

Animal Health Research Institute
Quena, Regional Laboratory

ENTEROBACTERIACEAE AND ITS ROLE IN DIARRHEA OF NEWLY BORN FRIESIAN CALVES IN QUENA GOVERNORATE, EGYPT

(With 2 Tables)

By

S. Z. MAHMOUD

(Received at 8/3/2007)

الميكروبات المعويه ودورها في أسهال العجول الفريزيان حد يته الولاده
في محافظه قنا، مصر

سيد زكى محمود

أجريت هذه الدراسة على ٨٠ عينة براز مأخوذه من عجول فريزيان حد يته الولاده (عمر يوم الى ثلاث شهور) من مزارع مختلفه في محافظه قنا منهم ٢٠ عجل فريزيان فى حاله صحيه جيده ظاهريا و ٦٠ عجل فريزيان كان يعانى من اسهال شديد ذو رائحه كريهه مع فقدان للشهيه وارتفاع فى درجه الحراره. وقد اجريت على جميع العينات الفحوص البيكتريولوجيه. كانت نسبة عزل الميكروبات المعويه ٦٤ (٨٠%) وقد تم عزل الميكروب القولونى المعدى بنسبه ٤٨,٧٥% وميكروب السالمونيلا بنسبه ٢٣,٧٥% من جميع العينات بالاضافه الى عزل ميكروب السيدوموناس بنسبه ٥% وميكروب الشيغلا بنسبه ٢,٥%. وقد كانت نسبة عزل ميكروب القولون المعدى والسالمونيلا والسيدوموناس والشيغلا من العجول المصابه كالاتى على الترتيب ٦١,٦% ، ٣٠% ، ٥% ، ٢,٥% ومن العجول الاصحاء ظاهريا كالاتى على الترتيب من ١٠% ، ٥% ، ٥% ، ٠% ، وقد تم اختبار حساسيه الميكروبات المعزولة لبعض المضادات البكتريه وكان اكثرها تأثيراً هو السيبروفلوكساسين والستربتومايسين وجينتاميسين واللينكوسبكتين.

SUMMARY

This study was carried out on 80 faecal samples collected from Friesian calves (one day to three months old) at Quena Governorate. Twenty calves were clinically healthy and 60 calves showed sever diarrhoea, with offensive odour and fever. Bacteriological examinations revealed that 64 out of 80 faecal samples (80%) were positive to Enterobacteriaceae. Bacterial isolates of the diseased calves were identified biochemically as; 37 (61.6%) strains of *E. coli*, 18 (30%) strains of *Salmonella spp.*, 3(5%) strains of *Pseudomonas spp.*, 2(3.3%)

strains of *Shigella spp.* and from the clinically healthy calves 2 (10%), 1(5%), 1(5%), 0% respectively. Sensitivity tests for the bacterial isolates revealed that the tested strains were sensitive to ciprofloxacin, lincospectin, gentamycin, and streptomycin.

Key words: Enterobacteriaceae, diarrhea, newly born calves

INTRODUCTION

Diarrhoea is one of the most principle causes of calves mortalities (El-Ged *et al.*, 1994). Diarrhoea in young pre-weaned calves causes remarkable disturbances in clinical signs and blood parameters (Blood *et al.*, 1983 and Hassaan *et al.*, 1985). The most serious enteric infection usually attack calves during the first ten-weeks of life (Smith *et al.*, 1980 and Hoiseith and Stocker, 1981). The infectious agents capable of causing diarrhoea in newlyborn calves are numerous and the most important enteropathogens are *E. coli* and *Salmonella* species. Newly born calves could be infected with *Salmonella* at time of parturition or sooner after birth (Jones *et al.*, 1983 and Peel *et al.*, 1990). Colibacillosis is the most common disease of calves during the first days of their life and manifested clinically by sever diarrhoea followed by rapid death (Blood *et al.*, 1983). The immune system of animals at young age is not well developed and the maternal immunity would not withstand variable infections (Holland, 1990; and Mahmoud, 1993). The results of many investigations showed that the greatest losses among animals occurs in the neonatal period (Snodgrass and Angus, 1983). Colibacillosis appear to be the most important disease of calves during the first few days of their life.

