PREVALENCE OF *EIMERIA* AND *CRYPTOSPORIDIUM* SPECIES IN CATTLE IN KAFR EL-SHEIKH PROVINCE

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ABSTRACT

This study was carried out, during 2011, to determine the prevalence of Eimeria spp. and Cryptosporidium spp. infecting cattle in Kafr El-Sheikh province. Faecal samples (n=698) were examined with flotation technique for Eimeria and modified Ziehl-Neelsen staining technique for Cryptosporidium spp. Oocysts. Eimeria spp. were in 28.94% and Cryptosporidium spp. in 34.1 % . Eleven Eimeria spp. were identified and their prevalence were E.ellipsoidalis (52.5%), E. cylindrica (34.2%), E. bovis (31.2%), E. zuernii (28.7%), E. subspherica (19.8%), E. alabmensis (6.9%), E. canadensis (3%), E. aubernensis (2%), E. wyomingensis (1.5%), E. brasiliensis (1%) and E.bukidnonensis (1%). Single infection of Eimeria species was found in 47.5% of the animals, while mixed infection of two and three species were found in 52.5% of animals. Cattle are grouped according to their age into four groups: <3, 3-6, 6-9, >9 months. Eimeria spp. infection were detected in 31.4%, 37.1%, 26.7% and 19.0%, while Cryptosporidium spp. infection was detected in 42.1%. 41.4%, 26.7% and 19% of the calves in the respective age groups. Peak prevalence of Eimeria spp. and Cryptosporidium spp. infection was observed during winter 33.3% and 39.9%, respectively. Eimeria and Cryptosporidium spp. infection were detected in 40.4% and 46.5% of diarrhoeic calves, respectively. There was higher prevalence of infection in diarrhoeic than in non-diarrhoeic calves.

INTRODUCTION

Coccidia are one of the most common intestinal parasites of large animals and are a cause of disease and production losses for animals in capacity ***(Fitzgerald, 1980 and Bürger, 1983)***.

Coccidiosis in cattle commonly occurs as subclinical and predisposing for great encomical losses due to reduced appetite, reduced body weight, impaired feed conversion, unthriftness, diarrhea, dysentery, anemia and increased susceptibility to other diseases ***(Bohrmann, 1991 and Thomas, 1994)***. The development of clinical coccidiosis in cattle mainly depend on factor like species of Eimeria, age of infected animal, number of oocysts ingested, presence of concurrent infections and type of production system and management practices ***(Ernst et al., 1984)***. More than 20 species of *Eimeria* has been described in cattle, yet only a few cause significant diseases; for examples, *Eimeria bovis* and *Eimeria Zuernii* cause sever mortality rates, whereas *Eimeria auburnensis* and *Eimeria alabamensis* are considered mildly pathogenic ***(Daugschies and Najdrowski, 2005)***.

Cryptosporidium parvum is the most frequently detected protozoan parasite in calves less than 3 weeks age ***(Moore and Zeman, 1991 and De la Fuente et al., 1999)***, where it considered being one of the main common causes of diarrhea at this age ***(Koudela and Bokova, 1997)***. However, Cryptosporidiosis should not only be considered from the perspective of animal health and production, its zoonotic character and the possibility that animals may act as a source of infection to humans, via foodstuff and water, should also be considered. Although the infection leads to few deaths, serious economic losses can occur due to costs involved in the treatment ***(De Graaf et al., 1999)***. Single infection

with *C. parvum* is usually present in diarrheic calves; however, mixed infection with other pathogens exaggerates the problem ***(Vanopdenbosch et al., 1979)***.

Treatment of Cryptosporidiosis is not effective in cattle and its control measures are mainly based on preventive measures rather than treatment ***(Woods et al., 1996)***.

Despite its worldwide distribution and zoonotic relevance, there are still few publications concerning Cryptosporidium infection in cattle in Egypt as ***(Iskander et al., 1987; El-Sherif et al., 2000; El-Dessouky and El-Masry, 2005; El-Sherbini and Mohamed, 2006)***.

