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Calves as a Reservoir of Some Diarrheagenic Agents for Human Contacts in El-Behira Province

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ABSTRACT

Calves remain one of the most important reservoirs of diarrheagenic agents to their human contacts. This study investigated the role of calves in transmission of *Salmonella*, *Escherichia coli* and *Cryptosporidium* spp., to human. Fecal samples collected from 120 diarrheic calves up to 6 months of age and 100 diarrheic stool samples collected from human contacts from El-Behira province, Egypt were examined. The detection of *Salmonella* and *E. coli* was done by conventional bacteriological methods, while the *Cryptosporidium* spp. oocyst was screened by modified Ziehl-Neelsen acid-fast microscopy. It was found that the detection rates of *Salmonella, E. coli* and *Cryptosporidium* spp. in diarrheic calves were 10, 20.8 and 9.2%, respectively while in human contacts were 5, 17 and 7%, respectively. Serological identification of isolates of *Salmonella* clarified the presence of *S. Enteritidis, S. Typhimurium, S. Meleagridis, S. Anatum* and *S. Lagos* while that of *E. coli* revealed the presence of serotypes O_{158}, O_{18} and O_{114}. Statistical analysis showed that there was a significant association between the rates of isolation of *Salmonella* in relation of different age groups in calves while there was no significant association between other variables. The results of this study confirmed the significant role of diarrheic calves as sources of human infection with diarrheagenic agents in El- Behira province that necessitate the need for establishing a plan for control of infectious diarrhea in calves.

1. INTRODUCTION:

Diarrhea is a major problem in livestock production in Egypt and throughout the world (Ibrahim, 2007). Infectious diarrhea is the most significant cause of morbidity and mortality in neonatal dairy calves throughout the world (Lanz et al., 2008). Calves are at greatest risk of developing diarrhea within the first month of life and the incidence of diarrhea decreases with age (Garcia et al., 2000). The pathogens most commonly incriminated in neonatal calf scours include protozoal (*Cryptosporidium parvum*) and bacterial pathogens (Enterotoxigenic *Escherichia coli* and *Salmonella* spp.) (Waltner-Toews et al., 1986). *E. coli* normally inhabits the intestinal tract of man and animals with a potential to produce from mild to severe pathological conditions and it is considered one of the major causes of diarrhea in cattle. Enterotoxigenic *E. coli* (ETEC) expressing K99 (F5) fimbriae and heat stable type Ia (STa) toxin is the leading bacteria causing calf diarrhea (Osman et al., 2013). *Salmonella* infection has a wide variety of clinical symptoms ranging from asymptomatic to clinical salmonellosis. Acute diarrheal disease is most common with *S. typhimurium* and systemic disease is associated with *S. Dublin*. Calves less than 3 weeks of age are commonly infected by *Salmonella*. The lesions frequently observed in affected calves involve the

pseudomembrane on the mucosa of the small intestine as well as enlargement of the mesenteric lymph nodes (Mead et al., 1999). Cryptosporidium is mostly prevalent and widespread in calves from 4 days to 4 weeks (De La Fuente et al., 1999 and Abd-El-Wahed, 1999).

Concerning human, diarrheal diseases are a major cause of illness and death in low and middle-income countries, where there are over 1.5 billion diarrhea cases that occur annually among children less than 5 years old, resulting in nearly 700,000 deaths (Walker et al., 2013). According to the World Health Organization (WHO), diarrheal diseases are the second leading cause of death (~760,000 per year) in children <5 years of age. Recent studies suggested that diarrheal diseases are the leading cause of childhood deaths in developing countries. Although the contribution of zoonotic pathogens to human diarrheal disease is significant (Zambrano et al., 2014), these pathogens are often overlooked, and their detection may be hindered by patterns of seasonality (Lal et al., 2012). In Egypt more than 50% of deaths among children lower than two years are due to diarrheal diseases (Ministry of Health, 1985).

