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ROLE OF CALVES AND LAMBS IN TRANSMITTING CRYPTOSPORIDIOSIS TO CHILDREN IN WEST DELTA REGION OF EGYPT

(With 7 Tables)

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دور العجول والحملان فى نقل مرض الكربتوسبورديوزس إلى الأطفال في منطقة غرب الدلتا بمصر

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تعتبر الأوليات المعوية المسببة للإسهال من أهم الأمراض المشتركة المنتقلة من الحيوانات المختلفة إلى الإنسان وعلى رأسها الكربتوسبوريديوم بارفم. يعتبر طفيل الكربتوسبوريديوم بارفم من أخطر هذه الأوليات المعوية وأكثرها انتشارا علي مستوي العالم يصيب الأطفال الصغار والحيوانات الصغيرة والطيور والزواحف ويتسبب في نزلات معوية قد تؤدى إلى الوفاة في مرحلة حديثي الولادة، أو من يعانون من تثبيط مناعي في الإنسان والحيوان كما يعتبر هذا الطفيلي من الأسباب الهامة للإسهال في العجول والحملان حديثة الولادة. وقد أجريت هذه الدراسة ببعض المناطق الريفية في محافظتي البحيرة والإسكندرية لتحديد مدى انتشار طفيل الكربتوسبوريديوم بارفم في كل من الأطفال والحيوانات الصغيرة ودراسة تأثير عدد من العوامل المختلفة فى انتشار المرض وعليه فقد تم تجميع عدد ٦٨٨ عينة بواقع ٢٤٠ عينة براز من أطفال أعمارهم أقل من ٦ سنوات من المترددين على أقسام الأطفال في مستشفيات الإسكندرية والبحيرة بالإضافة إلى ٢٤٨ عينة من العجول و٢٠٠ عينة من الحملان تحت ٣ شهور. وقد تم التعرف على حويصلات الكربتوسبوريديوم بارفم عن طريق صبغ مسحات الروث والبراز بصبغة الزيل نيلسن المعدلة وتم تأكيد نتائج العينات الايجابية باستخدام اختبارELISA على عينات البراز أو الروث مباشرة. وقد بلغ معدل الإصابة بطفيل الكربتوسبوريديوم بارفم في العجول والحملان والأطفال ٩,٣، ١٣,٥ و ١١,٧ % على التوالى وكانت معدلات الإصابة المسجلة في العينات المجمعة من محافظة البحيرة أعلى منها في محافظة الإسكندرية وقد يرجع ذلك إلى الطبيعة الريفية لمحافظة البحيرة. هذا وقد تم دارسة تأثير العمر والحالة الصحية وفصول السنة على معدلات انتشار المرض ومن النتائج يتضح أن روث بعض حيوانات المزرعة قد يكون مصدرا للعدوى بتلك الطفيليات وأنها

مازالت تلعب دورا محوريا في المحافظة على انتقال بعض الأوليات المعوية المسببة للإسهال إلى الأطفال في المناطق الريفية حيث الاتصال المباشر بين تلك الحيوانات والإنسان لذلك يجب إتباع بعض الإجراءات الصحية لمنع انتشار ونقل تلك الأمراض إلى الإنسان مثل الاهتمام بفحص الحيوانات على فترات وتقديم العلاج للحيوانات المصابة من أجل تقليل إفراز الأطوار المعدية في روث تلك الحيوانات مع ضرورة التخلص الصحي من روث الحيوانات بعد معاملتها كيميائيا باستخدام المطهرات مثل مستحضرات الجير الحي. هذا وقد تمت مناقشة هذه النتائج مناقشة علمية مستفيضة.

SUMMARY

A total of 688 fecal and stool samples were collected from calves, lambs and children through out all seasons of the year including: 248 (62/ season) fecal samples of pre-weaned calves collected from diarrheic and apparently healthy animals from individually owned animals attending the veterinarian clinics in rural areas of Alexandria and Behera Provinces, 200 (50/ season) fecal samples of pre-weaned lambs were collected from diarrheic lambs and 240 (60/ season) stool samples were collected from children under 6 years old (the age of school entry) attending pediatric hospitals in Alexandria and Behera Provinces during the period extended from December 2008 to November 2009. All collected samples were subjected to microscopical examination by using Modified Ziehl-Neelsen (MZN) staining technique for detection of *Cryptosporidium parvum* oocysts. More over, all positive samples were confirmed by using RIDASCREEN Cryptosporidium ELISA kits supplied by GmbH, Darmstadt, Germany. It was found that the overall percentage of C. parvum infection in fecal samples of calves, lambs and children was 9.3, 13.5 and 11.7 %, respectively and the incidence of *C. parvum* was higher in Behera Province than in Alexandria Province due to its rural nature. The effects of age, health condition and season of the year on the incidence of infection with *C. parvum* were studied and the public health significance of cryptosporidiosis was discussed.

