

SOME BACTERIAL CAUSES OF DIARRHOEA IN CALVES

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SUMMARY

Two hundred and fifty fecal samples from diarrhoeic calves (1-30 days old) at Menufia Governorate were subjected to bacteriological examination . Results revealed the isolation of E.coli., Sal. spp, Kleb.ozaenae, Proteus.vulgaris and Enterococcus . faecalis with incidence percentages of (28% , 2% , 3.2% , 1.6% and 1.2% respectively) . In vitro antimicrobial sensitivity of the isolates revealed that they were more sensitive to Norfloxacin , Gentamicin and Colistin sulphate.

INTRODUCTION

Diarrhoea in calves during the neonatal period (i.e. the first four weeks of life)is very common . Various microbiological agents have been associated with the syndrome and the fate of the affected calves is dependent on the severity of the biochemical change (Srivasta and Sharma 1983).

The economic loss occasioned by neonatal disease in young calves has been recognized for many years and it's apparent from bacteriological examination of such calves that there are many causes of this loss , however colibacillosis infection caused by *E.coli* is by far the most common (Lovell and Hughes 1935) .

E.coli has been associated with neonatal calf diarrhoea . particular strains of *E.coli* are more effective than other at inducing disease (Roy et al ., 1986).

Specific serotypes are the causative agents of colibacillosis , certain serovars are associated with the development of diarrhoea and dilatation of an isolated loop of intestine (Moon 1974).

The role of *E.coli* as apathogen is well known , and many of *E.coli* isolates have been associated with awide variety of diseases in animals.

These pathogenic *E.coli* include enterotoxigenic *E.coli* (ETEC) and enteropathogenic , enteroinvasive , and uropathogenic *E.coli* (Levin 1987).

Predisposing factors play an important role in the establishment of calf diarrhoea , example weakness of calf at birth , poor management , stress , faulty nutrition and lack of specific immunity (Rosenberger , 1987 and mottelib , 1981).

Diarrhoea caused by Salmonella is described under Salmonellosis (Steenkamer, 1966). *Kl. Ozaenae*, *Pr. Vulgaris* and *St. faecalis* play a role in calf diarrhea (Ramaswamy et al., 1992). Antibacterial biogram was important for treatment and control.

This study aimed to detect the bacterial causes of diarrhea in neonatal calves at Menufia Governorate through isolation, identification and evaluation of the antimicrobial sensitivity of the isolated bacterial agent.

MATERIALS AND METHODS

Fecal samples : Two hundreds and fifty Fecal samples were collected from diarrhoeic (buffaloe and cattle calves) (1-30 days olds). Fecal samples were collected in sterile corked tubes and transferred immediately to the laboratory (Animal Health Research Institute – Shebin El-kom) in ice box within few hours for bacteriological examination.

For isolation of gram (-ve) enteric bacteria from the obtained fecal samples. A loopful from each fecal sample was inoculated at 37c for 24 hours. Loopfuls from one peptone water tube was cultured on each of MacConkey's agar and blood plates (Cruickshank et al., 1975), then incubated at 37c for 48 hrs (Makie and McCartney, 1989). Loopfuls from the other peptone water tube were cultured on selenite-F-broth (Oxoid) at 37c for 48 hrs, then subcultured on S-S agar plates (Oxoid) and brilliant green phenol-lactose agar plates. Suspected colonies were morphologically examined and biochemically identified using API test strips. For isolation and identification of Gram positive bacteria, sheep blood agar plates were inoculated from peptone water tubes. The growing colonies were purified and biochemically identified according to (Holt et al. 1994).

Meanwhile the suspected *E.coli* isolates were identified serologically according to (Edwards and Ewing 1972) using specific polyvalent and monovalent antisera.

Antibiograms of the bacterial isolates was done according to (Fingold and Martin 1982) by using Muller Hinton agar media. Anti bacterial sensitivity discs were employed, the following antibiotic discs (Oxoid) were used. norfloxacin (10µg), flumequin (30µg), streptomycin (10µg), gentamicin (10µg), neomycin (30µg), colistin sulfate (10µg), tetracycline (30µg), erythromycin (15µg), and trimthoprim- sulfamethoxazole (1.25 + 23.75µg)

RESULTS

From Table (1): It is clear that the most prevalent isolates obtained from diarrhoeic calves were *E. coli* with an incidence of 28% followed by *Kleb.*

ozaenae , *Sal.spp*, *Proteus. vulgaris* and *Enterococcus.faecalis* with an incidence of (3.2% , 2% , 1.6% and 1.2 %respectively).