The present study aimed to investigate the prevalence of *Salmonella*, *E. coli* and *Cryptosporidium spp.* in diarrheic calves and human contacts in El-Behira province.

2. MATERIALS AND METHODS:

2.1. Samples:

Fresh fecal samples were collected from 120 diarrheic calves up to 6 months of age at El-Behira province. In addition, 100 samples of human stools were randomly collected from human in contact with diarrheic calves. All samples were transferred in an ice box to the laboratory with minimal delay for bacteriological examination.

2.2. Isolation and identification of *Salmonella* (ISO) 6579 (2002):

Briefly, each sample was enriched by inoculation in 225 ml of buffered peptone water (BPW), after incubation at 37°C for 16 to 20 h, 0.1 ml was inoculated into a tube containing 10 ml of Rappaport-Vassiliadis (RV) broth and was incubated at 37°C for 18 to 24 h. Each selective enrichment broths were streaked onto xylose lysine deoxycholate (XLD) agar. Six biochemically confirmed *Salmonella* isolates were serologically identified on the basis of somatic (O) and flagellar (H) antigens by slide agglutination using commercial antisera following Kauffman–White scheme (Popoff et al., 2004) at the Serology Unit, Animal Health Research Institute, Dokki, Egypt.

2.3. Isolation and identification of *E. coli* (Quinn et al., 2011):

All samples were inoculated into nutrient broth for 24h at 37°C aerobically. After that, swabs were streaked onto MacConkey agar and Blood agar. Lactose fermented colonies were randomly selected from each isolate and confirmed to be E. *coli* by standard biochemical tests. Colonies were subculture onto Eosin methylene blue agar for 24 h at 37°C for aerobically characteristic metallic sheen colonies of E. *coli*. Six isolates of *E. coli* were identified serologically by slide agglutination test using standard polyvalent and monovalent *E. coli* antisera according to Edwards and Ewing, (1986) at the Serology Unit, Animal Health Research Institute, Dokki, Egypt.

2.4. Detection of Cryptosporidium oocyst by modified Ziehl-Neelsen stains (Casemore, 1985):

A thin smear from feces or stool was prepared by adding a drop of water to small pieces of feces or stool, then mixed well and spread on the glass slides with the help of platinum loop. The smear was allowed to air dried and then fixed with absolute methanol for 5-10 minutes. The fixed smears were covered with modified Ziehl-Nelseen acid fast stain (Carbol Fuchsin) for 2 minutes. The smears were rinsed under tap water, then decolorized with 10% sulphoric acid for 1 minute, then rinsed with tap water. Finally, the smears were covered with counterstain (methylene blue) for approximately 1 minute, then rinsed with tap water, air dried and examined microscopically by oil immersion lens X100 to detect *Cryptosporidium* oocysts. The oocysts will appear as densely stained red bodies clearly distinguishable against a blue back ground.

2.4. Statistical analysis:

Frequencies were subjected to Qui^2 analysis to assess the significance between different variables (SAS, 2004).

1. RESULTS and DISCUSSION

The rate of isolation of *Salmonella* from diarrheic calves and humans in relation to age groups was recorded in Table (1). It was clarified that the total rate of isolation of *Salmonella* in calves and human was 10 and 5%, respectively.

The detection rate of *Salmonella* in calves was in agreement with the findings of Atwa et al., (2012) (9%) while it was lower than those of Moussa et al. (2010) (43.53%), Izzo et al., (2011) (23.8%), Youssef and El-Haig, (2012) (18.66%) and El-Seedy et al., (2016) (18.1%). On contrary, it was higher than that reported by Haggag and Khaliel (2002) (4%) and Younis et al. (2009) (4.09%). Also, it was found that there was a significant association between the prevalence of *Salmonella* in the age group (≤ 2 months) (16.36%) and the age group (> 2 - 6 months) (4.62%).

