

SOME STUDIES ON BACTERIOLOGICAL CAUSES OF ENTERITIS IN NEWLY BORNE CALVES

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ABSTRACT

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This study was initiated to isolation and identification of probable bacterial causes in the fecal samples of newly born calves suffering from enteritis in some farms of Assiut Governorate A total number of 80 calves were subjected to the study. These animals were randomly selected from some Assiut farms. Twenty-five calves were considered as the control group. These animals aged from one day to one month suffering from sever diarrhea, increase of body temperature (average 40.5°C) and increase in heart rate. Some calves have had loss of appetite, dehydration and dullness. Fifty- five diseased calves were classified according to its age into four groups, the first group included fourteen calves aged 1-5days old, second group included sixteen calves aged from 6-10days old, third group included twelve calves aged from 11-15days old and the fourth group included thirteen calves aged from 16-30days old. The bacteriological examination revealed that the incidence of enteric bacterial isolates in apparently healthy calves was 72% and it was lower than in diseased calves (100%). It is clear that the incidence of enteric bacteria was 100% in all fecal samples. There were a wide range of bacteria isolates from feacal samoles of both apparently healthy and diseased calves. The isolates were Escherichia coli (O157) from diseased calves in all four groups with different percentages (64.28%, 50%, 33.33% and 23.07%) respectively, Salmonella typhimurium (35.71%, 18.75%, 25% and 7.60%) respectively and Clostridium perfringens (78.57, 56.25, 58.33 and 61.53) respectively respectively. It was noticed that Clostridium perfringens was recorded with the highest incidence. Moreover, diarrhea become sever and have offensive odor in calves occurred mainly when a combination between the three bacteria (E. coli (O157), Salmonella typhimurium and Cl.perfringens). It was noticed that younger calves were more susceptible than the older ones. Pathoenicity test in mice revealed that E. (O157) 87% were highly virulent strains followed by Salmonella typhimurium 75% while Cl. perfringens were lower in their virulence (62.5%)

Key words: Enteritis, *E. coli*, *Sal. Typhimurium*, calves, enteritis, and *Cl. perfringens*
Introduction

بعض الدراسات عن المسببات البكتيرية للنزلات المعوية في العجول حديثة الولادة

ازهار محمد حسن

الهدف من هذه الدراسة الوقوف على اهم المسببات البكتريولوجية للنزلات المعوية في العجول حديثة الولادة تحت ظروف التربية في بعض مناطق محافظة اسيوط. استخدمت لهذه الدراسة ٨٠ عجل منها ٢٥ حالة سليمة ظاهريا و ٥٥ حالات تعاني من النزلة المعوية باعراض كانت عبارة عن الاسهال مع ارتفاع درجة حرارة الجسم (٤٠.٥ درجة مئوية). تم تقسيم العجول المريضة الى ٤ مجموعات شملت المجموعة الأولى العجول من عمر يوم الى ٥ ايام وكان عددهم ١٤ عجل و اشتملت المجموعة الثانية على عجل اعمارها من ٦-١٠ يوم وعددهم ١٦ عجل، والمجموعة الثالثة شملت على ١٢ عجل تتراوح اعمارهم ما بين ١١-١٥ يوم والمجموعة الرابعة اشتملت ١٣ عجل اعمارهم بين ١٦-٣٠ days. اوضحت الفحوص أن العزلات البكتيرية في حالات النزلة المعوية في العجول الاصحاء وكانت ٧٢٪ وكان أقل منه في العجول المريضة (١٠٠٪). من الواضح أن حالات البكتيريا المعوية و ١٠٠٪ في جميع عينات البراز. كانت هناك مجموعة واسعة من البكتيريا من العجول الاصحاء والمريضة. عزلات القولونية (O157) من العجول المصابة في جميع المجموعات (٦٤.٢٨٪، ٥٠٪، ٣٣.٣٣٪، ٢٣.٠٧٪) على التوالي، Salmonella التيفية الفأرية (٣٥.٧١٪، ١٨.٧٥٪، ٢٥٪ و ٧.٦٠٪) على التوالي، والمطثية الحاطمة (٧٨.٥٧، ٥٦.٢٥، ٥٨.٣٣، ٦١.٥٣) على التوالي على التوالي. لوحظ انه تم تسجيل كلوستريديوم بيرفرينجنز وفقا لأعلى وقد لوحظ أن الإسهال في العجول وقعت عندما كان هناك مزيج بين ثلاثة انواع من البكتيريا (القولونية O157، Salmonella التيفية الفأرية والحاطمة كلوستريديوم). لوحظ أن العجول الاصغر في العمر كانت اكثر تائرا من الاكبر سننا. اختبار Pathogenicity في الفئران أظهر أن القولونية (O157) ٨٧٪ منها سلالات شديدة الضراوة تليها التيفية الفأرية Salmonella ٧٥٪ و ٦٢.٥٪ كلوستريديوم بيرفرينجنز كانت أقل في حنتها.

