Prevalence of *Cryptosporidium andersoni* in cattle in Babylon province, Iraq

M. K. A. Altamimi[*], M. Th. S. Al- Zubaidi[*] and A. B. Hasan[**]
[*]Dep. of Parasitology/ College of Veterinary Medicine/ University of Baghdad
[**]Dep. of Animal Production/ College of Agriculture\ Al-Qasim green University

Abstract

A total of 396 fecal samples were collected from cattle of different ages ranged from 1 month to more than 3 years old from five different areas in the province of Babylon (Al-Mussaib, Al-Sadda, Abo-gharag, Al-Hilla and Al-Qasim). The fecal samples were examined by two laboratory tests (Modified Ziehl-Neelsen staining and Sheather's sugar flotation method). The species were identified as *Cryptosporidium andersoni* based on the morphology and micrometry of the oocysts. The overall prevalence of *Cryptosporidium andersoni* infection was 13.13%, and the infection rates in Al-Musayyib, Al-Sadda, Abo-gharaq, Al-Hilla and Al-Qasim areas were 7.38%, 15.74%, 21.05%, 8.45% and 2.42% respectively. The highest infection rate appeared in age group > 1- 2 years old cattle which was 24.39%, while no infection recorded in age group 1- 6 months old, and there was no significant difference found in prevalence rates between males and females.

انتشار طفيلي *Cryptosporidium andersoni* في ابقار محافظة بابل، العراق

محمد خضير عباس التميمي[*]، محمد ثابت صالح الزبيدي[*] وعايد بجعي حسن[**]
[*]فرع الطفيليات/ كلية الطب البيطري/ جامعة بغداد
[**]قسم الانتاج الحيواني/ كلية الزراعة/ جامعة القاسم الخضراء

الخلاصة

جمعت 396 عينة براز من ابقار بأعمار مختلفة تراوحت بين 1 شهر الى اكثر من 3 سنوات من خمس مناطق مختلفة في محافظة بابل (المسيب، السدة، أبو غرق، الحلة والقاسم). استخدمت طريقتين مختبريتين هما (صبغة زيل نلسن المحورة والتطويف بمحلول الشيذر السكري المشبع) للكشف عن اكياس بيض الطفيلي في العينات، كما استخدم شكل وأبعاد أكياس البيض كمعيار لتحديد نوع الطفيلي. بلغت نسبة الإصابة الكلية بطفيلي *Cryptosporidium andersoni* 13.13%، وكانت نسبة الإصابة في مناطق المسيب والسدة وأبو غرق والحلة والقاسم 7.38%، 15.74%، 21.05%، 8.45% و2.42% على التوالي. ظهرت أعلى نسبة إصابة بالطفيلي في الفئة العمرية اكثر من 1– 2 سنة وكانت 24.39%، بينما لم تسجل اي اصابة في الفئة العمرية 1– 6 شهر ولم يلاحظ وجود أي فرق معنوي في نسب الاصابة بين الذكور والاناث.

Introduction

Cryptosporidium spp. is a common protozoan parasite that mainly infects the gastrointestinal and respiratory tract of a wide range of vertebrates including humans and cattle throughout the world (1). Previous studies around the world have shown that cattle is the most common species of mammals known to be infected with *Cryptosporidium* spp., and is the main source of human infection with *Cryptosporidium* (2, 3, 4). Four *Cryptosporidium* species, namely *C. parvum, C. andersoni, C. bovis* and *C. ryanae,* can infect cattle and cause bovine cryptosporidiosis (5). *C. andersoni* is

commonly found in the abomasum of juvenile and adult cattle, its oocysts are larger than those of the other three *Cryptosporidium* species in cattle about (5.5 × 7.4 μm) (6), and has been associated with gastritis, reduced milk yield and poor weight gain (6, 7, 8, 9). *C. andersoni* also has been isolated from humans with diarrhea in England (10) and paediatric patients in Malawi (11). There are many different studies conducted in the world to find out the prevalence of *C. andersoni*, this study aimed to determine the prevalence of *C. andersoni* in cattle in Babylon province.

