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DIARRHOEA IN KIDS ATTRIBUTED TO ENTEROBACTERIA AND *CRYPTOSPORIDIUM*

(With 4 Tables and 1 Figure)

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الإسهال في صغار الماعز الناتج عن الإصابات البكتيرية المعوية والكريبتوسبوريديم

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شملت الدراسة عدد ١٨٦ حالة من صغار الماعز تتراوح أعمارهم من أسبوع ألي ٦ أسابيع منها ١٢٤ حالة تعانى من الإسهال الحاد و ٦٢ حالة سليمة ظاهريا من بعض المزارع الخاصة في محافظة الدقهلية . اظهر الفحص الإكلينيكي وجود درجات متفاوتة من الاسهالات المائلة للاخضرار أو مخاطية مدممة في بعض الحالات مع الهزال والجفاف المتوسط وشديد الحدة . بعد الفحص بوقت قصير نفق عدد ٢٢ حالة . أظهرت نتائج الفحص الطفيلي عن تواجد طفيل الكريبتوسبوريديم بنسبة ١٤,٥% في براز الحالات المريضة أما منفردا بنسبة ٧,٢٥% أو مشتركا بنسبة ٧,٢٥%. كذلك اظهر الفحص البكتريولوجى عن عزل ميكروب القولون الممرض من ١٧,٧٤% من الحالات المرضية أما منفردا (٩,٧٦%) أو مشتركا مع ميكروب البر وتيس (٣,٢٢%) أو طفيل الكريبتوسبوريديم (٤,٨٣%) . أظهرت النتائج أيضا وجود إصابة بالسالمونيلا بنسبة ٥,٦٤% في الحالات المرضية . وكانت نتائج الفحص لمحتويات الأمعاء للحيوانات النافقة عن وجود إصابة بالميكروب القولوني منفردا بنسبة ٣١,٨% أو مزدوجا مع طفيل الكريبتوسبوريديم بنسبة ١٣,٦% أيضا تم عزل ميكروب السالمونيلا بنسبة ٩,١% . كذلك وجود إصابة بطفيل الكريبتوسبوريديم مع ميكروب البر وتيس بنسبة ١٣,٦%. تم في البحث أيضا عمل أختبار حساسية للميكروبات المعزولة .

SUMMARY

Faccal samples from 124 untreated diarrheic kids at age 1-6 weeks old as well as 22 samples of intestinal contents from freshly dead animals were collected from intensively managed` private farms in El-Dakahlia

province. The samples were examined for Enteropathogens and Cryptosporidium in comparison with 62 samples from apparently healthy kids of the same age on the same locality. Enterotoxigenic E.coli was isolated from 17.74% samples of diarrhoeic kids either purely 9.76% or mixed with proteus mirabilis 3.22% or with *Cryptosporidium parvum* 4.83%. The isolated E.coli strains were serotyped as 13.6% E. coli O_{78} K_{80} (B-); 9.1% E.coli O_{125} K_{70} (B15) and 77.3% untypable strains. Salmonella species were detected in 5.64% samples of diarrhoeic kids and the majority of strains were identified as salmonella typhimurium 71.43%. *Cryptosporidium parvum* was found in 14.5% of diarrhoeic kids either alone 7.25% or mixed with bacteria 7.25%. Proteus species and klebsiella aerogenes were only the isolates detected in faeces of examined apparently healthy kids. The examination of intestinal contents of freshly dead diarrhoeic kids revealed that E.coli, Salmonella and Cryptosporidium were isolated alone or mixed in percentage 45.4%, 9.1% and 27.2% respectively. In-vitro- sensitivity pattern of isolated strains proved that ciprofloxacin, enrofloxacin and gentamycin were the most effective drugs.