INTRODUCTION

Neonatal calf diarrhea (NCD) is a common cause of illness (10.2%) and death in pre-weaning calves. A variety of enteropathogens have been implicated in many cases are currently idiopathic.

Enteritis in young calves considered generally to be the main hazard to calf health. Diarrhea as a symptom of enteritis is one of the major health problem in many farms associated with newly born calves which considerable number could be lost (Acress *et al.*, 1977, Quigley *et al.*, 1995, Wells *et al.*, 1996). Bispham *et al.* (2001) studied that within the species Salmonella Spp. there are more than 2,000 different serotypes, which include bacteria of tremendous medical and veterinary importance. The pathology of Salmonella infections can vary from mild enteritis to severe systemic salmonellosis and is largely dependent on the particular combination of serotype and host species. Most calves are affected with E. coli within the first 3 days of life. There are many types of E. coli some are normal flora; different types cause septicemia; others are invasive; Enterotoxigenic E. coli (ETEC) is the most common cause of newlyborn calf diarrhea.

Dehydration is usually severe and may cause death before diarrhea develops. The course of the disease is rapid and begins from weakness, diarrhea, dehydration, to death occurred within than 24 hours. Antibiotics rarely affect the outcome of this disease, while fluid support is critical to survival. Vaccination of dry cows and good colostrum feeding can eliminate this problem (Van Bost and Mainil, 2003). Hala *et al.* (2006) recorded that Salmonella spp. are an important cause of enteritis and infected calves are at risk of developing septicemia.

Infections usually occur in 5- to 14-day-old calves. Blood and casts of intestines may be seen in the feces. Calves are slow to respond to treatment and are often sick for 1 to 2 weeks. S. Typhimurium is a common cause of enteritis in newly born calves. The disease is typically localized to the intestine and characterized by acute diarrhea and abdominal pain. (Geric *et al.*, 2006) There are several types of CI. Perfringens; type C can be a cause of diarrhea. More typically, this causes sudden onset of weakness or death. Colic or nervous system signs may be seen before death. Postmortem examination has characteristic hemorrhage in the intestines.

Although *C. difficile* infection has been suggested as a cause of diarrhea and enteritis further published evidence is lacking (Alexander *et al.*, 2012).

The objective of this study was to investigate possible bacterial causes of enteritis in apparently healthy and diseased calves under local breeding conditions. Moreover, serological identification of *E. coli* (O157) isolates and pathogenicity of some bacterial isolates such as *S. typhimurium* and *Cl. Perfringens* in mice were also described.

