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STUDIES ON SOME ZOONOTIC ENTEROPATHOGENS CAUSING ACUTE DIARRHEA IN CHILDREN IN KALYOBIA PROVINCE

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ABSTRACT

One hundred stool samples were collected from young children aged below 5 years with acute diarrhea attended to health units of rural areas at Kalyobia province,Egypt, as well as 66 faecal samples were collected from different animal species and birds living in contact with children. Moreover, 50 water samples collected from water supply (piped or pumped). All the collected samples were examined for bacterial and protozoal pathogens. The recovered enteric pathogens were Shigella spp. (16.7%), Salmonella spp. (16.7%), Escherichia coli (66.7%), Entamoeba histolytica (30%) and Giardia lamblia (70%) from diarrheal children, while they were detected with percentages of 15.4%, 15.4%, 69.2%, 25% and 75% respectively in contact domestic animals but they were 0%, 22.2%, 77.8%, 0% and 100% respectively in birds. Meanwhile, they were 0%, 25%, 75%, 33.3% and 66.7% respectively found in the examined water samples. The occurrence of diarrhea was more evident in males (57.5%) than females (42.5%) and the majority of cases existed among children in the first 2 years of life. Moreover, fever was present in bacterial but not in protozoal diarrhea, vomiting was present in 50% of bacterial and in 20% of protozoal diarrhea and blood was present in the stools of some cases of Salmonellosis, Shigellosis and Ambiasis. The majority of diarrheal cases with positive enteropathogens had out-door (pumped) water supply (90%) and with positive history of animal contact (75%). The zoonotic importanc of enteropathogens as well as the suggested hygienic measures were discussed.

INTRODUCTION

Diarrhea is a significant public health problem and it is ubiquitous prevailing everywhere in the world, its annual incidence in Asia and Africa is much higher than that in the rest of the world **(Alikhani *et al.*, 2007).**

Diarrhea is one of the leading causes of morbidity and mortality among infants and young children due to repeated attacks and their lack of resistance to overcome infections. Among children under 5 years in developing countries, diarrhea accounts for 17% of all deaths and annually 4 million deaths caused by diarrhea occur in children under 5 years age **(United Nation, 2006).**

Acute diarrhea was defined as abnormal faecal discharge characterized by frequent at least 3 times per day liquid or semi liquid loose stools, accompanied by symptoms such as nausea, vomiting, fever and involving dehydration and electrolyte loss **(Urbina *et al.*, 2003)**.

Diarrhea can be caused by wide range of bacteria (e.g. Shigella spp., Salmonella spp. and Escherichia coli) or entero protozoa (e.g. Giardia spp. and Entamoeba histolytica) **(Martha, 2004)**. The infectious agents associated with diarrheal disease are transmitted chiefly through faecal-oral route **(Black, 2001).** An estimated 94% of the diarrheal burden of diseases is attributable to the environment, and associated with risk factors such as unsafe drinking water, lack of sanitation and poor hygiene **(Pruss-Ustun and Corvalan, 2006).** Moreover, animals and birds play a great role in the epidemiology of childhood diarrheal infection as animals and birds act as reservoirs of some enteric pathogens causing diarrhea. There are many risk factors for young children such as inadequate personal hygiene, childhood habits like nail-bitting and thumb-sucking and close contact with domestic animals **(O'Brien *et al.*, 2001).** The isolation of multiple enteric pathogens is common in many developing countries. This problem may reflect the high degree of faecal contamination in the environment, or the high probability of person-to-person transmission and poor hygiene measures in foods and water **(Mattar *et al.*, 1999).**

The aim of this study is to investigate the bacterial and protozoal organisms associated with acute diarrhea among children in rural areas at Kalyobia province and to throw some light on animals and or birds as reservoirs and water as a source of intestinal infectious agents, in addition, to conclude the available preventing measures to overcome such problem.

MATRIALS AND METHODS

The present study was carried out in the laboratory of zoonoses department, Faculty of Veterinary Medicine, Benha University,Egypt.