Key words: *Cryptosporidiosis, C.parvum, cales, lambs, children.*

INTRODUCTION

Cryptosporidium species are of great public health concern as they may cause infection and severe illness in human specially children. Infections are self- limiting in people with normal immune systems but

infection can be life threatening in people who have comprised immune system especially infection with *Cryptosporidium parvum* which always strongly associated with acute or chronic diarrhea in infants and HIV-infected adult patients worldwide as several recent waterborne outbreaks have shown, poses a significant threat to public health (Gabriela *et al.*, 2005). *C. parvum* is considered one of the most important biological contaminants in drinking water. Dairy calves are thought to be the primary source of *C. parvum* contamination in watersheds. Understanding the spatial and temporal variation in the risk of C. parvum infection in dairy cattle is essential for designing cost-effective watershed management strategies to protect drinking water sources (Szonyi *et al.*, 2010).

The incidence of *C. parvum* in calves, lambs and children was investigated by many authors including: Noordeen *et al.* (2000); Wade *et al.* (2000); El-Sibae *et al.* (2003); Zidan (2006); Castro-Hermida *et al.* (2007); Quilez *et al.* (2008); Coklin *et al.* (2009); Byomi *et al.* (2010); Daz-Lee *et al.* (2010).

Because of the public health significance of *C. parvum*, this work was applied to determine the incidence of *C. parvum* infection in calves, lambs and children in rural areas of Behera and Alexandria Provinces, compare using traditional methods in identifying *C. parvum* with using ELISA for detecting serological response, investigate seasonal patterns and age-associated trends of *C. parvum* in calves, lambs and children and throw a beam of light upon the public health significance of *C. parvum* and the role of calves and lambs in continuation of its transmission.

MATERIALS and METHODS

1. Collection of samples:

A- Calves:

A total of 248 fecal samples were collected from diarrheic (152) and apparently healthy calves (96) with age ranged from one day old to 3 months (pre- weaned calves) from individually owned animals attending the veterinarian clinics in rural areas of Alexandria and Behera Provinces (62/Season).

B- Lambs:

A total of 200 fecal samples were collected from diarrheic lambs (71 males and 129 females) ranging in age from one day old to 3 months (pre- weaned lambs) from individually owned animals attending the veterinarian clinics in rural areas of Alexandria and Behera (50/ Season).

C. Children stool specimens:

Stool specimens were collected from 240 diarrheic children (120 males and 120 females) under 6 years old (the age of school entry) attending pediatric hospitals in Alexandria and Behera Provinces (60/Season). Specimens were collected from cases in the same rural areas from which animal samples were collected.

Full history for each sample was taken including locality, age, health status and the characters of fecal samples then all samples are transferred directly as soon as possible to the laboratory of Animal Hygiene and Zoonoses Department, Faculty of Veterinary Medicine, Alexandria University and kept at 4 °C till examination.

2. Materials:

- **Modified Ziehl-Neelsen stain** was prepared as mentioned by Henrikson and Pohlenz (1981)
- **RIDASCREEN Cryptosporidium** ELISA kits supplied by GmbH, Darmstadt, Germany.

3. Methods:

- Preparation of collected samples: 5 g of each fecal and stool sample was placed in 2.5 % potassium dichromate solution and stored at 2–8°C until it is used in the ELISA test. If the material is not going to be used within 3 days, it is recommended to store it at –20°C. Multiple freezing and thawing of the sample must be avoided.
- Microscopic examination using MZN staining technique was performed according to Henrikson and Pohlenz (1981).
- ELISA was done using RIDASCREEN Cryptosporidium kits according to the manual supplied by GmbH, Darmstadt, Germany

RESULTS

Table 1: Incidence of *C. parvum* infection in examined fecal and stool samples of investigated species by MZN staining technique and confirmed by ELISA:

