

**EVALUATION OF PARENTERAL TREATMENT OF
COLIBACILLOSIS IN PRE-WEANING
BUFFALO CALVES USING AMOXICILLIN AND
CLAVULINIC ACID (SYNULOX®)**

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ABSTRACT

Thirty, 1-2 month old pre-weaning Egyptian buffalo calves in a private farm at Sharkia Governorate were divided into naturally diarrheic calves and non diarrheic calves were subdivided into two equal groups. The first group was treated intramuscular (1/M) with amoxicillin suspension (clamoxyl L.A ®) at a dose level of 1ml / 15Kg B.wt . and the second group was treated 1/M with amoxicillin potentiated clavulanic acid (Synulox ®) at dose of 1ml / 20 kg for 3 successive days. Rectal swabs were aseptically collected from diarrheic and healthy calves. Bacteriological examination of isolates revealed that *E.coli* strains were the main causative agent of diarrhea in diseased calves. Susceptibility of isolated strains to different antimicrobials was conducted and showed that amoxicillin potentiated with clavulanic acid was highly effective. Blood samples from studied calves before and after treatment were collected for biochemical and hematological studies. In diseased calves, a significant decrease in serum total protein with its albumin and globulin in addition to immunoglobulin, hemoglobin decreases also were recorded. While leukocytosis and significant increases in platelets were noticed. A non significant decrease in sodium with a significant decrease in potassium were recorded in diseased calves. Parenteral treatment of diarrheic calves with amoxicillin potentiated with clavulanic acid was more effective than amoxicillin alone in reducing the clinical symptoms and improvement of general health conditions.

INTRODUCTION:-

Colibacillosis is one of the most common causes of diarrhea , death and economic losses in calves farms (Wittum et al., 2002). Amoxicillin

is a broad – spectrum beta –lactam antibiotic can be recommended for treatment of calf diarrhea (Constable, 2004) .

Resistance to beta-lactam antibiotic in both Gram positive and Gram negative bacteria is mainly due to inactivation by beta-lactamases (Livermore, 1995). Clavulinate is a non competitive inhibitor of beta-lactamase produced by these bacteria (Yamamoto and Nakazawa, 1997). Therefore, combination of clavulanic acid and amoxicillin is highly effective against resistant bacteria (Simpson et al., 1998).

Oral amoxicillin has documented efficacy in the treatment of experimentally induced diarrhea (Palmer et al., 1997 and Bywater, 1977) but oral administration of amoxicillin was not effective in the treatment of naturally acquired diarrhea in beef calves (Radostitis et al., 1975). In addition, prophylactic and therapeutic antimicrobial administration in dairy calves revealed that In-feed antimicrobials were associated with higher level of multiple antimicrobial resistance of fecal *E.coli* than in calves not receiving In-feed antimicrobials (Berge et al., 2006). Therefore this study was designed for evaluation of parenteral treatment of colibacillosis in pre-weaning buffalo calves using amoxicillin and Clavulanic acid (Synulox, an injectable preparation) and its effects on clinical and some biochemical parameters.

MATERIAL AND METHODS:-

MATERIALS:

1- Animals: Thirty, 1-2 months old pre-weaning Egyptian buffalo calves in a private farm at Sharkia Governorate were used. Twenty calves out of them were divided into two groups:

A-Naturally diarrheic calves : They were 10 calves in the febrile stage of infection and displayed clinical symptoms as : depraved appetite, followed by profuse diarrhea staining the perineum and foul smelling fluidy faeces. Calves were dull and depressed but were responsive to stimuli. Rectal temperature was 40-41.5°C and remain high.

B- Non diarrheic 10 calves apparently healthy buffalo calves kept as a control group.

2- Drugs :

A- Commercial antibacterial discs : Were obtained from BioMerieux (France). These discs were Amoxicillin (30ug), Amoxicillin and clavulanic acid (30ug), Enrofloxacin (10ug) Ceftiofur Soduim (30ug), Flumequine (30ug), Gentamycin (10ug), Streptomycin (10ug), Ampicillin (10ug), and Doxycillin (30ug).