1-Collection of samples:

1-a-Childern's stool specimens:

A total of 100 fresh stool specimens were collected from young children aged less than 5 years, attended to health units of rural areas in some villages at Kalyobia province, Egypt. They were suffering from acute diarrheal episode less than one week duration and did not receive any chemo-therapy .Histories were established by the use of questionnaires elicting data from parents on the child's age, sex, presence of associated symptoms (as fever, vomiting, blood in faeces), water supply and contact with animals or birds in their houses. The stool specimens were collected in clean sterile disposable plastic containers ,labeled and transferred as soon as possible with a minimum of delay to laboratory for examination.

1-b- Animal's faecal samples:

Faecal samples were collected from 40 animal species (cattle, calves, buffaloes and dogs) and 26 birds (chickens and ducks) which were living in contact with children in their houses. The samples were collected and send to laboratory as mentioned previously.

1-c-Water samples:

Fifty samples were collected from either piped or hand pumped water in glass bottles, cleaned and sterilized in hot air oven to be examined.

2-Bacteriological examination for enteropathogens:

Stool and faecal samples were macroscopically examined for consistency ,blood and mucus. A loopful of each faecal samples (either from children, animals or birds) was inoculated on to nutrient broth and selenit F broth tubes. Then a loopful from the incubated broths was streaked on Macconkey agar plates and Salmonella shigella agar plates and incubated at 37°C for 24 hrs **(Koneman et al.,1993).** The non lactose fermenting as well as lactose positive colonies were picked up and subcultured on nutrient agar slope and incubated at 37°C for 24 hrs to obtain pure cultures .Such cultures were identified according to schemes described by **(Kauffmann1972,Finegold & Martin 1982 and Quinn et al., 2002).**

3-Parasitological examination for protozoal pathogens:

This was done by direct microscopic examination of the collected samples either unstained (Direct wet mount) according to **Levine (1985)** or stained by trichrome stain according to **Fleck & Moody (1988)** as the staining allow recognition of detailed organism morphology and help in identification of protozoa.

4-Examination of water samples:

The collected water samples were examined bacteriologically according to **American Public Health Association (1989)** and parasitologically according to **Carlose et al .,(1980).**

RESULTS AND DISCUSSION

Table (1): Enteropathogens isolated from diarrheal children, animal and or birds in contacts and water

Enteropathogens	Children (100)		Animals (40)		Birds (26)		Water (50)	
	No	%	No	%	No	%	No	%
Bacteria	30	30	13	32.5	9	34.6	16	32
Protozoa	10	10	4	10	1	3.8	6	12
Total	40	40	17	42.5	10	38.5	22	44

Table (2): Types and percentages of isolated enteropathogens:

Isolated from	No. of positive	Bacteria							Protozoa				
		Shigella spp.		*Salmonella spp.*		*E.coli spp*		Total	*E.Histolytica*		*G.lamblia*		Total
		No	%	No	%	No	%	No	No	%	No	%	No
Children	40	5	16.7	5	16.7	20	66.7	30	3	30	7	70	10
Animals	17	2	15.4	2	15.4	9	69.2	13	1	25	3	75	4
Birds	10	0	0	2	22.2	7	77.8	9	0	0	1	100	1
Water	22	0	0	4	25	12	75	16	2	33.3	4	66.7	6

Table (3): Frequency distribution of isolated diarrheal pathogens among 40 positive cases:

Characters	Exam. No	Positive		Bacteria		Protozoa	
		No	%	No	%	No	%
Sex							
Males	52	23	57.5	18	60	5	50
Females	48	17	42.5	12	40	5	50
Total	100	40	40	30	75	10	25
Age							
<1 years	35	15	37.5	12	40	3	30
1-2 years	30	16	40	12	40	4	40
3-5years	35	9	22.5	6	20	3	30
Total	100	40	40	30	75	10	25
Bloody stool							
Yes	4	4	10	3	10	1	10
No	96	36	90	27	90	9	90
Total	100	40	40	30	75	10	25
Vomiting							
Yes	34	17	42.5	15	50	2	20
No	66	23	57.5	15	50	8	80
Total	100	40	40	30	75	10	25
Fever							
Yes	6	6	15	6	20	0	0
No	94	34	85	24	80	10	100
Total	100	40	40	30	75	10	25
Animal contact							
Yes	66	30	75	22	73.3	8	80
No	34	10	25	8	26.7	2	20
Total	100	40	40	30	75	10	25
Water sources							
Pipe	35	4	10	2	6.7	2	20
pump	65	36	90	28	93.3	8	80
Total	100	40	40	30	75	10	25

