during the period from November, the first 2009 until 30th April, 2010. The study involved Al-Nasr station for dairy cattle and three regions in Baghdad; AL-Taji, AL-Shula, and AL-Gazaliya. The fecal samples were collected directly from the rectum in a plastic containers with a detailed history about age, sex and previous treatment and were transported to the parasitology laboratory which belongs to the college of veterinary medicine-University of Baghdad.

2- Oocyst detection:

Modified Zehil Neelson stain method (7) was used to detect the oocysts in the fecal sample in which thin smears of feces were made on a clean, grease free glass slide and air dried. Then the smears were fixed transiently over a flame, and stained with a strong carbol fuchsin solution for 5

minutes. The slide was heated until steam appeared but boiling was avoided, and an additional stain was poured if the slide subjected to dryness. After staining, the smear were washed in running tap water for 1-2 min, then decolorized in 5% sulphuric acid for 30 seconds. Again the smears were washed in tap water for 1-2 min and counter stained with 3% methylene blue for 1 min. Finally the smears were washed in tap water and air dried, and examined microscopically under oil immersion (100 ×) for *Cryptosporidium* oocysts.

3- Measuring of oocysts size

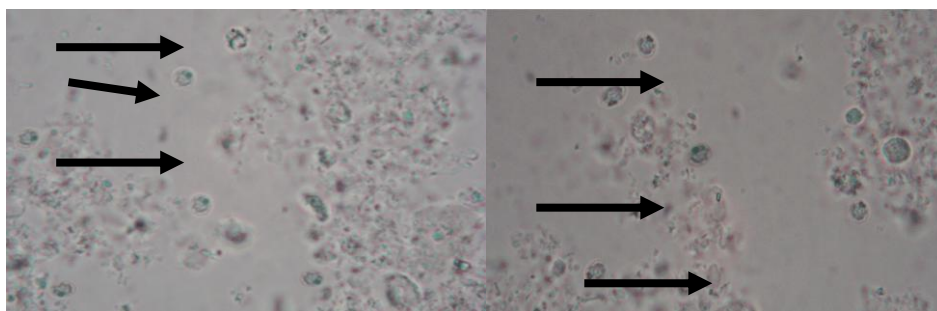
Ocular micrometer was used to measure the sizes of oocysts. Ten oocysts from each positive sample were randomly chosen from different location within the slide and measured. 4-Statistical analysis

The Chi-square test was used to analysis the overall prevalence data, and differences were considered significant when $P < 0.01$.

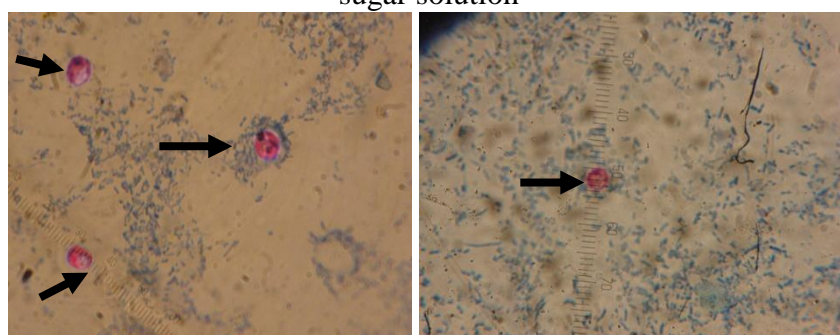
Result

During the present study, two types of oocysts were recognized and were identified as *C. parvum* and *C. andersoni* based on morphology and micrometry. One type of oocysts was spherical in shape, most commonly seen in the fecal samples of young calves (*C. parvum*). The other type was oval in shape, mostly found in fecal samples of older calves (*C. andersoni*). Micrometrically, smaller

oocysts with average length of $5.2 \pm 0.41 \mu\text{m}$ and width of $4.1 \pm 0.21 \mu\text{m}$ were considered *C. parvum*, whereas oocysts with average length of $6.9 \pm 0.72 \mu\text{m}$ and width of $5.8 \pm 0.87 \mu\text{m}$ were considered *C. andersoni* (Fig 1, 2). Both types appeared as densely stained red bodies against a dark blue back ground, with a clear halo around the oocyst (Fig 3, 4).