Key words: *Diarrhoea, Kids, Enterobacteria, Cryptosporidium.*

INTRODUCTION

Goats have become more popular in Egypt as they are relatively cheep, highly fertile and possess high efficiency in converting poor material to meat and milk. Furthermore, they have unique ability to adopt and maintain itself in harsh environments. Goats husbandry in our country has been practiced in fixed flocks, mobile flocks (grassing) and individual farmer's animals. Its numbers had been increased gradually since 1980 to reach three times in 1996 (3378000 heads) CAPMAS, 1996.

Under all management or production systems diarrhoea threatens virtually neonate kids in most every locate. It is caused by a combination of many risk factors. Interaction between environments (over crowding; transportation, inclement weather), nutritional imbalance; improper colostrum intake and virulence of pathogens provoke the imbalance of intestinal equilibrium resulting in diarrhoea. Also, immaturity of immune system of neonate kids increases the susceptibility to contagious and opportunistic infection (Radostits et al., 1994).

Several bacterial species may be involved in diarrhoea and losses of neonatal lambs and kids, the most important being is certain strains of E.coli that possessing virulent factors, salmonella species and campylobacter species. These pathogens are responsible for great mortality and various morbidity changes and at the same time constitute a hazard to public health. Cryptosporidium contribute to cause high morbidity and mortality in neonatal kids especially under stress conditions or in association with other enteropathogens (Moon and Woodmansee, 1986 and Matthews, 1999).

Several outbreaks and sporadic cases of diarrhoea were occurred in neonatal kids especially in intensive farm production and large flocks in our area at El-Dakahlia Province. Treatment of such cases were usually initiated before the exact etiological diagnosis was confirmed and including isolation of affected kids from healthy herd; fluid and electrolyte replacement and the use of antibiotics. Therefore, the present work was aimed to study the role of Enterobacteriaceae and Cryptosporidium as a causative agent of diarrhoea in kids. Also, to determine in vitro- the antibiotic sensitivity of isolated organisms.

MATERIAL and METHODS

Samples:

Faecal samples were collected aseptically from 124 untreated diarrhoeic kids, 1-6 weeks old located at intensively managed private flocks in EL-Dakahlia Province during the period (1999). Faecal samples were obtained by means of sterile probes introduced into the rectum of each kid, kept in sterile plastic bottles. Intestinal contents were collected in sterile containers from 22 freshly dead kids of this condition short time after examination. In addition, 62 apparently healthy kids of similar age on the same localities were also sampled. All samples were labeled and sent to laboratory for bacteriological and parasitological examination.

Enterobacteriaceae isolation and identification:

Each faecal sample was divided into 3 portions under aseptic condition. The first part was streaked directly onto predried surface of MacConkey agar (Oxoid, CM7); Eosin methylene blue agar (EMB, Oxoid, CM69) and Xylose lysine desoxycholate agar (XLD, Oxoid CM469). The plates were incubated at 37°C for 24 hours.

The second faecal part was inoculated into Rappaporte-Vassiliadis broth (R.V., Oxoid, CM669) and incubated at 43°C. After 24 hours

incubation, loopfules from R.V. enrichment were streaked onto XLD agar plate with incubation at 37°Cfor 24 hours.

The growing colonies on various plates were examined morphologically, picked up, Gram stained and purely subcultured on to blood agar plates. The suspected isolates were maintained on nutrient agar slops for identification using API20 (bio Merieux) and appropriate biochemical tests as described by Collins et al., (1995).

The identified E.coli strains were tested for enterotoxin production through grown the E.coli isolate in trypticase soya broth at 37°C in stationary culture overnight. Culture was centrifuged at 4000 rev/min for 20 min. The supernatant was tested using commercially VET-RPLA kits (reversed passive latex agglutination) from Oxoid (TD 0920A) following the manufacturer's direction.

The biochemically identified E.coli and salmonella strains were subjected for serological identification using available coli test sera agglutinating (bioMerieux) and diagnostic salmonella agglutinating antisera (DenkaSelken Co. LTD, Tokyo, Japan) according to mannufacturer's instruction.