B- Amoxicillin suspension (Clamoxyl L.A ®) from Pfizer-Egypt at a dose rate of 1ml / 15 kg.B.wt (10mg / kg. I.M).

C-Clavulanate –potentiated Amoxicillin (**Synulox ® suspension**) from Pfizer- Egypt for parenteral administration (1ml / 20 kg.B.wt.) (12.5mg / kg.I.M.) once daily for 3 successive days .

3- Media Nutrient broth ,nutrient agar ,MacConkey broth , MacConkey agar and EMB.

METHODS:-

1- Sampling : Rectal swabs were aseptically collected from diarrheic and healthy calves . Jugular blood samples were collected from healthy and diseased calves before and after treatment for serum separation and stored at –20 °c for biochemical determination . Blood samples were collected into EDTA contained tubes for hematological parameters

2- Bacteriological examination :

A- Isolation and Identification of the causative agents : Rectal swabs were aseptically collected from diarrheic calves and processed for isolation of *E.Coli* following the procedure of **Edwards and Ewing (1972)**. The isolated strains were identified on the basis of cultural, morphological and biochemical characteristics as described by **Krieg and Holt(1984)**.

B- Sensitivity tests : They were carried out using disc diffusion method (**Bauer et al.,1966**).

3- Parental treatment of diarrhea : Ten Egyptian buffalo calves suffered from diarrhea due to *E.coli* infection were divided equally into two groups:

The first group was injected intramuscularly with amoxicillin (clamoxyl® L.A) at a dose level of 1 ml / 15 kg.B.wt (15 mg / Kg.B.wt) and the second group was injected I.M with amoxicillin potentiated clavulanic acid (Synulox®) at a dose of 1ml / 20 Kg.B.wt. for 3 successive days .The different samples were examined before treatment and 7 days post treatment Ten apparently healthy calves were used as a control group .

4-Biochemical analysis:

1-Determination of total serum proteins and albumin according to **King and Watton (1959)**, while the globulin value from total serum protein value.

2- Determination of total immunoglobulin by zink sulphate turbidity test following **Mcewan et al (1970)**.

3- Determination of serum sodium and potassium by using atomic absorption spectrophotometer after **khan et.al.(1995)**.

5-Haematological examination:

R.Bcs., T.leucocytic count, hemoglobin concentration and packed

cell volume, were conducted after Coles (1986).

6- Statistical analysis : to obtained data were statistically analyzed by means of software computer programs according to Spsswin (1995).

RESULTS:-

Diseased calves showed depression, reluctant to move, anorectic, profuse watery diarrhea and high temperature (40-41.5°C).

The present data revealed that *E.coli* was the main bacterial cause of diarrhea in pre-weaning buffalo calves . Parasitological and viral causes were excluded . The results of antibiogram study were shown in Table(1). Isolated *E.coli* strains were highly susceptible to Amoxicillin potentiated with clavulanic acid and Eneofloxacin, Ceftiofur sodium, Flumequine, Gentamycin and Streptomycin were effective on 80% of the total isolates, mean while 20% of the isolates were resistance . Amoxicillin potentiated with calvulinic acid was more effective in reducing the clinical symptoms of diarrheic calves and improvement of general health condition when compared with diseased calves treated with amoxicillin alone. Treatment with both drugs resulted in decrease of body temperature from (40-41.5°C) to 38°C within 24 hours. The results. ohematological and serum biochemical pro-

file in clinically healthy and diseased calves before and after treatment were shown in Table 2 and 3, respectively.

DISCUSSION:-

Diarrhea in young pre-weaned calves is a syndrome of great aetiological complexity. In addition to the influence of varied environmental, managerial, nutritional and physiological factors. The common finding in calves with diarrhea are bacteremia and failure of passive transfer of immunoglobulins. It has diverted attention from the finding of numerous studies that calves with diarrhoea have coliform bacterial overgrowth of the small intestine. More recent studies have consistently documented that calves with naturally acquired diarrhoea, have altered small intestinal bacterial flora. Specifically, *E.coli* bacterial numbers are increased to 10000-fold in the duodenum, jejunum and ileum of calves (Constable, 2004).