So, the aim of the present study was to determine:

- Prevalance and seasonal dynamics of *Eimera* spp. infecting cattle.
- Morphological identification of *Eimeria spp*. oocysts.
- Prevalence and seasonal dynamics of *Cryptosporidium spp.* oocysts.

MATERIALS AND METHODS

1. Animals:

In the present study 698 cattle of various ages, sex and breeds distributed in Sakha farm or private animals owned by farmers in Kafr El-Sheikh province were examined for the presence of some enteric protozoan parasites during the period extend from the beginning of January to December, 2011.

2. Collection of faecal samples:

A total of 698 faecal samples were collected from rectum or immediately after defecation from individual animal in polyethelyne sac and labeled according to age, sex, date of collection and locality.

The faecal samples were transferred immediately to the laboratory of parasitology, Faculty of Vet. Med. Kafrelsheikh University.

3. Preparation and examination of faecal samples:

In the laboratory, the preparation and examination of the collected samples were conducted in the same day of collection.

The faecal samples were examined macroscopically three grades, solids, semi-solid and diarrhea.

4. Laboratory examination of the collected faecal samples for *Eimeria* spp.:

a. Flotation technique for detection of Eimeria species:

Examinations of faecal samples were done by concentration flotation technique according to Kruse and Pritchard (1982).

b. Sporulation of *Eimeria* species oocysts:

Eimeria oocysts from positively faecal samples were collected, purified and left to sporulate in 2.5% potassium dichromate according to ***William's (1969***).

c. Identification of different *Eimeria* species oocysts**:**

The recovered species of *Eimeria* were identified according to the morphological characteristics of the *Eimeria* species infecting cattle as describe by ***Christensen (1941), Pellerdy (1965), Joyner et al. (1966)*** and ***Levine (1973)***.

5. Laboratory examination of the collected faecal samples for *Cryptosporidium* species:

Modified Ziehl-Neelsen staining technique was carried out according to ***Henriksen and Pohlenz (1981)***.

RESULTS

Out of 698 examined faecal samples 28.94% and 34.1% were found positive for *Eimeria* spp. and *Cryptosporidium* spp., respectively. The rates of infection with *Eimeria* spp. and *Cryptosporidium* spp. were found higher in females (31.19% and 34.17% ,respectively) than males (25.19% and 33.97%, respectively) (Fig. 1).

Cattle group of 3-6 month old showed the highest rate of infection (37.1%) *with Eimeria* spp. while the highest infection rate of *Cryptosporidium* spp. (42.1%) was recorded among the cattle group of less than 3 month old. Figs. (2 and 3).

Concerning the seasonal dynamics of *Eimeria* spp. infection, the results in Fig. (4), revealed that cattle examined showed the highest rate of infection (33.3%) in winter, followed by spring (29.1%), summer (27.1%) and autumn (26.6%).

Concerning the seasonal dynamics of *cryptosporidium* spp. infection, the results in Fig. (5), revealed that cattle examined showed the highest rate of infection in winter (39.9%), followed by spring (36.6%), autumn (28.7%) and summer (24.7%).

Concerning the faecal consistency and its relation to *Eimeria* spp. and Cryptosporidium infection, the results in Fig. (6 & 7), revealed that cattle examined showed the highest rate of infection in diarrheic faeces 40.4% and 46.5%, respectively.

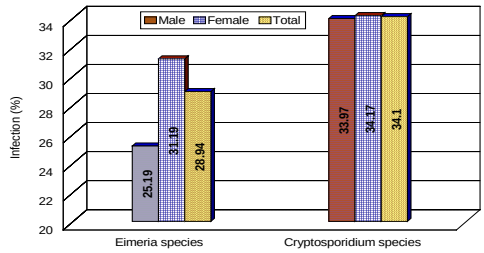


Figure (1): Prevalence of Eimeria and Cryptosporidium species in relation to the sex of examined cattle in Kafr El-Sheikh Province.