Parasitological examination:

The third potion of each faecal sample was used for detection of the parasitic infestation through:

a) Direct faecal smear (Soulsby, 1982).
b) Concentration flotation technique (Levine, 1985).
c) Staining of samples with Modified Ziehl-Nellsson (Henriksen and Pohlenz (1981).
d) Staining with Safranin-methylene blue (Baxby et al., 1984).

Also the same procedures mentioned above were done on intestinal contents samples from dead diarrhoeic kids.

In vitro antibiotic sensitivity test:

The disk diffusion technique was performed on isolated bacteria from diarrhoeic cases according to Finegold and Martin (1982). Ten chemotherapeutic disks kindly supplied by Oxoid were used and namely ciprofloxacin, enrofloxacin, clormphenicol, gentamycin, streptomycin, neomycin, ampicillin, erythromycin, amoxycillin and trimethoprim-sulphamethoxazole. The degree of sensitivity was determined and interpretated according to Oxoid Manual (1998).

RESULTS

The results are recorded in Tables 1, 2, 3 & 4 and Plate 1.

DISCUSSION

Various degrees of diarrhoea, depression, dehydration, weakness and loss of weight were noticed during clinical investigation of diarrhoeic kids. Also body temperature was usually elevated. Diarrhoea in some cases was offensive semifluid watery yellowish containing mucous and sometime tinged with blood. In others, faeces were foetid profuse clay to yellowish or grayish, some times mucoid or contained blood streaks. Short time after examination, 22 (17.7%) of diarrhoeic cases were found dead. As they were in poor condition with general emaciation of whole carcasses. These findings support many of previous investigations in a similar condition whose recorded mortality rates ranges from 16.27% to 31% among diarrhoeic kids (Vihan et al., 1990; Upadhaya et al., 1998 and Ghosh and Patutunda 1998).

The bacteriological examination of 124 faecal samples from diarrhoeic kids revealed 32 (25.8%) bacterial cultures belonging to family Enterobacteriaceae either alone 23 (18.5%) or mixed with Cryptosporidium in 9 (7.3%) samples (Table, 1). Shams et al. (1989) found that 22% of faecal swabs from diarrhoeic kids were positive bacteriologically which is slightly lower than the reported herin.

Results in Table (2) showed that E.coli was the dominant bacterial infection among diarrhoeic cases. It was isolated from 22 cases with an incidence of 17.74%. The organism was detected either alone in 12 (9.76%) or mixed with proteus mirabilis in 4(3.22%) or with Cryptosporidium in 6 (4.83%) cases. Such incidence of E.coli infection agreed to certain extent with those reported by Jiwa (1993) and Abo-EL-Hassan (1996). While high incidence was recorded by Munoz et al. (1996) and Yadava and Choudhary (1996).

In this study, all the isolated E.coli strains from diarrhoeic kids were enterotoxigenic produce heat labial enterotoxin when tested by VET-RPLA kits and serologically identified as 13.6% E.coli O_{78} K_{80} (B-), 9.1% E.coli $O_{125}K_{70}$ (B_{15}) and 37.3% untypable strains. (Table 3). The association of these serotypes with sheep and goats diarrhoea were reported by Karmy and Ragab (1983); Refai (1988) and Jiwa (1993).

Generally in E.coli infections, diarrhoea occurs through the effect of enterotoxins, which stimulate guanylate cyclase activity of the ileal epithelium (heat stable toxin ST) or adenyl cyclase activity of intestinal and capillary epithelium (heat labial toxin LT) resulting in

hypersecretion of electrolytes particularly Na^+ and HCO_3 and an increased diffusion of water into lumen of the intestine which resulted in acidosis and dehydration (Kaske, 1993).