(Fig 1, 2) Left *C. andersoni* (oval) and Right *C. parvum* (round) oocysts under sheather's sugar solution



(Fig 3, 4) Left *C. andersoni* and Right *C. parvum*, oocysts in modified ziehl – Neelsen staining

1-The prevalence of *Cryptosporidium* species in relation to the sex .

Out of 268 fecal samples screened, 95 (35.44%) samples were found positive for Cryptosporidiosis. The total prevalence ratio in males was 34.09% (30 positive samples out of 88 samples) compared with 36.11% (65 positive samples out of 180 samples) in females . The species prevalence revealed 23.88% of *C. parvum*, 5.59% for *C. andersoni*, and 5.97% for mixed infection with no significant differences (table 1).

2-The prevalence of *Cryptosporidium* species in relation to the age

Table 2 shows the prevalence of *Cryptosporidium* species in different age classes. Calves less than two months old

not only had the highest ratio of cryptosporidial infection, but also had the highest prevalence of *C. parvum*. Out of 96 samples 46 (47.91%) samples were found positive for cryptosporidiosis and out of these positive samples 41 (89.13%) samples were considered as *C. parvum*, 1 (2.17%) sample as *C. andersoni* and 4 (8.69%) as mixed infection ($P < 0.01$). Prevalence of *C. parvum* infection decreased gradually coincident with gradual increase in prevalence of *C. andersoni* infection with proceeding in age in such a manner that the age class between 12-24 months had the least prevalence ratio of cryptosporidial infection (18.18%) accompanied by

highest prevalence of *C. andersoni* infection (72.72%).

3-The prevalence of infection in relation to the months of the year .

April had the highest prevalence ratio of cryptosporidial infection (20 positive

samples out of 45 samples (44.44%)) with significant difference from all other months involved in this study, whereas November had the least prevalence ratio (25.58%) (table 3).

Table 1: The prevalence of *Cryptosporidium* species in relation to the sex

Sex	Total screened	No positive (%)	<i>C. parvum</i> (%)	<i>C. andersoni</i> (%)	Mixed infection (%)
male	88	30 (34.09)	23 (26.13)	4 (4.54)	3 (3.40)
female	180	65 (36.11)	41 (22.78)	11 (6.11)	13 (7.22)
total	268	95 (35.44)	64 (23.88)	15 (5.59)	16 (5.97)

Table 2: The prevalence of *Cryptosporidium* species in relation to the age

Age class month	Total screened	No positive (%)	<i>C. parvum</i> (%)	<i>C. andersoni</i> (%)	Mixed infection (%)
< 2	96	46 (47.91)	41 (89.13)	1 (2.17)	4 (8.69)
3-6	61	23 (37.7)	17 (73.91)	3 (13.04)	3 (13.04)
7-12	52	15 (28.85)	4 (26.67)	3 (20)	8 (53.33)
>12-24	59	11 (18.64)	2 (18.18)	8 (72.72)	1 (9.09)
Total	268	95 (35.44)	64 (67.36)	15 (15.78)	16 (16.84)

Table 3: The prevalence of *Cryptosporidium* in relation to the months of the year

Months	Total screened	No positive (%)	<i>C. parvum</i>	<i>C. andersoni</i>	Mixed infection
November	43	11 (25.58)	5 (11.62)	4 (9.30)	2 (4.65)
December	45	14 (31.11)	9 (20)	2 (4.44)	1 (2.22)
January	45	17 (37.78)	12 (26.66)	2 (4.44)	3 (6.66)
February	45	16 (35.55)	11 (24)	3 (6.66)	2 (4.44)
March	45	17 (37.78)	12 (26.66)	2 (4.44)	4 (8.88)
April	45	20 (44.44)	15 (33.33)	2 (4.44)	4 (8.88)
Total	268	95 (35.44)	64 (23.88)	15 (5.59)	16 (5.97)