The present work was carried out to clear up the role of bacteria belong to family Enterobacteriaceae in diarrhoea of neonatal calves at Quena Governorate

MATERIALS and METHODS

Materials:

1-Animals:

A total number of 80 Friesian calves aged from 1 day to three months old were monitored. Of these calves, 60 were suffered from profuse watery diarrhoea with offensive odour, fever, dryness and anorexia, and the remaindes were clinically healthy (control group).

2- Samples:

Faecal samples were collected in sterile labeled plastic bags from the rectum of diarrhoeic and healthy calves

Methods:

The collected samples were inoculated directly into nutrient broth and into selenite F broth and they were incubated for 18 hours at 37°C. A loopful from the incubated broth was streaked onto MacConkey agar and *Salmonella Shigella* agar plates, and incubated at 37°C for 24-48 hours (Pelton *et al.*, 1994). The suspected colonies were picked up, purified and biochemically identified as Cruickshank *et al.* (1975), Collee *et al.* (1996). Antibiogram for bacterial isolates were carried out on 22 isolates using the disc susceptibility test by diffusion methods according to Quinn *et al.* (1994). The antibacterial discs supplied by Oxoid with different concentrations included ciprofloxacin(30µg), rifampicillin (5µg), doxycillin (30µg), ampicillin (10µg), chloramphenicol (30µg), lincospectin (15+200µg), streptomycin (10µg), colistin sulphat (10µg), gentamycin (10µg), and flomoxacin (10µg) were used. The media used in sensitivity tests was nutrient agar (Oxoid) (CM0003) Lot/ 345492.

RESULTS

Isolation and identification of isolated organisms:-

The bacteriological examinations of the faecal samples collected from diarrhoeic and clinically healthy calves revealed the presence of *Escherichia coli*, *Salmonella Pseudomonas* and *Shigella* organisms. These microorganisms were isolated from the examined calves in a variable rate as shown in Table (1).

Furthermore, the sensitivity of the recovered microorganisms to some antimicrobial agents is recorded in Table 2.

Table 1: Bacteriological examinations of the examined calves.

The examined calves	Number of the positive cases to Enterobacteriaceae		Number of the positive cases to E.coli		Number of the positive cases to Salmonella spp.		Number of the positive cases to Pseudomonas spp.		Number of the positive cases to Shigella spp.	
	No.	%	No.	%	No.	%	No.	%	No.	%
Diarrhoeic calves (n=60)	60	100	37	61.6	18	30	3	5	2	3.3
Clinically Healthy calves (n= 20)	4	20	2	10	1	5	1	5	0	—
Total n=80	64	80	39	48.75	19	23.75	4	5	2	2.5

Table 2: Antibacterial sensitivity tests of some isolates (n=22 Strains)

	Ceprofl oxacin 30µg	Lincosp ectin 15+200µg	Strepto mycin 10µg	Genta mycin 10µg	Ampici lin 10µg	Colistin sulphat 10µg	Flam quin 10µg	Chloram phenicol 30µg	Doxy cillin 30µg	Refamp icillin 5µg
<i>E.coli</i> n=12	+++	+++	+++	++	+	+	+	++	+	+
<i>Salmon</i> <i>Ell</i> n=7	+++	++	++	++	-	+	+	++	-	-
<i>Pseudo</i> <i>Monas</i> n=2	++	++	+	+	--	-	-	--	-	-
<i>Shigella</i> n=1	++	++	+	-	-	-	-	--	-	-