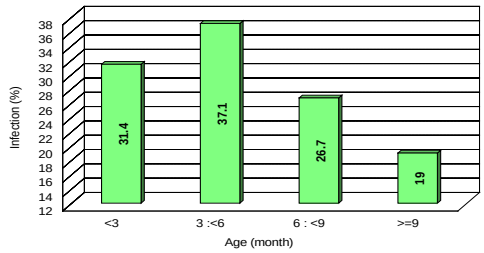


Figure (2) : **Prevalence** of Eimeria species in relation to age of examined cattle.

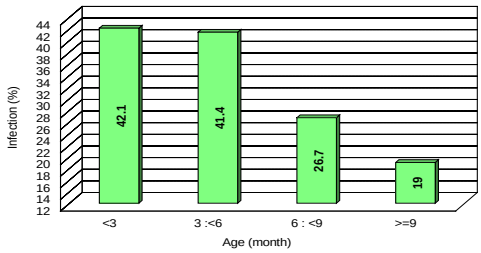


Figure (3) : Prevalence of Cryptosporidium species in relation to to age of examined cattle.

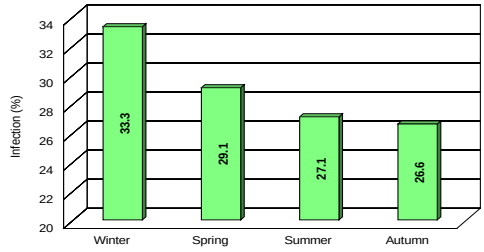


Figure (4) : Seasonal infection rate of Eimeria species in cattle in Kafr El-Sheikh Province.

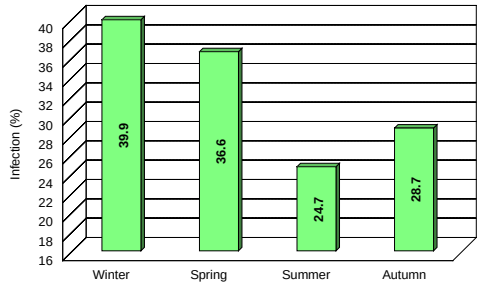


Figure (5) : Seasonal infection rate of Cryptosporidium species in cattle in Kafr El-Sheikh Province.

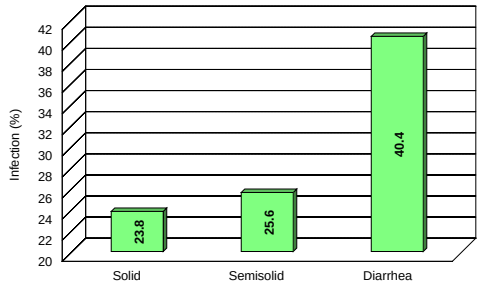


Figure (6) : The relation between the faecal consistency and Eimeria species infection in cattle in Kafr El-Sheikh Province.

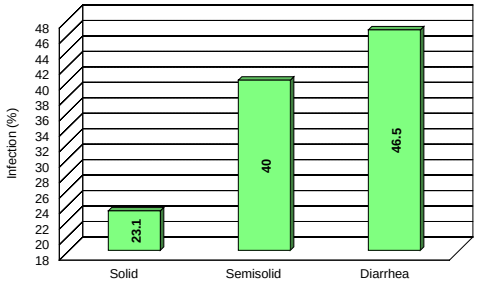


Figure (7) : The relation between the faecal consistency and Cryptosporidium species infection in cattle in Kafr El-Sheikh Province.

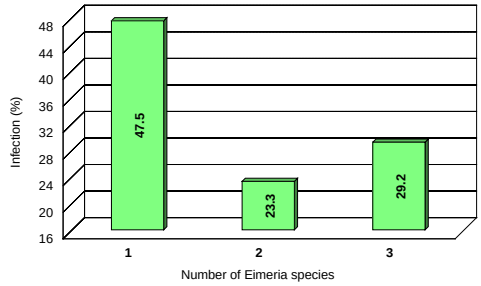


Figure (10) : The percentage of Eimeria species in infected cattle.

