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ROLE OF ENTERIC BACTERIA IN THE AETIOLOGY OF NEONATAL BUFFALO CALVES DIARRHOEA .

A. I. TANIOS ; E. R. ZAKI * ; R. EL-SHERNOBY** MONA A. EL-SHABRAWY*** and R. S. SELEIM**

Serology Unit , Animal Health Research Institute .
*Department of Buffaloe Diseases , Animal Health Research Institiute
**Microbiology Department , Animal Health Research Institute .
***National Research Centre , Dokki , Cairo , Egypt .

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SUMMARY

A total of 66 faecal samples were collected from diarrhoeic calves , aged from birth up to 2 months in El-Kalioubia and Giza Governorates. Bacteriological examination of faecal samples revealed that 23 samples were positive with an incidence rate of 34.85 % . Escherichia coli was the highly prevalent microorganisms with an incidence of isolation 34.85 % , followed by salmonella species and Yersinia enterocolitica (13.64 % and 7.58 % respectively). Seven cases of diarrhoeic calves harboured mixed infection (10.6 %) while 16 cases were single infection . All isolates were subjected to serological examination and the results showed that 3 isolates of Escherichia coli were typed by O:k serotypes including O111 (2 isolates), O119 (1 isolate) while 6 isolates were typed by K99 antisera. Nine isolates of Salmonella were typed as Salmonella typhimurium (5 isolates), Salmonella dublin (3 isolates) and Salmonella enteritidis (1 isolate). Five isolates of Yersinia enterocolitica were typed as O3 (3 isolates) and O9 (2 isolates) . Pathogenicity test was carried out using suckling mouse technique and the results showed that 12 isolates of Escherichia coli and all isolates of Yersinia enterocolitica were enterotoxigenic .

INTRODUCTION

Diarrhoea in calves is a syndrome of great aetiological complexity , in addition to the influence of varied environmental , managemental , nutritional and physiological factors .

The infectious agents capable of causing diarrhoea in the neonatal calves are numerous , the most important bacterial enteropathogens are enterotoxigenic Escherichia coli , salmonellae and Yersinia enterocolitica (Snodgrass et al. , 1986).

The role of bacterial agents is more complex in causing diarrhoea because they are associated with many attributes as certain proteins which has toxic and lethal effect on the host cells at a exquisitely low concentration like the enterotoxins produced by Escherichia coli . Other non proteinaceous toxic bacterial substances like the endotoxins (LPS) which are mainly lipoproteins and they are biologically active at much higher concentrations . Other proteins which are considered as major virulence factors as adhesins responsible for adhesion of the bacteria to the host cell like K99 , F41 , F17, F165 , 987 (Ganaba et al. , 1995 and Vazquez et al. , 1996) , many of these adhesins act as haemolysins and immunoglobulin proteases (Falzano et al. , 1993).

Salmonellae produce highly virulent enterobacterins , cytotoxins and siderophores which destruct the host cells and causes septicaemia. Moreover , the LPS is another factor which play a role in antiphagocytic capabilities (Yolkoyama et al. , 1998) . Invisins are highly virulent proteins secreted by Yersinia enterocolitica and is responsible for attachment of the bacteria on host cells as well as for tissue invasiveness . Other antigenic proteins V and W are responsible for resistance to intracellular killing after invasion and after intraphagocytic multiplication (Finlay and Stanley , 1997) .

The aim of this study was directed mainly to determine the presence and prevalence rate of some enteric pathogens of public health importance as Escherichia coli , salmonellae and Yersinia enterocolitica from untreated calves with diarrhoea before treatment .

MATERIAL AND METHODS

Samples :

A total of 66 faecal samples were collected from untreated buffalo calves aged from few days up to 2 months in El-Kalioubia and Giza Governorates during the period from October, 1998 up to January, 1999. The calves were suffering from profuse watery diarrhoea tinged with blood , fever and anorexia . Samples were collected before their treatment with different medication .

Isolation and identification :

Direct cultures onto MacConkey agar , blood agar, Minica polyvitex agar, Salmonella Shigella Xylose lysine dextrose and Cefsulodine Irgasan Novobiocin (CIN) agar for isolation of salmonellae , Escherichia coli and Yersinia enterocolitica The plates were incubated at 37(C for 24 ñ 48 hours for salmonellae and Escherichia coli and at 25 (C for 48 hours for the isolation of Yersinia enterocolitica (Adesiyun et al. , 1992) .

Indirect culturing in Selenite F-broth and tetrathionate broth as pre-enrichment fluid media and incubated at 37(C for 16 ñ 18 hours and an inoculum was streaked onto MacConkey and Salmonella Shigella agar for isolation of salmonellae . For Yersinia enterocolitica , cold enrichment method (Greenwood et al. , 1975) was applied on phosphate buffer saline at 4(C for 3 weeks and subculture onto Cefsulodine Irgasan Novobiocin (CIN) agar .