Materials and Methods

1. **Source and collection of specimens:** Fecal samples (~5 grams each) were collected from 396 pre and post-weaned calves, heifers and adult cattle of different ages ranged from 1 month to more than 3 years old during the period from the first of November 2012, until 30th May 2013. The study involved Al-Musayyib, Al-Sadda, Abo-gharaq, Al-Hilla, and Al-Qasim in the province of Babylon. The fecal samples were collected directly from the rectum in a clean plastic containers with a 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 Ziehl-Neelsen stain method (12) 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. After staining, the smear were washed in running tap water for 1-2 min, and 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 objective 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. *C. andersoni* size is about (5.5 × 7.4 μm) (6).
4. **Statistical analysis:** The Chi-square test was used to analysis the overall prevalence data, and differences were considered statistically significant when $P< 0.05$ (13).

Results

The prevalence of cryptosporidiosis was studied on the basis of the detection of oocysts in the fecal materials collected from 5 different areas in the province of Babylon. Out of 396 fecal samples screened, 52 (13.13%) animals were found positive for cryptosporidiosis by Modified Ziehl-Neelsen staining (Fig.2), and Sheather's sugar flotation methods (Fig.1), also study the morphological characters and micrometry of oocysts for species identification (Fig.3). Oocysts with average length of 6.9± 0.72 μm and width of 5.8± 0.87 μm were considered *C. andersoni*. The parasite appeared as densely stained red bodies against a dark blue back ground, with a clear halo around the oocyst (Fig.2).

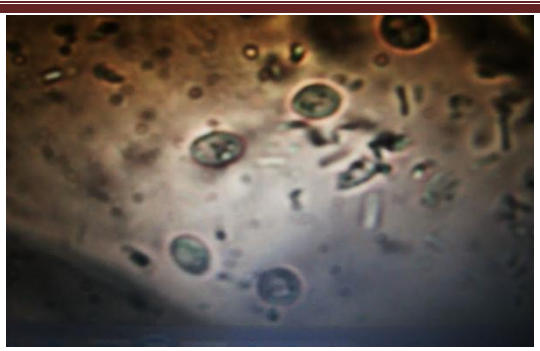

Fig.1. *C .andersoni* (oval) oocysts under Sheather's sugar solution ×100

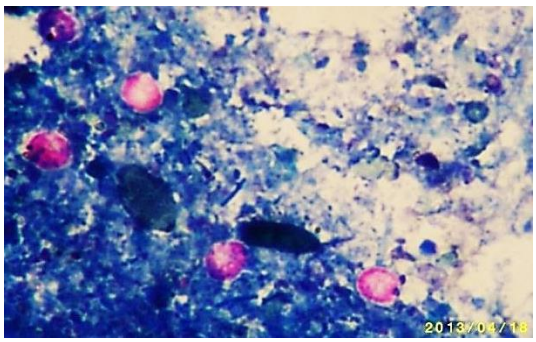

Fig .2. *C. andersoni* oocysts in modified Ziehl-Neelsen staining × 100

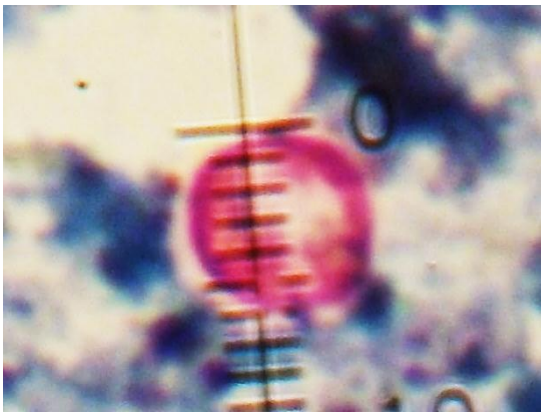

Fig. 3. *C. andersoni* micrometry of oocyst 100 ×12 (6.1× 5.6 µm in size)