Eleven *Eimeria* spp. were identified and their prevalence were *E. ellipsoidalis* (52.5%), *E. cylindrica* (34.2%), *E. bovis* (31.2%), *E. zuernii* (28.7%), *E. subspherica* (19.8%), *E. alabmensis* (6.9%), *E. canadensis* (3%), *E. aubernensis* (2%), *E. wyomingensis* (1.5%), *E. brasiliensis* (1%) and *E. bukidnonensis* (1%) (Fig.8, Fig.9 and Table1). Single infection of *Eimeria* species was found in 47.5% of the animals. Mixed infection of two and three species were found in 52.5% of animals, Fig. (10) and Table (2).

Table (1): Prevalence of single and mixed infection of Eimeria species in cattle in Kafr El-Sheikh Province.

Species	Single infection		Mixed infection		Total infection	
	No	%	No	%	No	%
Total	**96**	**47.5**	**106**	**52.5**	**202**	**28.94**
E. ellipsoidalis	21	10.4	85	42.1	106	52.5
E. cylindrica	6	3.0	63	31.2	69	34.2
E. bovis	19	9.4	44	21.8	63	31.2
E. zuernii	15	7.4	43	21.3	58	28.7
E. subspherica	4	2.0	36	17.8	40	19.8
E. alabamensis	14	6.9			14	6.9
E. canadensis	6	3.0			6	3.0
E. aubernensis	4	2.0			4	2.0
E. wyomingensis	3	1.5			3	1.5
E. brasiliensis	2	1.0			2	1.0
E. bukidnonensis	2	1.0			2	1.0

Table (2): Prevalence of mixed Eimeria species in cattle in Kafr El-Sheikh Province.

Species	Infected cattle	
	No	**%**
Total	**106**	**52.5**
E. ellipsoidalis + E. cylindrica	26	12.9
E. subspherica + E. cylindrica	12	5.9
E. subspherica + E. zuernii	9	4.5
E. ellipsoidalis + E. zuernii + E. bovis	19	9.4
E. ellipsoidalis + E. cylindrical + E. bovis	25	12.4
E. ellipsoidalis + E. subspherica + E. zuernii	15	7.4

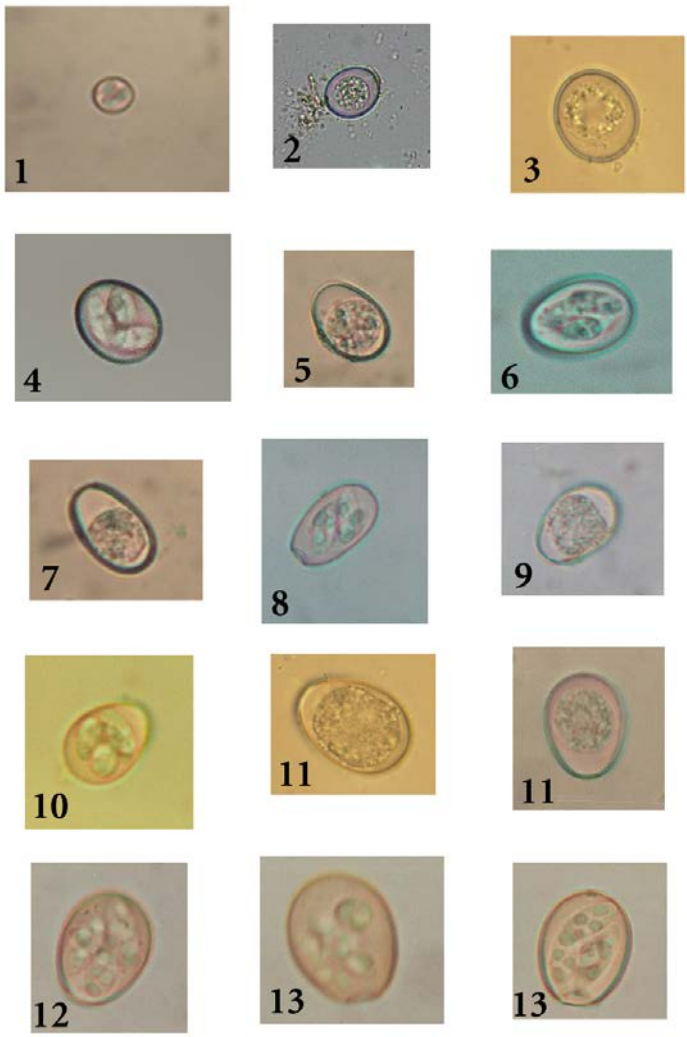
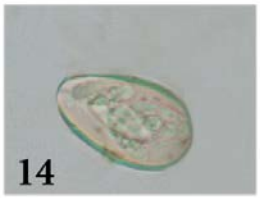