At least 3 suspected well separated colonies were subjected to oxidase and catalse tests (Konneman et al. , 1992) . Oxidase negative and catalse positive colonies were tested with API 20E Kit System (API # 2010) .

Isolates were serotyped using the available polyvalent and monovalent antisera by slide agglutination test according to Edwards and Ewing (1972). Escherichia coli isolates were identified serologically by using Wellcome Escherichia coli diagnostic antisera . Standard type culture strain of Escherichia coli (431 (101 : K30) 99 : F41 : N.M.) kindly supplied from National Animal Disease Center, USA used in the preparation of K99 antiserum. Serological identification of suspected salmonella serovars was carried out according to Kauffman-White scheme using Salmonella polyvalent and monovalent ìOî and ìHî antisera obtained from Wellcome Research Laboratories, Bechanham, England. Yersinia enterocolitica O-grouping antisera were obtained from DENKA SEIKEN Co. LTD , Tokyo , Japan.

Pathogenicity test :

All Escherichia coli and Yersinia enterocolitica isolates were tested for its ability to produce heat stable toxin by the infant mouse test (Pai and Morse , 1978 and Robins-Browne et al. , 1993) .

RESULTS

The faecal samples collected from diarrhoeic calves (66) showed that only 23 (34.85 %) were positive for enteric pathogens (Table 1) . The most prevalent isolates were Escherichia coli 16 cases (24.24 %) , followed by Salmonella 9 cases (13.64 %) and Yersinia enterocolitica 5 cases (7.58 %) (Table 2) .

Table (1): Incidence of enteric pathogens in faecal samples.

Total No Of samples	Positive		Negative	
	No.	%	No.	%
66	23	34.85	43	65.15

Table (2): Incidence of different pathogens in positive samples .

Isolate	No./total	%
Escherichia coli	16/66	24.24
Salmonella	9/66	13.64
Yersinia enterocolitica	5/66	7.58

The recovery of these pathogens was in single infection pattern in 16 cases (24.24 %) and in mixed infection pattern in 7 cases (10.61 %) (Table 3) . The distribution of isolates in correlation to age revealed that Escherichia coli was recovered as single infection in calves 0 -2 weeks of age while salmonellae and Yersinia enterocolitica were revealed from older calves at the age of 3 -4 weeks and 7 - 8 weeks respectively (Table 3).

Serotyping of the isolates showed that 3 Escherichia coli were typed by O:K antisera , 2 isolates belonged to O, 119 K69 and one isolate belonged to O111 : K58 . On the other hand , all isolates were typed by K99 hyperimmune sera and the results showed that 6 isolates were $K99^{+}$. Twelve isolates were enterotoxigenic with an incidence rate of 75 % (Table 4) .

Table (3): Pattern of isolation of different enteric pathogens in correlation to age .

Isolate	Type of infection	No. of isolates	%	Age (week)
Escherichia coli	Single 16 cases (24.24%)	9	13.6	0-2
Salmonella		5	7.6	3-4
Yersinia enterocolitica		2	3	7-8
Escherichia coli + *Salmonella*	Mixed 7 cases (10.61%)	4	4	7-8
Escherichia coli + *Yersinia enterocolitica*		3	4.6	7-8

Table (4): Serotyping and enterotoxin production of *Escherichia coli* isolates.

Serovar	O119:K69	O111:K58	Untypable	Total	
				No.	%
O:K antigen	2	1	13	3/16	18.75
K99	1	0	5	6/16	37.5
Enterotoxin	2	1	9	12/16	75

Table (5): Salmonella serovars isolated from diarrhoeic calves.

Salmonella serovar	No.	Antigenic scheme		
		O antigen	Phase	
			I	II
Salmonella typhimurium	5/9	1, 4, 5, 12	i	1, 2
Salmonella dublin	3/9	1, 9, 12	g, p	
Salmonella enteritidis	1/9	1, 9, 12	g, m	1,7

Table (6): Serovars and enterotoxin of *Yersinia enterocolitica* isolated from diarrhoeic calves.

	serovar	Isolated No.	No. of positive for enterotoxin
Yersinia enterocolitica	O3	3	3/5 (60%)
	O9	2	2/5 ((40%)

Using the polyvalent O , H as well as the monovalent antisera for Salmonella serotyping , 5 Salmonella typhimurium Gp B: 1,4,5,12 (i), 1,2 and 3 Salmonella dublin Gp D:1,9,12: g,p and 1 Salmonella enteritidis Gp C: 1,9,12 : g,m : 1,7 were identified serologically (Table 5) .