The results in Table (2) pointed out that Salmonella species were isolated from 7 (5.64%) faecal samples of diarrheic kids. This finding is nearly agree the observations of Kirby (1985) and Ismail and EL-Seedy (1989). High incidence (15%) was reported by Jiwa (1993) and low incidence (2.7%) was found by Munoz et al. (1996). On the contrary Abou-EL-Hassan (1996) failed to detect the organism from any rectal swabs from diarrheic neonate kids. Radostits (1994) reported that salmonella infection was asporadic cause of interitis and loss in neonatal goat kids.

The isolated salmonella strains were serotyped as 5(71.43%) Salmonella typhimurium and 2 (28.57%) untypable strains (Table 2). Salmonella typhimurium had been reported as the most common species isolated from diarrheic sheep and goats (Bulgin and Anderson 1981; Ismail and EL-Seedy 1989 and Matthews 1999).

Cryptosporidium parvum as showen in Plate (1) appeared to be the second most important cause of diarrhoea among examined neonatal kids as clear in Tables 1 and 2. It was found in faeces of 18 (14.5%) kids suffering from diarrhoea, either alone 7.25% or mixed with E.coli 4.83% or with Proteus mirabilis 2.41%. These findings agree to a large extent with those of Tzipori et al. (1982) and Abo-El-Hassan (1996), meanwhile high incidence had been reported by Munoz et al. (1996); Hilali et al. (1998) and Khalil, (2000) whose recorded an incidence of 42%, 55.6% and 46.5% respectively. Low incidence of Cryptosporidium among diarrhoeic kids was found by Gorman et al. (1990) who recorded an incidence of 10.5%.

The incidence of Cryptosporidium in diarrhoeic young animals might be due to young age which severely affected by the parasite whereas the young animals were immunologically immature and have a greater prevalence of infection and experience more sever illness than adults (Fayer, 1990).

In the present study *Cryptosporidium parvum* was responsible for the death of 6 (4.8%) kids suffering from diarrhoea when found mixed with E.coli and with proteus mirabilis (Table 2). Nagy et al. (1983) found that Cryptosporidium infection was responsible for 21% mortalities in diarrhoeic kids which is higher than the reported herin.

It is well known that Cryptosporidia adher to the microvillous borders of intestinal epithelial cells especially where it develops and

causes an atrophy of the intestinal villi with consequent malabsorption and hypersecretion (Chermette and Boufassa-Quzrot 1988).

Examination of intestinal contents of dead diarrhoeic kids for Enterobacteriacae are pointed in Table (2). It is clearly seen that E.coli was isolated in pure culture from 10 (45.5%) either singly 7 (31.8%) or mixed with Cryptosporidium 3 (13.6%). All isolated strains were serotyped E.coli O_{78} K_{80} (B-) (Table 3). Meanwhile, Salmonella typhymurium was isolated from 2 (9%) dead diarrhoeic cases. High mortalities in neonate kids due to these organisms were also reported by Vihan et al. (1990) and Jiwa (1993).

As clear in Table (2), E. coli; salmonellae and Cryptosporidium were absent in all faecal samples of examined apparent healthy kids meanwhile proteus spp and Klebsiella aerogenes were recovered from 12 (19.4%) and 6 (9.7%) faecal samples respectively but with non significant association as a single agent with diarrhoea. Similar observation have also been found by Munoz et al. (1996) and Matthelus (1999) who concluded that the isolation of Cryptosporidium was always significant in newly borne kids 1-4 weak old. Moreover, Abou-EL Hassan (1996) reported that only E.coli and Cryptosporidium showed significant difference associated with the diarrhoeic kids.

In vitro, the susceptibility distribution of each isolated pathogen to different antibiotics is presented in Table (4). The typical pattern of a highly effective compounds were observed for zones of ciprofloxacin, enrofloxacin and gentamycin for all tested three enteric pathogens. These findings coresponded with those reported by Adesiyum and Kaminjolo (1992) and Adesiyun et al. (1993).