1. **The prevalence of *Cryptosporidium andersoni* in relation to the sex**: Out of 396 fecal samples screened, 52 (13.13%) samples were found positive for *C. andersoni*. The total prevalence ratio in males was 11.76 (10 positive samples out of 85 samples) compared with 13.5% (42 positive samples out of 311 samples) in females. So there were no significant differences (P> 0.05)between males and females in the prevalence of *C. andersoni* (Table 1).
2. **The prevalence of *Cryptosporidium andersoni* in relation to the age:** The age wise prevalence was studied in five different age groups. Among 35 calves aged between 1-6 month, there were no positive cases for cryptosporidiosis. Of the 71 calves screened in the 7-12 month age group, 7 (9.85%) were found positive for cryptosporidiosis. Out of 82 animals from > 1- 2 years age group, the prevalence of *C. andersoni* was the highest and with significant difference (P< 0.05) from all other age groups, about 20 (24.39%). In 86 adult animals of > 2- 3 years age group, 14 (16.27%) were found to be positive for cryptosporidiosis, and among 122 adult animals of more than 3 years of age, 11 (9.01%) were positive for cryptosporidiosis (Table 2).
3. **The prevalence of infection in relation to the months of the year:** March had the highest prevalence ratio of cryptosporidial infection (12 positive samples out of 59 samples (20.33%), but no significant differences were found between all months involved in this study, whereas December had the lowest prevalence ratio (7.4%) (Table 3).

Table (1) The prevalence of *Cryptosporidium andersoni* in relation to the sex

Sex	Total screened	No. positive	prevalence %
Male	85	10	11.76
Female	311	42	13.5
Total	396	52	13.13

X^2 = 0.177, (p > 0.05)

Table (2) The prevalence of *Cryptosporidium andersoni* in relation to the age

Age class	Total screened	No. positive	prevalence %
1 – 6 months	35	0	0
7 – 12 months	71	7	9.85
> 1 year – 2 years	82	20	24.39
> 2 years –3 years	86	14	16.27
> 3 years	122	11	9.01
total	396	52	13.13

X^2 = 17.64, (p < 0.05)

Table (3) The prevalence of *C. andersoni* in relation to the months of the year

No.	Months	Total screened	No. positive	prevalence%
1	November	50	5	10
2	December	54	4	7.4
3	January	60	7	11.66
4	February	61	10	16.39
5	March	59	12	20.33
6	April	57	9	15.78
7	May	55	5	9.09
	Total	396	52	13.13

X^2 = 6.484 , (p > 0.05)

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* (5). Sporadic infections with other species have been reported (14). *Cryptosporidium 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 (15). Accordingly, in the present study, the diagnostic method used allowed the recognition of *C. andersoni* oocyst with certainty in cattle feces by microscopy due to its large oocyst size (6). A large number of epidemiological studies have been performed to estimate the prevalence of *Cryptosporidium* infections in cattle. The infection has been found worldwide, the present study revealed approximately similar ratio of prevalence compared with previous epidemiological study in Iraq (16). No sex preponderance was found in *C. andersoni* infection amongst the animals which is in accordance with many previous studies (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 exposed to similar condition, but the higher ratio revealed in females could be due to the stress of females during pregnancy, parturition and milking periods which make the females more susceptible to the infection (18). In the present study, an age-related observed in the prevalence of *C. andersoni,* which is the predominant species in juvenile and adult cattle (6). The

infection appeared in age group 7-12 month then increases gradually and reached the highest ratio (24.39%) in the age group > 1 year- 2 years then infection declines with increasing age. This is in accordance with almost all previous work which revealed a similar trend in infection versus age (19, 20, 21, and 22). 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 March than other months may be related to the suitable environmental factors which keep the oocysts alive (23).

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