The five isolated Yersinia enterocolitica strains belonged to serovar O3 (3/5 ; 60 %) and O9 (2/5 ; 40 %) , all serovars were positive for enterotoxin production (Table 6) .

DISCUSSION

Neonatal diarrhoea in calves is a complex disease of significant economic impact due to the heavy losses in fatality cases . The infection can be caused by a variety of infectious agents proliferating alone or in combination with other microorganisms (Acres et al. , 1979) .

The objective of this study was to determine the prevalence rate of different enteric bacterial pathogens associated with diarrhoea among newly born calves aged 0 - 2 months old. Faecal samples were collected from 66 untreated diarrhoeic calves and examined for the presence of Escherichia coli , salmonellae and Yersinia enterocolitica.

The results showed that 23 calves (34.85 %) were positive for the isolation of microorganisms

under choice of this article (Table 1). Out of these, 16 cases were Escherichia coli (24.24 %) , salmonellae (9 cases ; 13.64) and Yersinia enterocolitica (5 cases ; 7.58 %) (Table 2) . These findings are agree with Srivastava and Sharma (1983) who studied the pathogenic bacteria in diarrhoeic calves (4-30 days) and stated that Escherichia coli played a major role in causing the infection (36.2 %) followed by salmonellae which was incriminated for 10.8 % of the cases . Myers et al. (1984) isolated Yersinia enterocolitica from faeces of healthy beef calves in U.S.A. Tanios (1994) isolated Yersinia enterocolitica from apparently healthy cows with an incidence of 2.5 % while Davey et al. (1983) found this organism or related species in 50 % of the cows examined in Scotland. The difference in the isolation rate may be related to differences in sampling protocols, methodology and climatic variations .

In this study , it is interesting to mention that Escherichia coli was prevalent in calves aged from 0 - 2 weeks and salmonella species in 3 - 4 weeks old calves , while Yersinia enterocolitica was much higher in older calves (7 - 8 weeks) . This observation go hand to hand with that mentioned by Gunter and Shuldes (1985) who correlated the isolation of Escherichia coli and salmonellae to the age and explained that factors as the immune status of the animal and administration of colostrum . It was observed in this work that single infection of Escherichia coli or salmonellae were predominant at the first days of birth which may be attributed mainly to the effect of bacteriocins expressed by salmonellae which antagonise the multiplication and colonization of Escherichia coli. Shin et al. (1994) stated that the infection with Escherichia coli preceeds the infection with salmonella at the young ages and as the animal gets older the endothelium of the intestine develops resistance to the bacterial adhesins mainly the K99 whereas the salmonellae starts to dominate over the other pathogens in the older calves (2 - 8 months) when exposed to stress factors as transportation , confinement or intensive management.

Serological examination of all isolates showed high prevalence of untypable Escherichia coli serovars (Table 4) and this may attributed to the limited available number of O:K serotypes. Six isolates of Escherichia coli (37.5 %) were $K99^+$ and this result agrees with that mentioned by Fagan et al. (1995) who recorded a much higher incidence of Escherichia coli isolated from diarrhoeic calves had the K99 antigen pilus . On the other hand , Ganaba et al. (1995) found no isolate for K99 antigen from calves with diarrhoea. This variable rates of results may be attributed to the differences in age of calves examined and the husbandry conditions. Concerning the O:K serotypes, O119 : K69 was isolated from two calves while O111 : K58 was isolated from one calf . Vorster et al. (1994) mentioned that O119 : K69 play an important role in 4 outbreaks of diarrhoea in calves. Also O111 , was implicated epidemiologically as associated agent of calf diarrhoea

(Erganis et al. , 1989) .

Regarding salmonella serovars isolated in this study (Table 5) , Salmonella typhimurium was isolated from 5 cases , Salmonella dublin from 3 cases while Salmonella enteritidis was isolated from one calf . These serovars were also recorded by many authors such as (Fetisova, 1989 ; Konency et al., 1992 and Adesiyun et al., 1993) .

Serovars and enterotoxin production of Yersinia enterocolitica isolated from diarrhoeic calves (Table 6) . Two serovars were identified serologically O3 (3 isolates) and O9 (2 isolates) . These two serovars were also reported by Swaminathanh et al. (1982) and Adesiyun et al. (1992). Serovar O3 was isolated from two community outbreaks in Japan (Asakawa et al. , 1973) and serovar O9 was implicated in an outbreak in Finland (Toivanen et al. , 1973) . In this study , all five isolates of Yersinia enterocolitica were positive for enterotoxin production and this results agree with that reported by Kwaga and Iversen (1992) .

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