Table (2): demonstrates that the isolated bacteria were predominant at age (1-15 days old) with an incidence of 25.6% and 10.4% at (16-30 days old)

Table (3): Revealed that high incidence of single infection cases (33.6) and lower in mixed infection cases (2.4%)

Table (4) indicates mixed infection cases were : *E.coli* with *Kleb. ozaenae* , *E. coli* with *Sal spp* and *E.coli* with *Proteus. Vulgaris* with an incidence of 1.2% ,0 .8% and 0.4% respectively

Table (5): Shows the serotyping of *E.coli* strains isolated from the diarrhoeic calves . The following serotypes were identified as

O26:k60 , O111:k58 , O119:K69 , O86:K61 and untypable strains with percentage of 9.6%,5.2%,6.8%,3.2% and 3.2% respectively.

Table (6-8): Shows the results of the antibiogram of the different isolates in which Norfloxacin , Gentamicin and Colistin sulphate were the most effective antibiotics on the different types of the isolates *E.coli* , *Kleb. ozaenae* and *Sal.spp* with the percentage of 92.8% , 75% and 80% respectively for Norfloxacin and 85.7%, 62.5% and 60% respectively for Gentamicin and 78.6% , 50% and 80% respectively for Colistin sulphate .

DISCUSSION

Neonatal calf diarrhoea causes high economic losses not only due to high mortality rate but also produce decrease in weight gain which resulted from high losses in body fluids which exceeded 10% .

This study indicated the role played by some bacterial agents in the occurrence of calf diarrhoea .

The study gave informations about the antibiogram resistance of the isolated microbes which may help in protection of calves .

Neonatal calf diarrhoea is a syndrome referred by many *investigators* (*Fouad et al.*, 1976 ; *El- Sayed* 1987 and *Mettias* 1987).

The result given in table (1) revealed that the incidence of *E.coli* in the examined fecal samples was relatively high (28%) followed by *Sal.spp* , *Kleb.ozaenae* , *Proteus. vulgaris* and *Enterococcus. faecalis* were (2%,3.2%, 1.6%, and 1.2%, respectively).

E.coli was the main cause of diarrhoea affecting newly born calves younger than one week (*Jayappa et al .,1984*) .

In this concern (*Wilson and Miles* 1984)reported that *E.coli* O111 : K 58 possessed an adhesive properties .

Also (*Niazi and Refai* 1988)demonstrated that the *E. coli* O 111 : K 58 exhibited not only adhesive property but also enterotoxigenic property.

Also shown in table (2) : isolated bacteria especially *E.coli* were in higher incidence between (1-15days old) due to high susceptibility of calves in this age for infection , this observation agree with results obtained by *Ahmed (1975) ; Mettias (1987) and Nornin et al (1991)*.

As shown in tables (1-3) revealed that *Sal. spp.* play a role in calf diarrhea and this agree with the result obtained from *Steenkamer (1966)* observed that *Sal. typhimurium* appeared the main cause of Salmonellosis in calves.

Table (5) revealed the most important *E.coli* strains which isolated from diarrheic calves were O26, O119 and O 111 with incidences of (24% , 17% and 13% respectively) . These results agreed with *Roy et al . , 1986 ; Farid et al . , 1976 and Ahmed et al.,1980*.

In this study *Proteus. vulgaris* isolated with incidence of 1.6% and this agree with (*Sinha and Narayan 1968*) who isolated *Proteus.vulgaris* from calves suffering from diarrhoea and enteritis.

It is hoped that data obtained will Provide a scientific and systemic control of this major economic disease . A total of 83 isolates from examined diarrhoeic calves were tested for their in vitro susceptibility to 9 antibacterial agents using disc diffusion test . The antibiogram of the pathogens could be variable from place to place and from case to another . This may be explained due to the wide use of chemotherapeutic drugs (*Tanaka , 1970*).

Tables (6-8) shows the results of antibiogram of the different isolates in which Norfloxacin , Gentamicin and Colistin sulphate were the most effective antibacterials .These results agree with (*Signorini et al .,1992*).

From the above mentioned discussion we could conclude that diarrhoea problem in neonatal calves at Menufia Governorate may be partially attributed to some bacterial agents especially *E.coli*, *Sal.spp.*,*Kleb. Ozaenae* and *Enterococcus.faecalis*

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Table (1): Incidence of isolated bacteria from faecal samples.

Bacterial isolates	incidence	
	%	No
1- <i>E.coli</i>	28	70
2- <i>Sal.spp</i>	2	5
3- <i>kebl.ozaenae</i>	3.2	8
4- <i>Proteus.vulgaris</i>	1.6	4
5- <i>Enterococcus. faecalis</i>	1.2	3
Total	36	90

Table(2) : Prevalence of isolated bacteria as regards to calf age

Bacterial isolates	(1-15 days old)		(16 -30 days old)		Total	
	No	%	No	%	No	%
1- <i>E coli</i>	51	20.4	19	7.6	70	28
2- <i>Sal. Spp</i>	3	1.2	2	0.8	5	2
3- <i>Kleb. ozaenae</i>	5	2	3	1.2	8	3.2
4- <i>Proteus. Vulgaris</i>	2	0.8	2	0.8	4	1.6
5- <i>Enterococcus. faecalis</i>	3	1.2	0	0	3	1.2
Total	64	25.6	26	10.4	90	36