Moreover, the detection rate of *Salmonella* spp. in human stool samples in the present study was 5% (Table, 1). On the other hand, statistical analysis showed no significant association between the prevalence of *Salmonella* in the age group (≤ 15 years) (4.65%) and the age group (> 15 years) (5.26%). In addition, serological identification of four isolates of *Salmonella* obtained from calves revealed the presence of *S. Enteritidis*, *S. Typhimurium*, *S. Meleagridis* and *S. Anatum* while that of two isolates obtained from human samples clarified the presence of *S. Enteritidis* and *S. Lagos* (Table, 2).

This obtained results agreed with the finding of Samaha and Nossair, (2012) who examined 50 human stool samples and found that 2 out of 50 (4 %) of examined stool samples were Salmonella positive and the serological identification revealed the presence of *S. Enteritidis* while it was lower than that reported by Awadallah et al., (2013) (8.7%).

Differences of the prevalence rates of *Salmonella* in diarrheic calves in comparison to the previous studies could be explained in the light of species and geographical locations and hygienic measures, and these factors significantly influence the prevalence of salmonellosis in calves (Younis et al., 2009).

Identification of the possible causative agent in outbreaks of diarrhea is important to allow targeted preventative measures, such as vaccination, and identification of possible risk factors or sources of infection (Izzo et al., 2011). *E. coli* and *Salmonella* are the most common identified pathogens in scouring calves less than 2 months of age (Achá et al., 2004).

The rate of isolation of *E. coli* from diarrheic calves and humans in relation to age groups was recorded in Table (3). It was clarified that the total rate of isolation of *E. coli* in calves and human was 20.8 and 17%, respectively. Statistical analysis clarified non-significant difference between the different age groups under investigation in both calves and human contacts.

Table (1): The rate of isolation of *Salmonella* from diarrheic calves and humans in relation to age groups

Factors	No. of examined samples	Positive		Chi-square
		No.	%	
***Salmonella* in calves**				
≤ 2 months	55	9	16.36	(4.68)*
> 2 - 6 months	65	3	4.62	
Total	120	12	10.0	
***Salmonella* in human contacts**				
≤ 15 years	43	2	4.65	(0.02) NS
> 15 years	57	3	5.26	
Total	100	5	5.0	

* Significant at (P< 0.05) NS Not significant

Table (2): Serotypes and antigenic structure of *Salmonella* serogroups recovered from the examined samples of calves and human

Source of samples	*Salmonella* serotypes	Antigenic structure			Group
		Somatic (O) antigen	Flagellar (H) antigen		
			Phase I	Phase II	
Calves	*S. Enteritidis*	1,9,12	g,m	–	**O:9 (D1)**
	S. Typhimurium	1 , 4 ,[5],12	I	1,2	**O:4 (B)**
	S. Meleagridis	3,{0}{15}{15,34}	e, h	1,w	**O:3,10 (E1)**
	S. Anatum	3,{0}{15}{15,34}	e, h	1,6	**O:3,10 (E1)**
Humans	*S. Enteritidis*	1,9,12	g,m	–	**O:9 (D1)**
	S. Lagos	1,4, ,[5],12	I	1,5	O:4

Table (3): The rate of isolation of *E. coli* among diarrheic calves and human contacts in relation to age groups

Factors	No. of examined samples	Positive		Chi-square
		No.	%	
***E. coli* in calves**				
≤ 2 months	55	14	25.45	$(1.31)^{NS}$
> 2 - 6 months	65	11	16.92	
Total	120	25	20.8	
***E. coli* in human contacts**				
≤ 15 years	43	8	18.60	$(0.13)^{NS}$
> 15 years	57	9	15.78	
Total	100	17	17.0	

NS Not significant

Table (4): Serotypes and antigenic structure of *E. coli* serogroups recovered from the examined samples of calves and human

Source of samples	*E. coli*	
	Serotypes	No.
Calves	O_{18}	2
	O_{158}	3
Humans	O_{114}	1

Table (5): The rate of detection of *cryptosporidium* from diarrheic calves and human contacts in relation to age groups