The result of bacteriological examination of faecal samples revealed that *E.coli* is the most common bacterial isolates . Similarly to that obtained previously by El shaboury et al,(2000) and Ok et al (2009). The administration of antimicrobial agents that decrease small intestinal coliforms bacterial numbers in calves with diarrhea may prevents the development of bacte-

remia, decrease the morphological damage of small intestine (Constable, 2004), thereby facilitating digestion and absorption and increasing growth rate.

Osborne et al. (1959) concluded a good correlation between in vitro antimicrobial susceptibility of fecal *E. coli* isolates and clinical response to antimicrobial treatment, however other studies found no correlation between in vitro antimicrobial susceptibility of fecal *E. coli* isolates and clinical response to antimicrobial treatment (Boyd et al., 1974 and Bywater et al., 1978).

Based on the available data, it appears that antimicrobial efficacy is best evaluated by the clinical response of a number of calves to treatment, rather than the result of in vitro antimicrobial susceptibility testing performed on fecal *E. coli* isolates (Constable, 2004).

The present data revealed that the isolated strains of *E. coli* were highly sensitive to Amoxicillin potentiated with clavulanic acid and Enrofloxacin. Meanwhile amoxicillin without clavulanic acid was less effective than the potentiated one. Supporting this finding, Rolinson (1994) found that *E. coli* strains were susceptible to amoxicillin clavulanate with no firm evidence of increased resistance. In our study, amoxicillin potentiated with clavu-

lanate was clinically more effective than amoxicillin alone as exhibited by the rapid recovery of diarrheic calves.

A number of antimicrobial agents produce deleterious effects on small intestinal function and morphology when administered per os to healthy milk fed dairy calves (Shull and Fredeick, 1978). In addition, oral Amoxicillin has documented efficacy in the treatment of experimentally induced diarrhea (Palmer et al., 1977 and Bywater, 1977), but amoxicillin administered per os was not efficacious in the treatment of naturally acquired diarrhea in beef calves (Radostits et al., 1975) therefore the present study was directed to detect efficacy of parenteral administration of amoxicillin or amoxicillin potentiated clavulanate in naturally preweaning diarrheic calves. The obtained data demonstrated that both drugs were effective in treatment of calf diarrhea, however, amoxicillin potentiated clavulanate was better in shorting diarrhea duration and improvement of general health condition.

On the other hand, antimicrobial therapy may decrease the bacterial pathogen numbers but clinical efficacy is greatly impaired in the absence of adequate immune function (O'Mahony et al., 2002).

In comparison to healthy calves, the diseased (diarrheic) calves had significantly low serum total immunoglobulins (Ig) level this decline in Ig between 1-2 month age is attributed mainly to the catabolism of maternally derived antibodies i.e. catabolism of passive acquired Ig which overlap the rise in autogenous actively acquired Ig synthesized by calf itself. (Logan, et al., 1973).

Similar results have been observed by (Roy et al., 1997, Khadr and El-gharieb., 2001). Low serum immunoglobulins, was independent risk factors for increased morbid-days (Barnett et al., 2003).

Quigley et al.,(1995) and Rajala and Castren (1995) noticed absence of association between passive immunity status and diarrhea morbidity as immunoglobulin are more effective in preventing diarrhea when acting locally in the gastro intestinal tract.

The obtained data showed a significant decrease in total serum protein concentration with apparently normal hydration status in diarrheic calves, and the albumin and globulin were significant decreased when compared with healthy calves. A similar finding was recorded by Donovan et al (1998) and partially by Omran et al (2005). Hypoproteinaemia was suggested either reactive hypoglobulinaemia or failure of

passive transfer maternal antibodies (O'Mahony et al, 2002). The decreased serum total protein could be neglect a failure of transfer of maternal antibodies (Jonic, 1997), because immunoglobulin are the only class of serum proteins that increase markedly as a result of ingestion of colostrum (Tennant et al, 1969). Moreover, serum albumin level in diseased calves has been shown to vary (Schultze et al, 1971).