Investigates species	MZN			ELISA		
	NO	Positive	%	NO	Positive	%
Calves	248	23	9.3	23	22	95.7
Lambs	200	27	13.5	27	25	92.6
Children	240	28	11.7	28	28	100
Total	688	78	11.34	78	75	96.2

Table 2: Incidence of *C. parvum* infection in calves in relation to their health status:

Health status	No. of examined samples	Positive cases	%
Diarrheic calves	152	19	12.5
Apparently healthy calves	96	4	4.2
Total	248	23	9.3

Table 3: Incidence of *C. parvum* infection in total examined samples in relation to locality:

Locality	examined samples								
	Calves			Lambs			Children		
	No	+ve	%	No	+ve	%	No	+ve	%
Alexandria Province	124	10	8.1	100	11	11.0	120	11	9.2
Behera Province	124	13	10.5	100	16	16.0	120	17	14.2
Total	248	23	9.3	200	27	13.5	240	28	11.7

Table 4: Incidence of *C. parvum* infection in examined samples in relation to seasons of the year:

Seasons of the year	Examined samples								
	Calves			Lambs			Children		
	No	+ve	%	No	+ve	%	No	+ve	%
Winter	62	5	8.1	50	7	14.0	60	4	6.8
Spring	62	8	12.9	50	6	12.0	60	8	13.3
Summer	62	6	9.9	50	6	12.0	60	13	21.9
Autumn	62	4	6.5	50	8	16.0	60	3	5
Total	248	23	9.3	200	27	13.5	240	28	11.7

Table 5: Incidence of *C. parvum* infection in examined fecal samples of calves and lambs in relation to age groups:

Age group (month)	Calves			Lambs		
	No	+ve	%	No	+ve	%
< 1	122	11	9	84	19	22.6
1 - < 2	61	7	11.5	64	5	7.8
2 - < 3	65	5	7.7	52	3	5.8
Total	248	23	9.3	200	27	13.5

Table 6: Incidence of *C. parvum* infection in stool samples of children in relation to age groups:

Age group (year)	No. of examined samples	Positive cases	%
< 2	94	17	18.1
2 - < 4	75	7	9.6
4 - < 6	71	4	5.6
Total	240	28	11.7

Table 7: Incidence of *C. parvum* infection in examined stool samples of children in relation to history of animal contact:

History of children	No. of examined samples	Positive cases	%
Animal contact	148	22	14.9
Non animal contact	92	6	6.5
Total	240	28	11.7

DISCUSSION

Cryptosporidiosis remains among the most prevalent zoonotic pathogens in spite of the great strides made in its control and extirpation. It causes gastroenteritis and diarrhea in several species specially calves, lambs and human. The infection may be transmitted from man to man or from animals to man. Moreover, cryptosporidiosis is also of well known veterinary importance as it may cause economic losses in calves and lambs.

In the current study, the role of calves and lambs as a source of children infection with *C. parvum* was investigated.

The total incidence of *C. parvum* infection in examined fecal and stool samples collected from calves, lambs, dogs and children by MZN staining and confirmed by ELISA assay was presented in Table 1; firstly the obtained results revealed that the total incidence of C. parvum in calves fecal samples using MZN staining teachnique was 9.3 %. This result was lower to that obtained by Quilez *et al.* (2008) (57.8%), Byomi *et al.* (2010) (10.24 %). On the other hand, this result was higher that obtained Wade *et al.* (2000) (0.9 %), Castro-Hermida *et al.* (2007) (8.4 %), Coklin *et al.* (2009) (6.2 %). Moreover, Ralston *et al.* (2003) found nearly similar results. Secondly, the obtained results revealed that the total incidence of *C. parvum* in lambs was 13.5 %. This result was lower than that obtained by Ozmen *et al.* (2006) (66.7 %), Paoletti *et al.* (2009) (17.5 %) and Yang *et al.* (2009) (24.5 %), while it was higher than that obtained by Zidan (2006) (12.4 %), Castro-Hermida *et al.* (2007) (5.3 %) and Wang *et al.* (2010) (4.8 %). Finally, the obtained results in Table 1 revealed that the total incidence of *C. parvum* in children was 11.7 %. This incidence was higher than that obtained by Handousa *et al.* (1991) (10.9 %) and El-Sibae *et al.* (2003) (8 %) on the other hand this

incidence was lower than that recorded by Soliman (1992) (13.5 %), Dupont *et al.* (1995) (20 %) and Rozej *et al.* (2010) (47.5 %). Moreover, this result was nearly equal with that obtained by Zidan (2006) (11.95 %) and Freites *et al.* (2009) (11.8 %).

