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STUDIES ON ROLE OF *ENTEROBACTERIACEAE* IN NEWLYBORN FRIESIAN CALVES DIARRHOEA IN ASSIUT AND QUENA GOVERNORATES WITH SPECIAL REFERENCE TO THEIR SENSITIVITY TO SOME ANTIBACTERIAL AGENTS

(With 4 Tables)

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دراسات عن دورالميكروبات المعوية في إسهال العجول الفريزيان حديثة الولادة بمحافظتى أسيوط وقنا ودراسة حساسية هذه الميكروبات لبعض المضادات الحيوية

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أجريت هذه الدراسة على عدد ٦٠ عينة براز جمعت من عجول فريزيان حديثى الولادة (من عمر يوم إلى شهرين) من مزارع مختلفة بمحافظتى أسيوط وقنا وكانت تعانى من الإسهال وذلك بهدف إلقاء الضوء عن دور هذه الميكروبات المعوية كمسبب لاسهالات العجول حديثة الولادة وقد أسفرت الدراسة عن أن ٢٨ (٦٦،٤٦%) من هذه العجول كانت إيجابية للعزل الميكروبى بالميكروبات المعوية وقد تم عزل ٣٢ عترة من مجموعة الميكروبات المعوية صنفت بيوكيميائيا كالآتى: ٨ عترات (٢٥%) من الميكروب القولونى وقد صنفت سيرولوجيا فكانت ثلاث عترات منها O44 : K74 , ٥ عترات (١٥.٦٢%) من ميكروب البروتيس فلجاريس , ٤ (١٢.٥٠%) من ميكروب البروتيس ميرابيلس , ٤ (١٢.٥٠%) من ميكروب المورجانيلا مورجانى , ٢ (٦.٢٥%) من ميكروب الانتيروباكتر ايروجينز , ٣ (٩.٣٧%) من ميكروب الانتيروباكتر كلواكى , ٤ (١٢.٥٠%) من ميكروب السيتروباكتر دايفرسيس , ٢ (٦.٢٥%) من ميكروب الكلبسيلا اوكسى توكا وقد أجريت اختبارات الحساسية , وذلك باستعمال تسعة مضادات حيوية , على العترات المعزولة فكانت غالبية هذه العترات البكتيرية مقاومة بدرجة أو بأخرى لغالبية المضادات الحيوية المستعملة في الدراسة , وقد نوقشت الاهمية الصحية والاقتصادية لهذه المشكلة الحقلية الهامة.

SUMMARY

A total of 60 faecal samples were collected from newlyborn Friesian calves (age from 1 day old to 2 months) suffering from diarrhoea from different farms in Assiut and Quena Governorates. The study aimed to throw some light on the role played by *Enterobacteriaeceae* as a causative agent of diarrhoea in newlyborn calves. The study explained that 28 out of 60 (46.66%) calves were bacteriologically positive for *Enterobacteriaeceae*. 32 bacterial strains were isolated and identified biochemically as followed: 8 (25%) strains of *E.coli*, three of them were serotyped as O44:K74, 5 strains (15.62%) of *Proteus vulgaris*, 4(12.50%) of *Proteus mirabilis*, 4 strains (12.50%) of *Morganella morgani*, 2 strains (6.25%) of *Enterobacter aerogenes*, 3 strains (9.37%) of *Enterobacter cloacae*, 4 strains (12.50%) of *Citrobacter diversus*, 2 strains (6.52%) of *Kliebseilla oxytoca*. Sensitivity testing by using nine antibiotics was done against isolated strains resulting that the most of these bacterial strains were resistant. Also the public health and economic importance of that field problem was discussed.

Key words: *Enterobacteriaeceae, Friesian calves, diarrhoea*

INTRODUCTION

Diarrhoea is the most important cause of calf mortality in Egypt (Bymoi *et al.*, 1996). It can be attributed to infection with a single or multiple agents.