MATERIALS and METHODS

Animals:

A total of 80 balady cow calves were investigated in this study. Calves were distributed over different areas (Dayrout, Manfolot, el- Sahal) cattle farms. These farms have been troubled and suffered from diarrhea in newly born calves. These animals were selected randomly. The samples were collected at December 20, 2011. Calves (25) were subjected to careful clinical and laboratory examinations and used as a control. These animals were fed on their mothers. The studied cases were of different sex and their ages ranged from one day – one month. Calves classified into four groups according to their age, first group included fourteen animals their age from 1-5 days, second group included sixteen animals of age from 6-10 days, third group included twelve animals with age from 11-15 days and fourth group included thirteen animals of age from 16-30 days.

Sampling:

Samples from feces and intestinal contents were collected from the rectum using sterile swabs from 55 diarrheic calves showed signs of diarrhea. as well as 25 of clinical healthy calves (control). The samples from calves were collected in plastic bags and were transported refrigerated to the laboratory where were processed within 4 hours of collection. Samples were then immersed in nutrient broth about 24 hours, until bacteriological examination. Isolation and Identification of *E- Coli* (O157) and *Sal. typhimurium* (Quinn *et al.*, 2002).

E- Coli (O157)

Samples were cultured nutrient broth and MacConkey broth and for 24 hours at 37°C and then a loopful was taken and cultured onto the following solid media; nutrient agar, 5% sheep blood agar and MacConkey agar. All inoculated plates were incubated at 37°C for 24 hours then the colonies were identified.

Serotyping of the isolated strains of *E- Coli* was done according to Edward and Ewing (1972).

S. typhimurium

Enrichment of fecal samples were processed in Rappaport broth (at 37°C for 18 h) and Selenite brilliant green broth (at 42°C for 18 h) followed by incubation of the enrichment cultures on modified brilliant green agar (Oxoid) containing 120 mg of sulfadiazine/liter.

Isolation and purification of *Cl Perfringens* Willis (1977): Samples from feces of calves with clinical symptoms of enteritis were inoculated into tubes of freshly prepared cooked meat medium and incubated anaerobically for 24 hours at 37°C. A loopful of inoculated fluid medium was streaked onto neomycin sulphate blood agar with 5% sheep blood plates for isolation of *Cl perfringens*. The streaked plates were incubated anaerobically for 24 hours at 37°C using a Gaspak anaerobic Jar. Suspected colonies of *Cl perfringens* that were characterized by double zone of hemolysis were sub cultured onto egg yolk agar plates. The colonies that grew only in anaerobic condition, non motile, catalase negative and lecithinase producer were picked up and purified for further identification test.

Identification of *Cl. Perfringens*:

Typical colonies were identified as described by Murray *et al.* (2003) depending on characteristic colonial morphology, hemolysis activity, gelatin hydrolysis, fermentation of glucose lactose, sucrose, maltose and mannitol, indole test, urease production, litmus milk test and lecithinase production.

Pathogenicity of the recorded bacterial isolated in mice:

It was carried out according to Koneman *et al.* (1996) where 32 mice, 26-28 days old were used to investigate the pathogenicity and were classified into 4 groups, each contained 8 mice which received 1ml/mouse of an isolate cells suspended in sterile saline containing 7.5×10^8 CFU/ml for (E-Coli

(O157), *Sal. typhimurium* and *Cl. Perfringens*. Mice were inoculated I/P and kept separately. Last group was kept as a control and injected only with sterile saline. All mice were kept under observation for 7 days. The number of dead mice was recorded and dead mice were subjected to bacteriological investigated for re-isolation of inoculated strains.

RESULTS

Table 1: Percentages of *E. coli* isolated from diseased calves.

Animal groups	Total group number	Number of <i>E. coli</i> isolated	Percentages (%)
First group	14 (diseased calves)	9 (cases)	64.28
Second group	16 (diseased calves)	8 (cases)	50.00
Third group	12 (diseased calves)	4(cases)	33.33
Fourth group	13 (diseased calves)	3(cases)	23.07

Table 2: Percentages of *Sal Typhimurium* isolated from diseased calves.