Results recorded in **Table (1)** illustrated the percentage distribution of the enteropathogens isolated from diarrheal children in relation to incontact animals or birds and from water sources. It is evident that the bacterial pathogens were detected with a percentage of 30%,32.5%,34.6% and32% in diarrheal children, in contact animals, birds and water respectively, while the protozoa were detected with a percentage of 10%,10%,3.8% and 12% in the previously mentioned examined samples respectively. Regarding types of isolated pathogenic bacteria causing acute diarrhea in children, Shigella spp. were encountered in 5% of all examined children representing 16.7% of bacterial pathogens **(Table 2)** .Concerning Salmonella spp., it was detected in 5% of all examined children representing 16.7% of bacterial pathogens. Meanwhile, E.coli was encountered in 20% in the examined children representing 66.7% of bacterial pathogens. These results substantiate what had been reported by **(Taha, 1989; El-Sherbini, 1992; Haggag et al.,2005;Olesen et al., 2005 and Abu-Elamreen et al., 2008)** but higher than obtained by (**El-Sheikh & El-Assoli 2001; Basil, 2002; Shebib et al.,2003 and Khan Mohammed et al.,2005)** and lower than recorded by **(El-Wakeil,1990;Urbina et al.,2003 and Patel et al.,2008).**

The detection of Shigella, Salmonella and E.coli among children in rural areas may be due to poor sanitary conditions, ingestion of food or drinking water contaminated by excreta of animals and or birds, presence of arthropods especially flies which acting as mechanical vector of such organisms. Moreover, E.coli is a normal inhabitant of the intestinal tract of man and animals and is the commonest organism that may cause severe outbreak of diarrhea and gastroenteritis. Concerning the pathogenic protozoa causing diarrhea.,Entamoeba histolytica was encountered in 3% of examined children and 30% of protozoal organisms but Giardia lambilia was found in 7% of them and represented 70% of protozoal organisms .These results were in accordance with **(El-Sherbini, 1992; Shebib *et al.,* 2003; Urbina *et al.,* 2003 and Rai *et al.,* 2005)** but lower results were recorded by **(El-Sheikh&El-Assouli 2001;Olesen *et al.,* 2005 and Abu-Elamreen *et al.,* 2008)**. The high incidence of Giardia lamblia was explained on the basis of easy transmission and greater chance of exposure to infection due to lack of hygiene, as well as faecal contamination of water supplies from animal sources and over crowding **(VanKeulen *et al.,* 2002& Shebib *et al.,* 2003) .** Regarding bacterial and protozoal enteropathogens detected in different domestic animals and birds in contact with diarrheal children ,Shigella spp., Salmonella spp., E.coli, Entamoeba histolytica and Giardia lamblia were detected with a percentage of 15.4%,15.4%,69.2%,25% and 75% from the totally detected enteropathogens in domestic

animals, while they were 0%,22.2%,77.8%,0%, and 100% respectively in birds in their houses **(Table 2).** These results were more or less similar to those mentioned by **(El-Taher & Ismail 1981;Gross,1983; Gilman et al., 1988& Taha, 1989).**