Calf diarrhoea is one of the most common diseases which is multifactorial in cause and involves an interplay between enteropathogenic bacteria, viruses, protozoa and the immunoglobulin status of the calf. (Radostits *et al.*, 1994).

The statistical data recorded in the animal reports of the Egyptian General Organization for Veterinary Service showed high mortality rates among calves (47.2%) and 75% of the total mortalities were due to enteric infections (Abou-Zaid and Nasr, 1995).

El-Ged, *et al.* (1994) indicated that diarrhoea is a clinical entity which causes serious economic losses as it may lead to calf mortality, weight loss or even late growth.

The members of the *Enterobacteriaceae* are geographically wide spread and may are widely distributed throughout the environment in soil, water, on plants as well as in the intestines of animals and humans (Quin *et al.*, 1994).

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

Therefore, the aim of this study is to isolate and identify the bacterial causes of calf diarrhoea belonging to *Enterobacteriaceae* group and antibiogram for isolated bacterial strains.

MATERIAL and METHODS

Material:

1- Animals:

60 newlyborn friesian calves (39 males and 21 females) aged from 1 day to two months old were suffering from moderate to severe watery diarrhoea. Those animals and their dams did not receive vaccines against etiologic agents of enteritis such as rota, corona and *Escherchia coli.*

2- Samples:

Faecal samples were collected from the rectum of diarrhoeic calves to avoid more contamination. Each sample was put in a clean dry plastic bag and labeled.

Methods:

Each sample was examined bacteriologically for *Enterobacteriaceae* on the basis of Koneman *et al.* (1994) and Quinn *et al.* (1994). It was streaked directly on the surface of MacConkey's agar at 37°C for 24-48 hours. At the same time, Salmonella was detected by enriching faecal samples on selenite F broth at 37°C for 18 hours, then subcultured on MacConkey's agar and SS agar plates.

Suspected colonies for *Salmonella* and other *Enterobacteriaceae* were purified and preserved for further identification. Identification of different isolates were made mainly on the basis of morphology and biochemical reactions.

Serological identification of the isolates, that produced biochemical reactions simulating *E.coli* was carried out after their purification by determination of the group antigens using slide agglutination test, against *E.coli* antisera obtained commercially from AG, Marburg Germany and following the instruction of the manufactures.

Antibiogram sensitivity testing was carried out using the disc and agar diffusion method according to Quinn *et al.* (1994). Nine different antibiotic discs, supplied by Oxoid were used. These antibiotics were neomycin (30μg), Gentamycin (10μg), Kanamycin (30μg), chloramphenicol (30μg), ampicillin (10μg), Nalidixic acid (30μg), penicillin (10μg), erythromycin (15μg) and flumequine (30μg).

RESULTS

The obtained results are shown in Tables 1-4

DISCUSSION

Calf scour is a complex syndrome because it has been difficult to determine the role of many different infective agents which have been isolated from the faeces and tissues of affected ones (Acres *et al.*, 1975). However predisposing factors in such cases play an important role in the establishment of calf scour (Amrousi *et al.*, 1971).

Table 1 shows that diarrhoeic calves positive for *Enterobacteriaeceae* were totally 28 (46.66%) for Assiut and Quena Governorates, and that higher incidence may reflect (to some extent) the picture of farms that included the tested diarrhoeic calves. Also it means that *Enterobacteriaeceae* play an important role as a cause of diarrhoea in newly born calves only or with other factors such as rota & corona viruses and *Cryptosporidium parvum* (Navada, *et al.*, 2000 and Azhar, 2000).

Also, Table 1 shows that the incidence of infection with enteric microorganisms in Assiut Governorate was (59.52%) and it is higher than that of Quena Governorate (16.66%). That result may be attributed to difference in hygienic measures adopted in different farms especially in Assiut farms also it may be due to over crowdeness, bad management

and law immune status of the animals in Assiut farms which lead to higher incidence of infection.