Animal groups	Total group number	Number of <i>Sal Typhimurium</i>	Percentages (%)
First group	14 (diseased calves)	5 (cases)	35.71
Second group	16 (diseased calves)	3 (cases)	18.75
Third group	12 (diseased calves)	3 (cases)	25.00
Fourth group	13 (diseased calves)	1 (cases)	7.60

Table 3: Percentages of *Cl. perfringens* isolated from diseased calves.

Animal groups	Total group number	No of <i>Cl. perfringens</i> isolated	Percentages (%)
First group	14 (diseased calves)	11(cases)	78.57
Second group	16 (diseased calves)	9 (cases)	56.25
Third group	12 (diseased calves)	7 (cases)	58.33
Fourth group	13 (diseased calves)	8 (cases)	61.53

Table 4: Pathogenicity of some enteric bacteria isolated from calves the virulence of bacteria isolated from calves, as demonstrated by the sequence of mortality in mice in seven days. *E. Coli* (O157) were highly virulent strain 87.5% followed by *Sal. typhimurium* 75.0% while *Cl. perfringens* were lower in their virulence 62.5%.

Bacterial Isolates	No. of dead mice/day							Total	
	1	2	3	4	5	6	7	No.	%
<i>Escherichia coli</i> (O157)	0	1	2	0	3	0	1	7/8	87.5
<i>Salmonella typhimurium</i>	1	0	1	0	1	3	0	6/8	75.0
<i>Clostridium perfringens</i>	0	1	1	0	2	1	0	5/8	62.5

Table 5: That percentage of enteritis were higher in diseased calves 100 % which represented by 55 diseased calves classified according their age into four groups than apparently healthy calves 72% which represented by 25 App. healthy calves.

Animals	Fecal samples		
	No.	+ ve	Percentages %
App. Healthy calves	25	18	72
Diseased calves	55	55	100
Total	80	73	91.25

DISCUSSION

E. coli was isolated from from diseased calves in all groups with different percent ages (64.28%, 50%, 33.33% and 23.07%), *Sal. typhimurium* (35.71%, 18.75%, 25% and 7.60%) and *Cl., Perfringens* (78.57, 56.25, 58.33 and 61.53), As shown in (Tables and figures (1), (2), and (3) respectively).

The results presented in Table (1) showed the percentages of *E.coli* (O157) from diseased calves. It was noticed that First group which included 14 diseased calves and aged from one to five days old was higher than other groups, while fourth group which included 13 diseased calves and aged from 16-30 days old were lower. These results come in accordance with that of Nahed (2004). The obtained result coincided to a large extent with West (1977) who mentioned that, calf scours is often attributed to *E.coli* is clinically complicated by microbes like *P. vulgaris*. Elsa *et al.* (2004)

reported that *E.coli* (O157) play an important role in 4 outbreaks of calf enteritis.

The results presented in Table (2) showed the percentages of *Sal typhimurium* isolated from diseased calves. It was noticed that First group which included 14 diseased calves and aged from one to five days old was higher than other groups, followed by the third group, then, second group and the fourth group was the lower. These results come in accordance with Nahed (2004) and (Geric *et al.*, 2006).

The results presented in Table (3) the percentages of *Cl. perfringens* from diseased calves. It was noticed that first group was higher than other groups, (78.57%) followed by the fourth group 61. (53%), then, third group (58.33%) and the second group was the lower (56.25%). These results were not agreement with Geric *et al.* (2006).

It was noticed from Table (4) that the virulence of bacteria isolated from calves, as demonstrated by the sequence of mortality in mice was as follows *E- Coli* (O157) were highly virulent strain followed by

S. typhimurium while *Cl. perfringens* were lower in their virulence. They may be due to pathogenic nature of the strain or the presence of virulence associated plasmid or production of endo or exotoxins. These results coincided to some extent with those of Hala and Abu-Zaid (2006) who found that *E. Coli* (O157) and O78 *Sal. enteritidis* and *Sal. typhimurium* were highly pathogenic to mice.

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