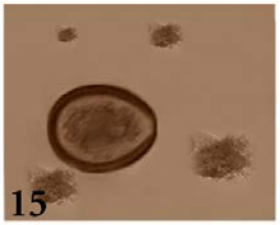
Figure (8):

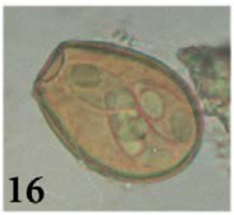
1. Sporulated oocyst of *E. subspherica* X 40
2. Unsporulated oocyst of *E. subspherica* X 100
3. Unsporulated oocyst of *E. zuernii* X 100
4. Sporulated oocyst of *E. zuernii* X 100
5. Unsporulated oocyst of *E. ellipsoidalis* X 100
6. Sporulated oocyst of *E. ellipsoidalis* X 100
7. Unsporulated oocyst of *E. cylindrica* X 100
8. Sporulated oocyst of *E. cylindrica* X 100
9. Unsporulated oocyst of *E. alabamensis* X 100
10. Sporulated oocyst of *E. alabamensis* X 100
11. Unsporulated oocyst of *E. bovis* X 100
12. Unsporulated oocyst of *E. bovis* X 100
13. Sporulated oocyst of *E. canadensis* X 40

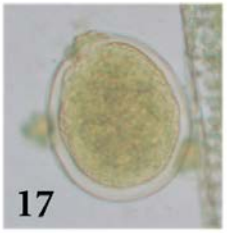
Cryptosporidium oocysts were identified by staining with modified Ziehl-Neelsen technique, Fig. (9), the oocysts appeared round or spherical in shape. The wall was smooth and the oocysts appeared as densely stained red bodies on green background with accentric dot or vacules.

The dimensions of the detected oocysts were 2.8-5 x 3.2-5.8 µm with an average of 3.99 x 4.7 µm.









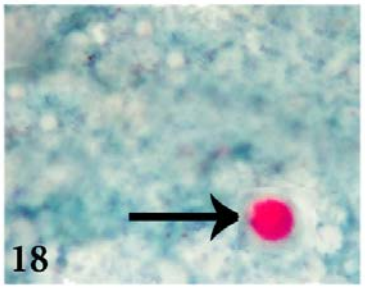


Figure (9):

14. Sporulated oocyst of *E. aubernensis* X 40
15. Unsporulated oocyst of *E. bukidnonensis* X 10
16. Sporulated oocyst of *E. wyomingensis* X 40
17. Unsporulated oocyst of *E. brasiliensis* X 10
18. Cryptosporidium oocyst stained with modified Zeihl- Neelsen stain X 100

DISCUSSION

The result of the present study revealed that, 28.94% from the examined cattle found to be infected with Eimeria species. These results was similar to that obtained by ***Waruiru et al. (2000)*** in ***Kenya, Hasan et al. (2010)*** in Iraq and *Toaleb et al.* (2011) in Egypt. On the other hand, the result in the present study disagreed with those of ***Arslan and Tüzer (1998)*** in Turkey and ***Lassen et al. (2009)*** in Estonian. This variation was most likely attributed to the differences in agro-ecology management, and husbandry practices of the study animals in different countries.