Moreover, it was evident that bacterial isolates were 32 isolated from 28 calves, it means that there was mixed infection in 3 cases, two cases, each with two isolates and the other case with three isolates.

As shown in Table 2, the isolated bacteria were 8 strains (25%) *E.coli*, 5 strains (15.62%) *Proteus vulgaris*, 4 strains (12.50%) *Proteus mirabilis*, 4 strains (12.50%) *Morganella morgani*, 2 strains (6.25%) *Enterobacter aerogenes*, 3 strains (9.37%) *Enterobacter cloacae*, 4 strains (12.50%) *Citrobacter diversus* and 2 strains (6.25%) *Kliebsiella oxytoca*. Those isolated bacteria were also isolated in several studies on calf diarrhoea in different percentages by El-Ged *et al.* (1994); Abd-El-Salam *et al.* (1998); Draz, *et al.* (1999); and Nguyen *et al.* (2000).

It was evident through the obtained results that *E.coli* strains represents the major percentage of Enterobacteriaecae (25% of all bacterial strains) isolated from faecal mater of diarrhoeic calves. Moreover *E.coli* play great role in the problem of calf diarrhoea and frequently isolated by several authors as Abd-El-Salam *et al.* (1998); Fuente, *et al.* (1998); Draz, *et al.* (1999) and Azhar (2000) in percentages 56.25%, 11.9%, 79.4% and 38.26% respectively. That variation in isolation percent may be attributed to that environmental factors which play a dangerous role in maintaining the infectious agents responsible for diarrhoea in calves where that *E. coli* was isolated from soil, water and air of the farms (Draz *et al.*, 1999).

Isolated *E. coli* strains were identified serologically as 37.5% *E.coli* O44:K74 and 62.5% untypable strains (Table 3). The association of that serotype with calf diarrhoea was reported by Lysenko and Andereeva (1966). Furthermore it was found through the study that *E. coli* O44: K74 (3 strains) were isolated from 3 diarrhoeic calves (aged between 2 days to 8 days old) and that form of diarrhoea known as colibacillosis of calves in the first two weeks of age.

Table 4 shows the results of sensitivity of antimicrobial agents used in this study. As shown in Table 3, the most bacterial isolates showed vriable degrees of resistance against antimicrobial agents, and that result was supported by El-Ged *et al.* (1994) and Urassa *et al.* (1997) who mentioned that resistant forms of bacteria were produced from the wide use of chemotherapeutic agents, which has resulted in most gram positive and gram negative bacteria continuously developing resistance to the antimicrobials in regular use at different time periods. On other

hand neonatal diarrhoea in calves is often treated with antimicrobial drugs, however, antibiotic therapy is frequently ineffective, partly due to the presence of drug resistant strains and the failure to identify drug sensitivity (Orden *et al.*, 2000). Also some reports suggested that pathogenic *E. coli* strains are more likely than non-pathogenic strains to be resistant to antimicrobial agents (Gyles *et al.*, 1977).

In the present study it was evident that *Morganella morgani* was sensitive to gentamycin, nalidixic acid and erythromycin, also *P.mirabilis* and *P. vulgaris* were sensitive to gentamycin, while Citrobacter diversus were sensitive to erythromycin and flumequine. On other hand most of pathogenic *E.coli* (O44:K74) and other untypable *E.coli and Proteus vulgaris* were moderatly sensitive to flumequine, ampicillin, neomycin and chloramphenicol respectively . These results are partially in agreement to that obtained by Asma *et al.* (1996)

In conclusion calf diarrhoea is still serious problem due to its special property that multi factors are responsible and the difficulty to determine the definite cause, so more efforts must be done to overcome that problem or at least minimize the great effects to possible lesser extent. Such efforts include medical care of dams especially in last months of pregnancy, firm control programs of vaccination, avoid the misuse of antibiotics, also complete hygienic measures must be followed in the farms to protect newlyborn calves from time of birth untill weaning.