Regarding serum sodium and potassium levels of diarrheic calves in the present study, there is no significant change in the level of sodium between groups, meanwhile a significant hypokalemia were recorded. This finding might be attributed to the electrolytes loss into the bowel lumen (Sweeney, 1999) causing water loss (Kane et al, 1984) and reduction of electrolytes in the serum (Wakwe and okon, 1995 and Sweeney, 1999).

In contrast, a hyperkalemia in diarrheic calves may be due to dehydration and peripheral circulatory failure that results in an aerobic oxidation, liberation of lactic acid and occurrence of acidosis that leads to drawing of potassium from cell to plasma causing hyperkalaemia (Radostitis, et al, 1975).

Meanwhile, Mehnichuk, et al (1987) recorded that the value of sodium and potassium in the serum

of sick animals remain unchanged which testifies to the deep disturbances of water salt metabolism in tissues.

Treatment of diseased calves with amoxicillin potentiated clavulanate resulted in improvement of biochemical values toward the normal levels, however, the effect of the latter drug was more prominent.

The hematological investigation revealed a significant decrease in hemoglobin concentration of diseased calves; these observations may be attributed to the decrease in plasma volume (Schlerka, et al, 1995 and Carmalt et al, 2000).

Hematological examination revealed leukocytosis compared with healthy calves these hematological parameters regained its normal values after treatment with amoxicillin or amoxicillin potentiated clavulanate.

It could be concluded that parenteral treatment of diarrheic calves due to *E.coli* strains with Amoxicillin potentiated clavulinic acid is highly effective in reducing the clinical symptoms and improvement of general health condition.

Table (1) :- Susceptibility of *E.coli* isolates from diarrheic calves to different antimicrobials.

Antimicrobials	Disc potency	Mean Zone of inhibition (mm)	Sensitivity %	Resistance %
Amoxicillin (Am)	30	15.3 ± 2.9	50	50
Amox.+ clavulinic acid (Amc)	30	21.1 ± 1.85	90	10
Ampicillin (Amp)	10	13.8 ± 2.54	50	50
Ceftiofur sodium (Eft)	30	18.6 ± 2.23	80	20
Doxy cyclin (Do)	30	17.4 ± 1.65	30	70
Enrofloxacin (En)	10	27.0 ± 1.9	90	10
Flumequine (UB)	30	25.5 ± 2.39	80	20
Gentamycin (CN)	10	24.1 ± 0.77	80	20
Streptomycin (S)	10	21.3 ± 1.65	80	20

Mean= zone of inhibition, ± standard error n=10

Table (2):- Effect of parenteral administration of Amoxicillin or Amoxicillin and clavulanic acid on blood picture of diarrheic pre-weaning buffalo calves caused by colibacillosis (n=5).

Animal Parameter	Apparently healthy calves (control)	Diarrheic pre-weaning buffalo calves			
		Before treatment with Amoxicillin	7-days post treatment with Amoxicillin	Before treatment with Amoxicillin + clavulanic	7-days post treatment with Amoxicillin +clavulanic
Hemoglobin (gm/dl)	11.8± 0.17a	9.8± 0.13c	10.94± 0.4b	9.8± 0.21c	11.08± 0.15b
R.B.Cs (10 ⁹ /ul)	8.23± 0.11b	9.26± 0.18a	8.17± 0.1b	9.48± 0.09a	8.31± 0.1b
W.B.Cs (10 ³ /u l)	9.3± 0.08c	12.79± 0.25a	9.43± 0.1c	13.04± 0.35a	10.2± 0.01b
P.C.V %	40± 1.14ab	38.2± 1.23bc	42.2± 1.7ab	34.3± 1.25c	44± 1.56a
Platelets (10 ³ /u l)	377.8± 19.4c	715.2± 12.5a	377.6± 16.8c	756± 13.47a	474.6± 1.03b

Table(3):-Effect of parenteral administration of Amoxicillin or Amoxicillin and clavulanic acid on some biochemical values of diarrheic pre-weaning buffalo calves caused by colibacillosis (n=5).