Table (3): The incidence of single & mixed infection cases from faecal samples

Cases of infection	Isolated bacteria	
	No	%
1- Single infection cases	84	33.6
2- Mixed infection cases	2.4	6
Total	90	36

Table (4) incidence of bacterial isolates from mixed infection cases (6)

Isolated bacteria	Incidence	
	%	No
<i>E.coli</i> & <i>kleb.ozaenae</i>	3	1.2
<i>E.coli</i> & <i>Sal.spp</i>	2	0.8
<i>E.coli</i> & <i>Proteus. vulgaris</i>	1	0.4
Total	6	2.4

Table (5): Serotyping of *E.coli* strains isolated from faecal samples

Serotype	Incidence	
	No	%
O 26 : K 60	24	9.6
O 111 : K58	13	5.2
O 119 : K69	17	6.8
O 86 : K61	8	3.2
Untypable strains	8	3.2
Total	70	36

Table(6) Results of antibacterial sensitivity of *E.coli* isolated from diarrheic calves

Antibacterial agents	Sensitive		Resistant	
	No	%	No	%
Norfloxacin	65	92.8	5	7.2
Flumequin	47	67.1	23	32.9
Streptomycin	22	31.4	48	68.6
Gentamicin	60	85.7	10	14.3
Neomycin	25	35.7	45	64.3
Colistin sulphate	55	78.6	15	21.4
Tetracycline	35	50	35	50
Erythromycin	17	24.3	53	75.7
Trimethoprim & Sulphamethoxasole	25	35.7	45	64.3

% was calculated to the number of isolates (70)

Table 7: Results of antibacterial sensitivity of *Klebsiella. ozaenae* isolated from diarrheic calves.

Antibacterial agents	Sensitive		Resistant	
	No	%	%	No
Norfloxacin	6	75	2	25
Flumequin	3	37.51	5	62.5
Streptomycin	2	25	6	75
Gentamicin	5	62.5	3	37.5
Neomycin	3	37.5	5	62.5
Colistin sulphate	4	50	4	50
Tetracycline	2	25	6	75
Erythromycin	3	37.5	5	62.5
Trimethoprim & Sulphamethoxasole	2	25	6	75

% was calculated to the number of isolates(8)

Table(8) Results of antibacterial sensitivity of *Sal. spp* isolated from diarrheic calves).

Antibacterial agents	Sensitive		Resistant	
	No	%	%	No
Norfloxacin	4	80	1	20
Flumequin	3	60	2	40
Streptomycin	2	40	3	60
Gentamicin	3	60	2	40
Neomycin	4	80	1	20
Colistin sulphate	4	80	1	20
Tetracycline	3	60	2	40
Erythromycin	1	20	4	80
Trimethoprim & Sulphamethoxasole	1	20	4	80

% was calculated to the number of isolates(5).

الملخص العربي

بعض الاسباب البكتيرية للإسهال في العجول

السيد محمد السيد الدهشان

قسم البكتريولوجي - معهد بحوث صحة الحيوان المعمل الفرعي بشبين الكوم

قامت هذه الدراسة لغرض استبيان المسببات البكتيرية المصاحبة للإسهال في العجول حديثة الولادة، واعتمدت الدراسة على فحص 250 عينة إسهال من عجول صغيرة من عمر 1-30 يوم من محافظة المنوفية، وكانت نسبة الميكروبات الفردية (33.6%) فيما كانت نسبة الميكروبات الخليط (2.4%).

وكانت نسبة الميكروبات المعزولة نتیجتها كالتالي : الميكروب القولوني (70 معزولة) و ميكروب السالمونيلا (5) و الكليسيلا المعوية (8) و ميكروب بروتيس فولجارس (4) و الميكروب السبحي (3).

تم تصنيف معظم عترات الميكروب القولوني المعزول في هذه الدراسة الى المجموعات 26:0 (9.6%) و 111 (5.2%) و 119 (6.8%) و 86 (3.2%). و كانت أعلى نسبة عزل العترات الميكروب القولوني (28%) و السالمونيلا (2%) و الكليسيلا المعوية (3.2%) و بروتيس فولجارس (1.6%) و الميكروب السبحي (1.2%). و قد سجلت توزيع عدوى الميكروبات المسببة للإسهال بالنسبة لعمر العجول حديثة الولادة و وجد أعلى نسبة إصابة كانت تتراوح في عمر من 1-15 يوم و كانت النسبة 25.6% بينما كانت 10.6% عند عمر 16-30 يوم .

كما اشتملت الدراسة على دراسة حساسية الميكروبات الأكثر شيوعا لعدد (9) من المضادات الحيوية و مركبات السلفا و أثبتت النتائج أن معظم المعزولات المختبرة ذو حساسية عالية للنورفلوكساسين و الجنتاميسين و سلفات الكوليستين .