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Table 1: Locations of studied diarrhoeic calves and percentages of microbiologically positive ones with *Enterobactereiaceae.*

Locality		Examined Diarrhoei calves	Positive calves culturally		Bacterial isolation data of diarrhoeic calves			
Governorate	Farm		No.	%	Calves with one isolate	Calves with Two isolate	Calves with Three isolate	No. of Bacterial Isolates
Assiut	Bani-mor	19	11	57.89	9	1	1	14
	Agri. Fac. Farm	6	5	83.33	5	1	-	7
	El-Hammam	3	3	100.0	3	-	-	3
	El-Awamer	9	5	55.55	5	-	-	5
	Private	5	1	20.0	1	-	-	1
Total		42	25	59.52	22	2	1	29
Quena	Fresian Farm	18	3	16.66	3	-	-	3
Total		60	28	46.66	25	2	1	32

Table 2: Isolalated Enterobacteria from diarrhoeic calves.

Bacterial isolates	No.	%
Eshcerchia coli	8	25
Proteus vulgaris	5	15.62
Proteus mirabilis	4	12.50
Morganella morganii	4	12.50
Enterobacter aerogenes	2	6.25
Enterobacter cloacae	3	9.37
Citrobacter diversus	4	12.50
klebseilla oxytoca	2	6.25
Total	**32**	**99.99**

Table 3: Serotyping of isolated *E. coli* strains.

E. coli strains	Enteropathogenic *E. coli* strains O44:K74		Untypable *E. coli* strains	
	No.	%	No.	%
8	3	37.5	5	62.5

Table 4: Antibiotic sensitivity testing for isolated *Enterobacteriacae* from faecal samples.

	E.coli O44:K74 No. (3)			Other *E. coli* No. (5)			*C.diversus* No. (4)			*P.vulgaris* No. (5)		
	R	I	S	R	I	S	R	I	S	R	I	S
Neomycin (30 µg)	1	1	1	1	3	1	3	1	-	1	4	-
Gentamycin (10 µg)	1	1	1	2	2	1	1	2	1	1	1	3
Kanamycin (30 µg)	-	3	-	2	1	2	3	1	-	4	1	-
Chloramephni-col (30 µg)	1	2	-	-	3	2	3	1	-	5	-	-
Ampecillin (10 µg)	1	2	-	1	3	1	3	1	-	5	-	-
Nalidixic acid (30 µg)	-	2	1	2	2	1	3	-	1	1	2	2
Penicillin (10 µg)	1	2	-	4	1	-	3	1	-	1	4	-
Erythromycin (15 µg)	1	2	-	3	1	1	-	1	3	4	1	-
Flumequine (30 µg)	-	3	-	2	1	2	1	-	3	3	1	1

	P.mirabilis No. (4)			*E.aerogenes* No. (2)			*E.cloacae* No. (3)			*K.axytoca* No. (2)			*M.morgani* No. (4)		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Neomycin (30 µg)	2	2	-	1	1	-	1	2	-	1	1	-	3	1	-
Gentamycin (10 µg)	2	-	2	-	1	1	1	-	2	1	1	-	-	1	3
Kanamycin (30 µg)	2	2	-	2	-	-	2	1	-	1	-	1	1	2	1
Chloramephni-col (30 µg)	4	-	-	2	-	-	1	2	-	1	-	1	4	-	-
Ampecillin (10 µg)	4	-	-	2	-	-	3	-	-	2	-	-	3	1	-
Nalidixic acid (30 µg)	4	-	-	2	-	-	3	-	-	1	1	-	1	-	3
Penicillin (10 µg)	4	-	-	1	-	1	1	-	2	1	-	1	2	2	-
Erythromycin (15 µg)	2	2	-	1	1	-	3	-	-	1	-	1	1	-	3
Flumequine (30 µg)	2	1	1	2	-	-	3	-	-	-	2	-	1	2	1

R (-ve) = Resistant
I (+ve , ++ ve) = Intermediatory
S (+++ ve) = sensitive
No. = No. of isolates examined