+++ = Sensitive +, ++ = Intermediat --- = Resistance

DISCUSSION

Enterobacteriaceae play an important role as a cause of diarrhoea in neonates. Several studies were carried out and showed that the most important bacterial infection are *E. coli* and *Salmonella spp.* (Mottelib 1972). Amer *et al.* (1985) and Hassaan *et al.* (1985) reported that *E. coli* was the main cause in the pathogenesis of enteritis in calves at Upper Egypt. The bacteriological examinations of the present study revealed that the isolation rate of *E. coli* and *Salmonella* was (48.75%) and (23.75%) from diarrheic and healthy calves respectively. These results agreed with those mentioned by Abd El - Salam *et al.* (1998), Asma *et al.* (1996), Farid *et al.* (1992) and Mousa *et al.* (2001). They elucidated that the isolation rates of *Salmonella* organisms from diarrhoeic and healthy calves were 43.53%, and 27.69% respectively. The isolated *E. coli* and *Salmonella spp.* from the diarrhoeic calves were 61.6% and 30%, respectively while from healthy calves were 10% and 5% respectively. These results agreed with those reported by El-Ged *et al.* (1994) and Aisha (2001). The obtained results indicated that *E. coli* was the main bacterial cause of enteric infection in newly born calves while *Salmonella* organisms formed the second cause of enteritis in calves. *Pseudomonas spp.* and *Shigella spp.* were isolated from the diarrhoeic and the healthy calves with a rate of 5% and 2.5% respectively and from diarrhoeic calves the isolation rate was 5%, 3.3%, respectively. From healthy calves the isolation rate of *Pseudomonas spp.* was 5%. These results agreed with that reported by Mottelib (1972), Amer *et al.* (1985), and Snodgrass *et al.* (1986). Hala (2002) mentioned that *Pseudomonas spp.* is an opportunistic pathogen of medical and veterinary importance due to its capability of infecting human, animals and poultry. The infection with this organism has been regarded as one

of the serious economic losses and considered as one of the most dangerous disease which affects animals (Hick *et al.* 1991). *Shigella* and *Pseudomonas* organisms recorded the lowest incidence in diarrhoeic calves and they were found in form of mixed infection either with *E. coli* or *Salmonella* (Abd El-Salam *et al.*, 1998). The results of sensitivity tests using different antibacterial discs revealed that the tested isolated strains (22 isolates) were sensitive to ciprofloxacin, lincospectin, gentamycin and streptomycin but resistance to colistin, flomoxacin, ampicillin and doxycillin. These resistance of bacteria to antibiotic may be due to wide use of antibiotic (El-Ged *et al.*, 1994).

REFERENCES

- Abd El-Salam, M.N.; Ali, A.A and Ehsan, A. Hassan (1998):* Field investigation on some trace elements, vitamins and biochemical value in buffalo with diarrhoea in Assiut Governorate. Assiut Vet. Med. J. 39, 78: 157 -167.
- Aisha, R. Ali (2001):* Comparative studies on diarrhoea caused by *E. coli* in farm animals. J. Egypt Vet. Med. Assoc. 61 No. 6C: 39-49.
- Amer, A.A.; Hassaan, N.K.; El-Sebaie, A.; Bayoumi, A.H. and Ibrahim, A.H. (1985):* Studies on an outbreak of colibacellosis among Holstein Friesian calves in the newvally. Egypt: 1-clinical, hematological aetiological and pathological alteration. Assiut Vet. Med. J. 14, 27: 151-157
- Asma, O. Aly; Zamzam, H. Abd El-Wahed; Kohilo, Kh. and El-Sheikh, A.R. (1996):* Some studies on clinical, hematological and biochemical changes in diarrhoeic neonatal buffalo calves with reference to hygienic conditions. Assuit Vet. Med. J. 35, 69: 91-104.
- Blood, D.C.; Radastits, O.M. and Handerson, S.A. (1983):* Veterinary Medicine. A textbook of the disease of cattle, sheep, pigs, goats and horses 6th ed., Bailliere, Tindall.
- Collee, J.G.; Marmion, B.P.; Fraser, A.G. and Simmons, A. (1996):* Mackey and MacCartney Practical Medical Microbiology, 4th ed. Churchill Living Stone, N. Y. London.
- Cruickshank, R.; Dugid, J.R.; Marmion, B.P. and Swain, R.H.A. (1975):* Medical Microbiology: The practice of medical microbiology .12th ed. Vol. 2, Churchill, Livingstone, London.