Animals Parameter	Apparently healthy calves (control)	Diarrheic pre-weaning buffalo calves			
		Before treatment With amoxicillin	7-days post treatment with Amoxicillin	Before treatment with Amoxicillin + clavulanic	7-days post treatment with Amoxicillin +clavulanic
Total Protie (gm/dl)	6.5± 0.07a	5.4± 0.08c	6.2± 0.04b	5.5± 0.1c	6.4± 0.08a
Albumin (gm /dl)	3.27± 0.07a	2.62±0.06c	2.92± 0.17b	2.9± 0.43bc	3.47± 0.08a
Globulin (gm /dl)	2.87± 0.06a	2.35±0.09b	2.87± 0.065a	2.37± 0.08b	2.83± 0.07a
Total Ig (mg /dl)	1.9± 0.08a	1.6± 0.09b	1.73± 0.14ab	1.44± 0.08b	1.92± 0.012a
Sodium (m mol/l)	137.1±11.8ns	120± 8.5ns	132.8± 8.0ns	120± 8.1ns	134.8±8.95ns
Potassium (mmol/l)	4.68± 0.08a	3.61± 0.1c	4.2± 0.09b	4.07± 0.06b	4.9± 0.3a

N.s = No significant difference between groups

Mean values have different letters are significantly different from each other and vice versa and the highest mean value is represented with the letter (a) followed by the letters (b,c,.....etc.).

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الملخص العربي

تقييم علاج الإصابة بالميكروب القولوني العصوي في عجول الجاموس قبل الفطام باستخدام الأموكسيسيلين مع حمض الكلافولينيك (سينيولوكس) عن طريق الحقن
 أمينة السيد فرس، ماجدة معدوح محمد، أحلام السيد عبد اللطيف، رفعت خضري محمد .

معهد بحوث صحة الحيوان الزقازيق

تم تقسيم (عدد ثلاثون) عجل جاموسى عمر 1-2 شهر قبل الفطام فى احدى المزارع الخاصة بمحافظة الشرقية، الى عجول مصابة بالاسهال الطبيعى و عجول غير مصابة و سليمة . قسمت العجول المصابة بالاسهال الطبيعى الى مجموعتين متساويتين. عولجت المجموعة الأولى باموكسيسيلين طويل المفعول (كلاموكسيل) ® بجرعة 1 مللى لكل 15 كج من وزن الجسم والمجموعة الثانية عولجت بالاموكسيسيلين + حمض الكلافونيك (السينولوكس) بجرعة 1مللى لكل 20كج من وزن الحيوان ولمدة 3 أيام متتالية. ثم أخذت مسحات معقمة من المستقيم من كل من العجول المصابة بالأسهال والسليمة . و كشف الفحص البكتريولوجى من أن السلالات القولونية كانت للعامل الرئيسي المسبب للإسهال فى العجول المريضة . أجريت اختبارات حساسية السلالات المعزولة لمضادات الجراثيم المختلفة ، وأظهرت أن أموكسيسيلين مع حمض كلافولينيك كان فعالا للغاية.

تم أخذ عينات دم من العجول قبل وبعد العلاج لأجراء للدراسات البيوكيميائية والدموية ومقارنتها بالسليمة قبل العلاج وبعده . لوحظ انخفاض ملحوظ فى تركيز البروتين الكلى مع الزلال والجلوبولين المناعي فى العجول المريضة بالإضافة إلى نقصان الهيموجلوبين مع زيادة عدد الكريات البيضاء والصفائح الدموية زيادات كبيرة . بينما كان النقص فى الصوديوم غير مؤثر وعلى العكس كان النقص فى البوتاسيوم فى العجول المريضة معنوياً مقارنة بالعجول السليمة .

وكان العلاج بالأموكسيسيلين مع حمض الكلافونيك أكثر فعالية من الأموكسيسيلين وحده فى الحد من الأعراض المرضية وتحسين الصحة العامة للعجول المريضة .