Concerning the identification of common bacterial pathogens isolated from diarrheal cases, as well as animals and birds revealed Shigella. sonni and Shigella flexneri, while Salmonella typhimurium and Salmonella enteritidis and Enteropathogenic E. coli (EPE. coli) were the most commonly identified. The previously mentioned results showed that the enteropathogens (bacteria & protozoa) isolated from diarrheal children were the same isolated from animals and birds in contact with children and this indicated the probability of cross infection between animals and birds to contact children in their houses. It has been observed that animals and animal excreta play an important role in diarrheal diseases.The environmental hazards of freely living animals and birds inside the houses with the continuous excretion of dropping on the ground which in turn contaminated the hands of crawling children who subsequently contracted the infection **(Last et al., 1980).** Also **Davies *et al* (1983)** mentioned that the licking of home pets to infants gives an easy opportunity to share protozoal infection. Moreover, Giardia lamblia from animals were the same structure to that of human **(Owen *et al*., 1979).** From the zoonotic point of view, animals act as reservoir of certain etiologic agents of infectious diarrhea as Entamoeba histolytica, Salmonella and different form of pathogenic Ecoli **(Engleberg *et al.,* 1982).**

The bacteria and protozoal enteropathogens detected in water supply (piped or pumped) were Salmonella, E.coli ,Entamoeba histolytica and Giardia lambilia with a percentage of 25%, 75%, 33.3% &66.7% respectively. These results in agreement with **(Lifshitz *et al.,* 1988)** who stated that water-borne bacterial infections include some strains of Ecoli, Salmonella & others. and **Salata & Aucott, 1992** who recorded that the transmission of amebiasis, giardiasis included water borne route. Moreover, children with practice of using hands during meals and the contamination of water used for hand washing before meals could be the source for infection **(Midzi *et al*., 2000).**

Table (3) demonstrated the distribution of diarrheal pathogens among 40 positive cases, it is evident that the occurrence of diarrhea was more in males (57..5%) than in females (42.5%).Similar findings were reported by **(El-Sherbini 1992, Shebib *et al.,* 2003 and Rai *et al.,* 2005).** It appears to be associated with more active and outdoor wandering nature of male children **(Ishiyama *et al*., 2001).** Concerning the age distribution among 40 casas,15 were

less than 1 year old ,16 were of 1-2 years old and 9 were of 3-5 years old with the corresponding percentages 37.5%,40% & 22.5%.These finding indicated that the majority of diarrheal cases existed among children in the first two years of life and this may be attributed to the poor defense mechanisms against infection in this age group and beginning of supplementary food beside breast feeding **(Shebib *et al.,* 2003).** Moreover, the infant trials to compensate the milk feeding by inserting the figers into the mouth at weaning which facilitate the ingestion of all forms of infective agents **(Yoeli *et al*., 1972).** Also **table (3)** indicates that only 4 cases of diarrhea with positive enteropathogens had blood in their stools ,2 cases were Shigella , 1 case was Salmonella and 1 case was Entamoeba histolytica. This is in agreement with **Riley *et al.,* (1983)** who stated that the infectious agents with bloody diarrhea are Shigella, Salmonella and Entamoeba histolytica due to their invasive nature to the intestinal wall. **Rennels and Levine (1986)** found that in shigellosis, atypical clinical course was biphasic beginning with watery diarrhea followed with in 24 hrs by tenesmus and dysentery, during the later stage, organisms invade the colonic mucosa causing inflammation and hemorrhage, while **Lifshitz *et al* (1988)** reported that a typical episode of Salmonellosis involved watery diarrhea accompanied by fever ,some infections could result in dysentery like disease with bloody stools. In addition to stools are blood stained with mucus and few leukocytes in severe Entamoeba histolytica infections **(Mahmoud, 1987).**

This study revealed that 34% of diarrheal children had vomiting at attendance and 42.5% of diarrheal cases with positive stool enteropathogens presented with vomiting. According to types of stool pathogens, higher frequency of vomiting was observed among bacterial cases (50%) than protozoal cases (20%). These are in accordance with **(Rennels & Levine 1986)** who found that vomition in cases of Shigella might associated with tenesmus and colic and **Buchino *et al*., (1984)** mentioned that vomiting might occur with Salmonella gastroenteritis.