Discussion

Cryptosporidiosis is an emerging zoonotic disease of global importance caused by apicomplexan protozoan parasite(1). Four species of *Cryptosporidium* are identified to infect cattle, *C. parvum*, *C. bovis*, *C. ryanae* and *C. andersoni* (8, 9). Sporadic infection with other species have been reported (10). *C. bovis* and *C. ryanae* are morphologically similar to *C. parvum*. The size difference between these two species and *C. parvum* are too small for reliable species determination by microscopy, and differentiation must be done by molecular analysis(11). Accordingly, in the present study, the diagnostic method used allowed the recognition of two types of oocyst. So, many oocysts that have been identified as *C. parvum* may be *C. bovis* or *C. ryanae*. However, even with the application of molecular analysis in species determination, *C. parvum* has been the dominated species isolated from calves (12, 13, 14). A large number of epidemiological studies have been performed to estimate the prevalence of *Cryptosporidium* infection in cattle. The infection has been found worldwide, but reported prevalence range from 0 – 100% (11). The present study revealed approximately similar ratio of prevalence compared with some previous epidemiological studies(15, 16). No sex preponderance was found in *Cryptosporidium* infection amongst the calves which is in accordance with many previous studies (15, 16, 17). It is obvious from this study and from many other studies that there is no significant difference in *Cryptosporidium* infection ratio between males and females when they are bred in the same place and expose to similar condition, because neither males nor females have factors facilitate or impede infection while the other gender lacks them. The overall prevalence of *Cryptosporidium* infection declines with increasing age. This is in accordance with almost all previous work which revealed a similar trend in infection versus age (18,

19). This trend may be due two reasons: The first is the insufficiency of immune system of the neonate calves, and the second reason is the exposure of the calves to high number of oocysts that shed from recently calving cows (16). Virtually all infections in calves 5 weeks of age and younger are caused by *C. parvum*, although low levels of *C. bovis* and *C. andersoni* were found in some calves (1). This high peak prevalence possibly reflects the highly infections nature of this species which exploits immature immune status for their advantages (9). The number of infections with *C. parvum* dropped gradually in postweaned calves. However, because there was no molecular facilities to identify *C. bovis* and *C. ryanae*, it is reasonable to suppose the presence of these two species especially in postweaned calves, and the results of the present study concerning *C. parvum* may be change if such facilities are available. *Cryptosporidium andersoni* is the only *Cryptosporidium* species that can be identified with certainty in cattle feces by microscopy due to its large oocyst size. It is the dominant species in cattle older than 12 months (1). To our knowledge this is the first study in Iraq which involved the prevalence of *C. andersoni* infection. This species infects cattle with only one report in human identified 3 persons out of 2414 infected with *C. andersoni* (20). Although infected cattle do not usually show clinical signs related to the infection, the economic losses associated with this infection is high due to retard growth of animals and decreasing milk production (21). The highly tendency of *C. andersoni* to infect adult cattle may be related to the development of abomasums (which is the site of infection for this species) in adult animals. The higher prevalence of infection in April than other months may be related to the suitable environmental factors which keep the oocysts alive and /or increase the number of insects which play a critical role in the transmission of the infection (22).

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انتشار بعض انواع طفيلي الابواغ الخبيثة في بغداد

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الخلاصة

جمعت 268 عينة براز من عجول بعمر اسبوع واحد - سنتين من محطة النصر لتربية الابقار بالاضافة الى ثلاث مناطق في بغداد (التاجي ، الشعلة، الغزالية). استخدمت صبغة زيل نلسن المحورة في الكشف عن اكياس بيض الطفيلي في العينات ، كما استخدم شكل وابعاد الكيس كمعيار لتحديد نوع الطفيلي. بلغت النسبة الكلية للاصابة بالطفيلي 35.44% ولم يسجل فرق معنوي في نسب الاصابة بين الذكور والاناث، فيما اظهرت نسبة الاصابة انخفاضاً تدريجياً مع تقدم العمر وكان النوع *C. parvum* هو السائد في الاعمار اقل من 6 اشهر والنوع *C. andersoni* في الاعمار الاكبر من ذلك .