Factors	No. of examined samples	Positive		Chi-square
		No.	%	
Cryptosporidium in calves				
≤ 2 months	55	7	12.72	$(1.54)^{NS}$
> 2 - 6 months	65	4	6.15	
Total	120	11	9.2	
Cryptosporidium in human contacts				
≤ 15 years	43	5	11.62	$(2.48)^{NS}$
> 15 years	57	2	3.50	
	100	7	7.0	

NS Not significant

The detection rate of *E. coli* in calves was similar to those obtained by Joon and Kaura (1993) in India (23%) while it was lower than the findings of Haggag and Khaliel, (2002) (82%) and Osman et al., (2013) (63.6%), Hassan, (2014) (50%) and El-Seedy et al., (2016) (75.6%), while it was higher than those of Viring et al. (1993) in Sweden (11.5%) and Azzam et al. (2006) (5.4%). The differences of the prevalence rates of *E. coli* in diarrheic calves may be attributed also to the geographical locations and management practices as well as hygienic measures.

Regarding the frequency of detection of *E.coli* in human contacts (17 %), it was nearly similar to those reported by Byomi et al., (2017) (14.2%). This is not a surprise because it has been reported that EPEC strains and other pathogenic and toxigenic strains of *E. coli* were more prevalent in developing countries where poor hygienic practices are more prevalent than in developed countries. On contrary, it was lower than that that recorded by Awadallah et al., (2013) (64%) and EL-Alfy et al. (2013) (31.4%) while it was higher than that reported by Diab, (2014) (7.2%). The variation in the prevalence rates of *E. coli* from one study to another may be accounted for differences in number and health status of human cases, localities and hygienic measures.

In addition, serological identification of five isolates of *E. coli* obtained from calves revealed the presence of O_{18} (2 isolates) and O_{158} (3 isolates) while that of one isolate obtained from human samples clarified the presence of O_{114} (Enteropathogenic *E. coli*) (Table, 4).

E. coli species comprise intestinal and extraintestinal pathogens. The intestinal pathogens are also known as diarrheagenic *E. coli* (DEC) of which six categories have been characterized: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggreg-ative *E. coli* (EAEC), and diffusely adhering *E. coli* (DAEC) (Nataro and Kaper 1998). Recently EPEC has been divided in typical EPEC (t-EPEC) and atypical EPEC (a-EPEC) (Trabulsi et al., 1996).

Regarding *Cryptosporidium* spp. detection in calves and humans, the obtained oocysts were

morphologically confirmed as *Cryptosporidium* spp. oocysts similar to those described in livestock animals and humans in many previous reports (Fayer and Xiao, 2008, Hassanain et al., 2011 and Amer et al., 2013). In calves, only 9.2% of faecal samples were positive microscopically for *Cryptosporidium* oocyst (Table, 5). This result was lower than Ghoneim et al., (2017), Amer et al., (2010) and El-Seify et al., (2012) who found the prevalence of *Cryptosporidium* oocysts in calves in Egypt is 30.4, 30.2 and 34.1%, respectively by microscopical examination. Our findings agree with Mahfouz et al., (2014) who reported overall prevalence of 7.07% in cattle and nearly similar to those reported by Helmy et al., (2013) who reported 15%. Regarding *Cryptosporidium* oocyst detection in human stool samples, they were detected in 11.6% of stool samples of children less than 15 years old by microscopical examination, this result much lower than those reported by Ghoneim et al., (2017) who recorded a prevalence of 27% in children (6-12 years). The overall prevalence in our study was 7% which is compatible with the findings of Helmy et al., (2013) who reported 6.7% prevalence rate. The variation of *Cryptosporidium* prevalence between this study and other previous studies may be attributed to differences in age, breed of calves, season of sample collection, environmental settings, husbandry management as well as tools used for detection of *Cryptosporidium* oocysts in fecal samples (Ibrahim et al., 2016).

On conclusion, calves still constituted a major reservoir for diarrheagenic agent to human so a suggestive control plan should be established to control investigated agents in calves in order to protect human contacts.

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