The obtained results showed that the prevalence of *Eimeria* infecting female cattle was higher than that among the male one where the infection rates reached 31.19% and 25.19%, respectively as also recorded previously by ***Rehman et al. (2011)***. This result might be attributed to the female animal usually subjected to many stress condition as pregnancy, lactation, calving and little managemental care. Also most of males were breed for fattening purpose under good hygienic conditions.

The results in this study clarified that young age of cattle were more susceptible for *Eimeria* infection than old one. The most susceptible age was 3-6 month as the percentage of infection was 37.1% as it mentioned before by ***Lentze et al. (1999)***, ***Daugschies and Najdrowski (2005)*** and *Lassen et al.* (2009). These results could be attributed to calves age 3 months discontinue a milk diet and passive immunity drops. While the old animals are likely to have a developed immune system and experienced *Eimeria* infections.

The present data recognized that winter season was the most suitable season for *Eimeria* infection as the infection rate of *Eimeria* reached to 33.3% and it was followed by winter, spring, autumn and finally the summer season where the infection rates were 29.1%, 27.1% and 26.6%, respectively. These results agreed with ***El-Sherif and Aboel-hadid (2005)*** in Egypt and disagreed with that of ***Vercrysee (1982)*** who stated that no seasonal fluctuation in the prevalence of *Eimeria* species. From the present results, it was concluded that *Eimeria* infection was more abundant and common during winter season, this might be attributed to a good environmental condition during this season as good weather with moderate temperature that was suitable for the oocyst and accelerate its sporulation process.

The present data recognized that *Eimeria* infection in relation to consistency of faeces was higher in diarrheic than non diarrheic calves. This was in line with the observations of ***Chibuanda et al. (1997); Svensson (2000) and Kennedy (2001)***, all of which stated that development of clinical disease depends on the number of oocysts ingested.

Eimeria species identified in the present study have been previously reported by ***Abebe et al. (2008), Klockiewiez et al. (2007) and Lassen et al. (2009)***. *E. ellipsoidalis, E. cylinderica, E. bovis* and *E. zuernii* were recorded as the highest prevalent coccidian species which is accordance with reports of ***El-Sherif and AboEl-Hadid (2005)***.

The results in this study recognized the prevalence of *Cryptosporidium* species among cattle, as its total prevalence was 34.1%, these results were nearly similar to those obtained by ***Huetink et al.***

(2001) in the Netherlands and ***Watanabe et al. (2005)*** in Taiwan. These results were lower than that obtained by ***Silverlas et al. (2010)*** in Sweden and ***Bhat et al. (2012)*** in India. The variation between the different prevalence rates of the disease may be attributed to the system of rearing and management in addition to the level of hygienic measures applied. The other workers examined only diarrhoeic cases or apparently healthy or examination of neonatal or un-weaned animal only. While in the present study, examination was carried out in cattle at different ages, diarroeic and apparently health.

Regarding to the age, the present results recognized that calves less than 3 months were more susceptible for Cryptosporidial infection than the adult ones (9 month old)where the infection rate was 42.1% and 19.0%, respectively. These results agreed with those of ***De La Fuente et al. (1999)*** and ***Wade et al. (2000)***. On contrary, Cryptosporidiosis was recorded in older bovine calves ***(Maddox-Hyttel et al., 2006)***. These results could be attributed to the presence of calves with their dams especially during the lactation period where the adult act as carrier for *Cryptosporidium* oocysts or might be due to the underdevelopment immunity of calves to the parasitic infections.