It is noticed that only 6% of all diarrheal children presented with fever at attendance and all diarrheal cases with fever had positive stool bacterial enteropathogens and there is no fever presented in all protozal cases .This is in agreement with **Feigin & Stoller (1992)** who stated that fever was commonly associated with bacterial diarrhea and the protozoan diarrhea is not accompanied with fever except in some cases with amebic dysentery

Concerning animal contact, it is evident that the infection among diarrheal childern with history of animal contact was higher (75%) than in children with no history of animal

contact (25%). This indicates the importance of animal contact in spreading intestinal infections either bacterial or protozoal. Regarding water supply, infection among diarrheal children use pumped water sources was higher(90%) than children use piped water source (10%). Water forms a potential danger in rural areas, which is no in-door water source in most houses and water is obtained from either public tap or pump water that could be contaminated at several routes either from source contamination or during storage in the houses **(Soliman,1985).**

In conclusion, the results of this study revealed that the exposure of children to various enteric pathogens in rural areas may be indicative of poor access to quality drinking water, lake of knowledge about hazards of animals and or birds in houses and lack of education and personal hygiene. So the suggested measures for prevention and control of diarrhea among children in rural areas required sanitary disposal of human ,animal and birds excreta, personal hygiene, hand washing after defecation and before eating, Health education especially educate mothers about caring children under 2 years of age as it is the most susceptible age group and parents should be made aware of association with animals, thus hygienic precautions should be emphasized to control of animals or birds gaining access to contaminate living houses and lastly, protection of food and water supplies from faecal contamination.

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الملخص العربي

دراسات على بعض الميكروبات المعوية المشتركة المسببة للإسهال الحاد فى الأطفال فى محافظة القليوبية

لبنى محمد على سالم* و نجوى شعبان محمد على**

*** قسم الأمراض المشتركة – كلية الطب البيطرى.– جامعة بنها.**

**** قسم الطفيليات - كلية الطب– جامعة بنها.**

فى هذه الدراسة تم تجميع عدد ١٠٠ عينة براز لأطفال تقل أعمارهم عن ٥ سنوات مصابة بالإسهال الحاد من الوحدات الصحية فى بعض القرى الريفية بمحافظة القليوبية وبالإضافة إلى تجميع عدد ٦٦ عينة براز من الحيوانات والطيور المخالطة لهؤلاء الأطفال ، كما تم تجميع ٥٠ عينة من مياه الشرب. تم فحص هذه العينات للكشف عن وجود البكتريا أو البروتوزوا المسببة للإسهال وقد أسفرت النتائج عن وجود كلاً من الشيجلا (١٦,٧%) ، السالمونيلا (١٦,٧%) ، الإيشريشياكولاى (٦٦,٧%) ، الأنتاميباهستوليتكا (٣٠%) ، والجارديالامبليا (٧٠%) فى الأطفال ، بينما جاءت نسبة العزل لنفس المسببات فى الحيوانات المخالطة كالآتى ١٥,٤% ، ١٥,٤% ، ٦٩,٢% ، ٢٥% ، ٧٥% على التوالى فى حين كانت فى الطيور المخالطة صفر% ، ٢٢,٢% ، ٧٧,٨% ، صفر% ، ١٠٠% على التوالى ، بينما تم عزلها من مياه الشرب بنسبة صفر% ، ٢٥% ، ٧٥% ، ٣٣,٣% ، ٦٦,٧% على التوالى. هذا وقد أوضحت الدراسة أن نسبة الإسهال فى الذكور (٥٧,٥%) أعلى من الإناث (٤٢,٥%) وأن أعلى نسبة للإسهال فى الأطفال فى عمر سنتين وأن ارتفاع الحرارة كانت أكثر فى البكتريا بينما اختفت فى حالات البروتوزوا وأن ٥٠% من حالات البكتريا كانت تعانى من قىء أما كانت نسبته فى البروتوزوا ٢٠% وقد ظهر دم فى البراز فى بعض حالات الشيجلا ، السالمونيلا ، والأنتامييباهستوليتكا. هذا وقد وجد أن أغلب الحالات الإيجابية (٩٠%) كانت تستخدم مصدراً خارجياً للماء وكذلك كان أغلب الحالات (٧٥%) مخالطاً للحيوانات. وقد تم مناقشة مدى خطورة هذه الميكروبات وكذلك الطرق المقترحة لتجنب مخاطرها.