Finally, it must be strongly stressed that E.coli; salmonellae and Cryptosporidium enteropathogens have an important role in the diarrhoeic syndrome and death among neonate kids in the area of study. Therefore, the minimization of these infections relies on comprehensive control programs that incorporate for minimizing environmental stress and optimizing nutrition. Also allowing sucking before rearing; vaccination pregnant does with potentiated and effective vaccine (Wihan, 1993).

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Table (1): Incidence of Enterobacteriaceae and Cryptosporidium among examined kids

Condition of kids	Number of examined kids	Number of positive cases for						Overall incidence
		Enterobacteriaceae		Cryptosporidium		Dual infection		
		No.	%	No.	%	No.	%	
Diarrhoeic	124	23	18.5	9	7.25	9	7.25	33.1
Apparently healthy	62	18	29.03	-	0	-	0	29.03
Dead after short time of examination	22	9	40.9	-	0	6	0	68.2

Table (2): Prevalence of bacterial species and Cryptosporidium in examined kids

Causative agents	Condition of kids					
	Diarrhoeic		Dead		Apparently healthy	
	No.	%.	No.	%	No.	%
Escherichia Coli	12	9.76	7	31.8	-	0
*Salmonella spp.**	7	5.64	2	9.1	-	0
Klebsilla aerogenes	-	0	-	0	6	9.7
Proteus mirabilis	-	0	-	0	5	8.1
Proteus myxofaciens	-	0	-	0	7	11.3
Cryptosporidium parvum	9	7.25	-	0	-	0
Cryptosporidium parvum + E.coli	6	4.83	3	13.6	-	0
Cryptosporidium parvum + P.mirabilis	3	2.41	3	13.6	-	0
E.coli + P. mirabilis	4	3.22	-	0	-	0
Total	41	33.06	15	68.2	18	

* Salmonella typhymurium (5). Untypable (2).

Table (3): Serological identification of isolated *E.coli strains

Serotype	Diarrhoeic kids		Dead kids	
	No.	%	No.	%
E. coli O_{78} K_{80} (B-)	3	13.6	-	0
E.coli O_{125} K_{70} (B15)	2	9.1	-	0
Untypable E.coli	17	77..3	10	100
Total	22		10	

* All were produce LT toxin.

Table (4): The in-vitro susceptibility of 36 bacterial strains isolated from faecal samples of examined diarrhoeic kids to some antibiotics.

Antibiotic discs and their potency	*E. coli (22)**		*Salmonella (7)**		*Proteus mirabilis (7)**	
	Sensitive isolates	Activity percent	Sensitive isolates	Activity percent	Sensitive isolates	Activity percent
Ciprofloxacin 5μ g	22	100	7	100	7	100
Enrofloxacin 5μ g	22	100	7	100	7	100
Chlormphenicol 30μg	16	72.7	6	85.7	2	28.5
Gentamycin 10μg	22	100	7	100	7	100
Streptomycin 10μg	-	0	-	0	1	14.2
Erythromycin 15μg	-	0	-	0	-	0
Neomycin 30μg	10	54.4	-	0	5	71.4
Ampicillin 10μg	-	0	-	0	2	28.5
Amoxycillin 25μg	-	0	-	0	2	28.5
Trimethoprim-sulpha-methoxazole 1.25 - 23.75μg	18	81.8	2	28.5	1	14.2

(*) Number of tested strains

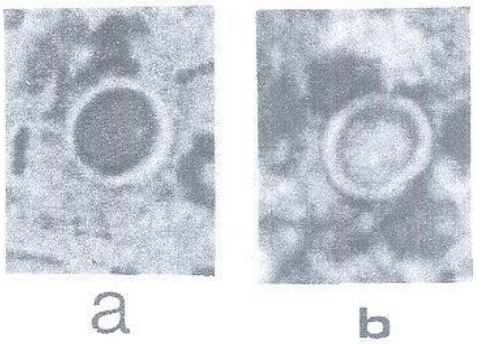

Plate (1)

(a) Oocyst stained with a modified Ziehl- Neelson x 1250

(b) Oocyst stained with Safranin- methelene blue x1250