A significant association between the season and the prevalence of *Cryptosporidium* spp. infection was recorded in this study. Winter recorded the highest prevalence (39.9%), while summer recorded the lowest (24.7%). It is suggested that the prevalence of infection by *Cryptosporidium spp*. is not only related to the presence of calves at risk but also related to the presence of the suitable climatic condition for viability and spread of the parasite. In winter, the temperature in our

locality is suitable for viability, survival and survival of *Cryptosporidium* oocysts. Similar findings were recorded by ***Tzipori (1983) and Lefay et al. (2000)***. These results were supported by ***Anderson (1986)*** who found that warm temperature of 18 to 29ºC, had been partially responsible for loss of the infectivity. However, ***Garber et al. (1994)*** attributed the high prevalence of Cryptosporidiosis in winter to the presence of large number of calves at risk a result of concentration of calving in winter months. On contrary, In California, ***Atwill et al. (1999)*** found that calves were at high risk of contracting infection by *Cryptosporidium* spp. during May month and they attributed this to the greatest contact with the source of infection, lowering animal resistance of environmental conditions that might favor transmission of the infection during this month. Contrary to all recorded results, absence of seasonality in the presence of Cryptosporidium infection has been described ***(Wade et al., 2000 and Castro-Hermida et al., 2002)***.

In regard to clinical signs, as previously mentioned, the main clinical sign of Cryptosporidiosis is diarrhea. The infection rate in diarrheic calves was 46.5%. This result came in accordance with carried out in cattle by ***Atwill et al. (1999) and Castro-Hermida et al. (2002)***. Coexistence of *C. parvum*, rotavirus, corona virus and Salmonella, among others, in calves of less than one month of age, with diarrhea was recorded ***(De la Fuente et al., 1999)***.

The morphological features of *Cryptosporidium* oocysts as detected by modified Ziehl-Neelsen technique reported in the present study were similar to those described by ***Henricksen and Pohlenz (1981)***.

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معدلات إصابة الأبقار بالايميريا والكربتوسبورديم في محافظة كفرالشيخ

أظهرت نتائج الدراسة الحالية ومن خلال فحص 698 عينة براز أبقار جمعت من محافظة كفر الشيخ خلال عام 2011م لتحديد الإصابة بالايميريا عن طريق (التعويم بمحلول الملح المركز) والكربتوسبورديم عن طريق صبغة زيل نيلسن. وجد أن نسب الإصابة الكلية بالايميريا 28.94% والكربتوسبورديم 34.1% وقد تم تشخيص 11 نوع من الايميريا كالتالي:

E.ellipsoidalis (52.5%), *E. cylindrica* (34.2%), *E. bovis* (31.2%), *E. zuernii* (28.7%), *E. subspherica* (19.8%), *E. alabmensis* (6.9%), *E. canadensis* (3%), *E. aubernensis* (2%), *E. wyomingensis* (1.5%), *E. brasiliensis* (1%) *and E.bukidnonensis* (1%).

وقد وجد أن الإصابة الفردية بنوع واحد من الايميريا كانت 47.5% بينما الإصابة المختلطة بنوعين أو ثلاثة كانت 52.5%.

وقد تم تصنيف الأبقار حسب العمر إلي أربع مجموعات أقل من ثلاثة شهور، 3 – 6 شهور ، 6–9 ، وأكبر من 9 شهور وكانت نسب الإصابة بالايميريا كالتالي ,31.4% 26.7%, 37.1%, بينما الإصابة بالكربتوسبورديم كانت كالتالي42.1%، ,41.4% ،26.7% و 19%.

وقد لوحظ ارتفاع نسبة الإصابة بالايميريا 33.3% والكربتوسبورديم 39.9% في فصل الشتاء وقد وجد أن الإصابة بالايميريا والكربتوسبورديم في الحيوانات المسهلة أعلي من غيرها فكانت الإصابة بالايميريا 40.4% والكربتوسبورديم 46.5%.