The result of comparison between the performance of the enzyme linked immunoassay (ELISA) with MZN staining technique in diagnosis of *C. parvum* infection in different animal species and children was demonstrated also in Table 1. All positive cases by MZN staining technique were retested by commercial ELISA kits that detected all positive cases of children (100 %) then calves (95.7 %) and lambs (92.6 %) with overall sensitivity of 96.2 %. These results agreed with Marques *et al.* (2005) and Zidan (2006) who concluded that the commercial ELISA and MZN technique proved to be valuable, sensitive and specific diagnostic tools for *C. parvum*.

The health status of calves under investigation affected the incidence of *C. parvum*. This illustrated in Table 2 where the incidence of C. parvum in diarrheic calves (12.5 %) was higher than apparently healthy calves (4.2 %). This result agreed with Naciri *et al.* (1999) who recorded the presence of *C. parvum* oocysts in the faeces of diarrheic suckling and dairy calves in France at the percentages of 34.7 and 2.4 %, respectively, while *C. parvum* were detected in faeces of non diarrheic suckling and dairy calves at a percentages of 13.8 and 3.9 %, respectively, Uga *et al.* (2000) who reported that calves infected with *C. parvum* had a significant higher rate of diarrhea (33 %) than non infected calves (8 %) suggesting that *C. parvum* infection is the likely cause, Zidan (2006) recorded an incidence of 14 % in diarrheic calves and 4.8 % in apparently healthy calves and Daz-Lee *et al.* (2010) found that a high number of infected animals suggesting the parasite *C. parvum* as a major parasitic disease agent of neonatal calves with diarrhea in dairy farms of the Metropolitan Region of Chile.

The data presented in Table 3 firstly, highlighted the incidence of C. parvum in calves in the two investigated provinces. The incidence was higher in Behera Province (10.5 %) than in Alexandria Province (8.1 %) that may reflect the rural nature of Behera Province as compared with Alexandria Province where hygienic conditions might be suspected. This result agreed with Zidan (2006) who recorded an incidence of 10.94 % in Behera Province that confirmed the endemic nature *C. parvum* infection of calves of Behera Province that may represent a zoonotic threat to human being in this province which need effective control

measures to eliminate that zoonotic threat. Data presented in Table 3 secondly, clarified that the incidence of *C. parvum* in lambs was higher in Behera Province (16 %) than in Alexandria Province (11 %). The higher incidence in Behera than in Alexandria similar to that of calves' incidence that may note that calves and lambs may act as reservoirs of *C. parvum* infection. This result was higher than that obtained by Zidan (2006) who recorded an incidence of 11.8 % in Behera Province that also confirmed the endemic nature *C. parvum* infection of lambs of Behera Province.

The incidence of *C. parvum* in the examined stool samples of children collected from different localities was present also in Table (3). The incidence of *C. parvum* in examined stool samples from children of Behera Province (14.2 %) was higher than in Alexandria Province (9.2 %). This result was in agreement with results of examination of calves and lambs from the same localities that reflected the close association between the incidence of infection in animals and children that highlighted the zoonotic significance of *C. parvum.* These results confirmed by Hira *et al.* (1989) who stated that contaminated water supplies and contact of animals such as sheep and goats were of the known risk factors facilitating transmission. Moreover, Keusch *et al.* (1992) indicated that persons at greatest risk were immunocompromised adults and children, especially those with AIDS, children in day care and travelers to endemic regions. The obtained result of *C. parvum* incidence in children of Alexandria Province under investigation was lower than that recorded by Soliman (1992) who detected a prevalence of 13.51% among school children in a rural area in Alexandria Province while the obtained result of *C. parvum* incidence in children of Behera Province under investigation was nearly similar to that obtained by Zidan (2006) (13.84 %).