- El-Ged, A.; El-Sayed, Z.M.; Khalid, A.; Abd-El-Gaber, G.; Abd-El-Rahman, M. and El-Bardisy, M.M. (1994):* Studies on the role of colostridial organisms and other bacteria in calf diarrhoea with special reference to their susceptibility to some antibacterial agents. *Assiut Vet. Med. J.* 30, 60: 194-207.
- Farid, F.A.; Kaldas, T.Y.; Soheir, Sh.E.; Naeal, Gh.K. and Saad, K.M. (1992):* Prevalence of *Escherichia coli* K99 in diarrhoeic newborn calves. *Proc.5th Sci. Cong., Faculty. Vet. Med., Assiut University.*
- Hala S. Ibrahim (2002):* Detection and control of *Pseudomonas aeruginosa* in feed Stuffs with special reference to ELISA. *J. Egy. Med. Assoc.* 62, 6c, 55-66.
- Hassaan, N.K.; El-Sebaie, A. and Amer, A.A. (1985):* Studies on an outbreak of colibacillosis among Holstein Friesian calves in the newvally Egypt. *Assiut. Vet. Med. J.* 14, 27: 161- 168.
- Hicks, C.V.; Eberhart, R.J.; Erskine, R.J. and Spencer, S. (1991):* Interaction between *Pseudomonas aeruginosa* and iodophoregermicides. *App. Environ. Microbiol.*, 57(2): 568-572.
- Hoiseth, S.K. and Stocker, B.A. (1981):* Aromatic-dependent *Salmonella typhimurium* are nonvirulent and effective as live vaccines, *Nature*: 238--239.
- Holland, R.E. (1990):* Some infectious causes of diarrhoea in young farm animals. *Clin. Microbiol. Rev.*, 3 (4), 345- 375.
- Jones, P.W.; Collins, P.; Brown, T. H. and Atiken, M.U. (1983):* *Salmonella saintpaul* infection in two dairy herds. *J. Hyg. Causl.*, 91: 243- 257.
- Mahmoud, A.R. (1993):* Clinicopathological studies on some diseases of cattle calves and buffalo calves. Ph.D. Thesis. Faculty Vet. Med. Cairo University.
- Mottelib, (1972):* A study on the changes of blood in buffalo calves suffering from enteritis due to different causative agents. MVSC Thesis, Fac. Vet. Med. Assiut Univ. Egypt.
- Mottelib, A.A.; Ammar, A.M. and Haroun, E.M. (1992):* Some studies on enteritis in young lambs. *J. Egy. Vet. Assoc.* 52 1, 1-11.
- Moussa, I.; Awad, W.S.A. and Ibrahim, A.K. (2001):* Using Fim A Gene Amplification assay for diagnosis of *Salmonella* infection in diarrhoeic and contact healthy calves. *J. Egypt Vet. Med. Assoc.* 64, 5, 81-90.

- Peel, J.E.; Kolly, C.; and Siegnthalei (1990):* Prophlactic effect of recombinant Bovine interferon -1 on acute *Salmonella typhimurium* in calves. *Am. J. Vet. Res.*, 51: 1059- 1099.
- Pelton, J.A.; Dilling, G.W.; Smith, B.P. and Jang, S. (1994):* Comparison of a commercial antigen capture ELISA with enrichment culture for detection of *Salmonella* from faecal samples. *J. Vet. Diagnost. Invest.*, 6(4): 501- 502.
- Quinn, P.J.; Carter, M.E.; Markey, B.K. and Carter (1994):* Clinical Veterinary Microbiology. Mosby-Year Book Europe Limited London England 1st.Ed
- Smith, B.P.; Habasha, F.G.; Reina Guerra, M. and Hardy, A.J. (1980):* Immunization of calves against Salmonellosis. *Am. J. Vet. Res.* 41: 1947-1951
- Snodgrass, D.R. and Angus, K.W. (1983):* Diseases of sheep. First Ed. pp. 43-48, Blackwell Scientific Puplication, London.
- Snodgrass, D.R.; Terzolo, H.R.; Sherwood, D.; Campbell, L. Menzies, J.D. and Synge, B.A. (1986):* Aetiology of diarrhoea in young calves. *Vet. Rec.*119 (2): 31-34.