The seasonal distribution of *C. parvum* in calves was recorded in Table 4 where the highest incidence of *C. parvum* was observed in spring season (12.9 %), followed by summer (9.9 %) then winter and autumn (8.1 %) and (6,5 %), respectively. This distribution was agreed with results obtained by Wade *et al.* (2000); Byomi (2010) who found insignificant association between infection with *C. parvum* in calves and season of the year. On the other hand, these results disagreed with Mann *et al.* (1986); Sahal *et al.* (2005) who recorded that the occurrence of the disease was more common in winter (56.4 %) than during other seasons (autumn 0 %. summer 15.4 % and spring 28.2 %). The seasonal

distribution of *C. parvum* in lambs was also recorded in Table 4 where the highest incidence of *C. parvum* was observed in autumn season (16 %), followed by winter (14 %) then similar incidence in both spring and summer (12 %). The effect of season on *C. parvum* infection in children was presented in Table 4 that revealed a higher incidence in summer season (21.9 %), followed by spring (13.3 %) then winter and autumn (6.8 %) and (5.0 %), respectively. This seasonal distribution was agreed with results obtained by Tzipori (1983); Mata *et al.* (1984); Marc *et al.* (1990) who reported that the higher incidence of infection was during warmer months (May to August) and Zidan (2006) who recorded an incidence of 18.46 %, 14 %, 10.71 % and 6.25 % during summer, spring, winter and autumn, respectively. These results suggested that the incidence rates were slightly higher during spring and summer seasons than during autumn and winter seasons. On the other hand, these results disagreed with Baxby and Hart (1986) who found that the peak incidence of Cryptosporidiun infection was in the late winter and early spring and Siwila *et al.* (2011) who reported that Cryptosporidium infections were significantly more common in the wet compared to the dry season.

The effect of age of calves and lambs on the incidence of *C. parvum* infection was illustrated in Table 5. Concerning calves, the highest incidence was observed in the age group (1- <2 months) (11.5 %) followed by the age group (<1 month) (9 %) and finally the age group (2- <3 months) (7.7%). This result disagreed with Zidan (2006) who found that the highest incidence was in the age group (up to 1 month) (15.2 %) followed by the age group (1- 2 months) (5 %) and Inpankaew *et al.* (2010) who found that calves less than 2 months old were more frequently infected by *C. parvum* than others. This result proved that the age of calves was an important factor affecting the incidence of *C. parvum* infection in calves. McCluskey *et al.* (1995) indicated that the period of prevalence of cryptosporidiosis was 100% in calves from 1-30 days of age. This proved that infection occurred early in the neonatal period and the environment was heavily contaminated with oocysts. Initial exposure to infective oocysts appears to occur in the maternity pen or shortly after placement in outdoor cages. However the role of the apparently healthy carriers in the epidemiology of the disease has more recently described. Excretion of oocysts has been found in apparently healthy adult cows (Villacorta *et al.*, 1991; Scott *et al.*, 1994). Concerning lambs, the highest incidence was observed in the age group (<1 months) (22.6 %) followed by the age group (1- <2 month) (7.8 %)

and finally the age group (2- <3 months) (5.8 %). This result agreed with Olson *et al.* (1997) in Canada, Majewaska *et al.* (2000) in West-Central region of Poland, Noordeen *et al.* (2000) in SirLanka and Zidan (2006) who found that the highest incidence was in the age group (< 1 month) (19.63 %) followed by the age group (< 2 months) (8.75 %). This study indicated that there was a clear association between infection with *C. parvum* and the age of lambs.

The effect of the age group distribution on the infection rates with *C. parvum* infection in was illustrated in Table 6. The highest incidence was observed in the age group (<2 years) (18.2 %) followed by the age group (2- <4 years) (9.6 %) and finally the age group (4- <6 years) (5.6 %). This result agreed with Zidan (2006) who found that the highest incidence was in the age group (<2 years old) (20.2 %) followed by the age group (3- 4 years) (7.5 %) then the age group (< 6 years old) (5.5 %). These results indicated that the age of children at risk is an important factor in *C. parvum* infection. These results were in agreement with results obtained by Das *et al.* (1993) who observed that the highest detection rate of Cryptosporidium oocysts was in the first two years of life in both diarrheic and control children and Henry *et al.* (1995) who concluded that the highest risk of persistent diarrhea was in children of 2-3 years.

Incidence of *C. parvum* infection in examined stool samples of children in relation to history of animal contact was illustrated in Table 7. It was clear that animal contact affected incidence of *C. parvum* in children where incidence was higher in children with history of animal contact (14.9 %) than others with no history of animal contact (6.5 %). This finding was in agreement with Soliman (1992); Younis (1995); Zidan (2006) who recorded an incidence of 16.7 % in contact group and 4.2 % in non contact group. Theses results confirmed the association between the influence of contact with animals and infection with *C. parvum* as most cases inhabited rural communities in Behera and Alexandria Provinces.

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