COMPARISON OF TREATMENT TRIALS IN INDUCED *E. COLI* DIARRHOEA: AFFECTS ON SOME SERUM BIOCHEMICAL PARAMETERS

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ABSTRACT

Study was conducted on 24 calves of one-week-age, randomly divided into four equal groups. First three groups were experimentally infected with *E. coli* and given treatment in various combinations, while group four was taken as uninfected untreated control. Results obtained on different parameters showed an overall lower ($P<0.05$) creatinine in calves treated with antibiotic+electrolytes and those treated with electrolytes alone. Overall mean serum sodium, urea and immunoglobulins revealed non-significant difference, while pH, potassium and AP were lower ($P<0.05$) in all treatment groups. Overall mean serum ALT was lower ($P<0.05$) in calves treated with antibiotic+electrolytes, while AST was higher ($P<0.05$) in calves treated with antibiotic alone than control group. Serum total protein and globulins were higher ($P<0.05$) in calves treated with electrolytes alone than control group, while albumin was higher ($P<0.05$) in all treatment groups. Significant relationship of serum sodium with ALT ($r = 0.49$; $P<0.05$), AP ($r = 0.42$; $P<0.05$) and creatinine ($r = 0.52$; $P<0.01$) in normal, while no such relationship was observed in treatment/diarrhoeic calves. However, the increase in body weight in control calves correlated with better clearance of creatinine ($r = -0.56$; $P<0.01$) and was similar in antibiotic+electrolytes treated calves ($r = -0.56$; $P<0.0001$).

Key words: Buffalo calves, *E.coli* diarrhoea, antibiotic, electrolytes, serum enzymes.

INTRODUCTION

It has been reported that calf mortality of about 20 per cent can reduce net profit of up to 38 per cent and thus contribute to considerable economic losses directly to the farmer and indirectly to the country. The incidence of calf mortality in cattle and buffalo has been reported to range from 29.1-39.8 per cent in Pakistan (Afzal *et al.*, 1983). In the first month of age, diarrhoea accounts for 3.6-6.3 per cent mortality (Khan and Khan, 1991). Diarrhoea in calves is a syndrome of great aetiological complexity. The most frequently encountered organisms are entropathogenic *E. coli* strains and rotavirus (Khan and Khan, 1997).

The diarrhoeic calves show extreme metabolic acidosis, haemoconcentration and hypofunction of both kidney and liver (Maach *et al.*, 1992). Serobiochemical changes include hyponatraemia, hypochloraemia, hyperkalaemia, hypoglycaemia (Maach *et al.*, 1992) and hypoproteinaemia (Aly *et al.*, 1996). Treatment of experimentally infected calves with enterotoxigenic *E. coli* (09; K30; K99) showed that caloric oral rehydration solution with glutamine is more effective in correcting plasma extracellular fluid and blood volume (Brooks *et al.*, 1997). Michna *et al.* (1996) used 8.4 per cent sodium bicarbonate solution intravenously and found improvement in the general health of the calves in 3-24 hours, while Holck *et al.* (1994) reported that treatment with danofloxacin (1.25mg/Kg i.m.) significantly improved the clinical conditions and resulted in fewer mortalities. Aly *et al.* (1996) treated diarrhoeic calves with 20% ampicillin in combination with fluid therapy and observed great improvement in clinical symptoms, blood picture and blood serum constituents. As evident from the above studies various remedial measures have been suggested in overcoming the problem in calves. Present study was an attempt to see the effect of various treatments on serum electrolytes, protein fractions, liver and kidney functions in induced diarrhoea by *E.coli* (local strain) in buffalo male calves and also to find relationship between electrolyte imbalance and serum enzymes with reference to treatments. It was also aimed to compare treatment protocols.

MATERIALS AND METHODS

The study was carried out on 24, one-week-old male buffalo calves. These calves were randomly divided into four groups, having six calves in each. Initial blood samples from all the experimental calves were collected twice, within 48 hours before infection, i.e., at 24 hour's interval. Faecal samples of these calves were examined for parasites and enteropathogenic *E. coli* and only negative calves to these were included in the study.

Induction of infection, treatment protocol and composition of rehydration solution has been described elsewhere (Javed *et al.*, 2000). First group (G-1) was treated with rehydration solution plus kanamycin, the 2nd group (G-2) with kanamycin and the 3rd group (G-3) with rehydration solution till the recovery or maximum up to six days. The 4th group (G-4) served as control.

All the calves were kept under identical conditions of feeding and management. They were given 2 kg milk, thrice daily and water *ad libitum*. The study was conducted during October 1990 and the calves were kept in sheds during the night, while in the open under the sun in the noon hours.

Blood samples of about 3 ml without anticoagulant were collected from each calf both in the morning and evening time. Blood samples were collected from all the groups at 12 hours interval for first four days and at 24 hour interval for next two days. Blood was allowed to clot and serum was separated by centrifugation which was stored at -20 °C till further use.

Serum studies included serum total proteins, albumin, globulins, A:G ratio, immunoglobulin, sodium, potassium, creatinine, urea and enzymes including alanine transaminase, aspartate transaminase and alkaline phosphatase. Serum total proteins were determined by biuret method as described by Oser (1976). Albumin was determined by bromocresol green dye binding method as described by Varley *et al.* (1980). Globulins in the serum were estimated by subtracting the concentration of albumin from the total proteins. A:G ratio was determined by dividing albumin with globulin. Immunoglobulins were estimated in ZST units as recommended by McEwan *et al.* (1970). Sodium and potassium were determined by flame photometric method as described by Tennant *et al.* (1972). Serum creatinine and urea were determined spectrophotometrically by using commercially available kits (Randox Laboratories Ltd. Curmlin, UK Cat No.CR 524, UR 2316). Serum enzymes including alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (AP) were determined spectrophotometrically by using commercially available kits of (Randox Laboratories Ltd. Curmlin, UK Cat.No.AL 1205, AS 1204and AP 502). Data thus obtained were subjected to analysis of variance technique and means were compared by LSD and DMR on personal computer by using SAS 6.2.1 statistical software package (Anonymous, 1996). Pearson correlation coefficients between various parameters were also obtained.

RESULTS AND DISCUSSION

Due to diarrhoea, various serobiochemical changes take place including decrease in sodium (Dalton *et al.*, 1965, Tennant *et al.*, 1972, Deshpande *et al.*, 1993; Radositis *et al.*, 1994; Doll and Trenti, 1996), increase in potassium (Dalton *et al.*, 1965; Tennant *et al.*, 1972; Groutides and Michell, 1990; Sahal *et al.*, 1993; Radositis *et al.*, 1994; Kaske, 1994; Doll and Trenti, 1996), and increase in serum total protein (Radositis *et al.*, 1994). However, increase in sodium, decrease in potassium (Radositis *et al.*, 1994) and serum total protein has also been reported (Aly *et al.*, 1996; Joshi *et al.*, 1997). While increase in blood urea nitrogen (BUN), creatinine (Tennant *et al.*, 1972; Groutides and Michell, 1990; Sahal *et al.*, 1993; Brooks *et al.*, 1997), AST, ALT and AP (Grodzki *et al.*, 1991; Lechowski, 1996) have been reported.

Overall higher serum proteins, globulins and serum immunoglobulins observed in calves treated with antibiotic+electrolytes than those treated with antibiotic alone and control calves during present study (Table 1) suggest better functioning of the organs including liver and immune system resulting in the rise in serum proteins and immunoglobulins against *E. coli*. These results confirmed the finding of Aly *et al.* (1996) about decrease in serum proteins due to diarrhoea with subsequent improvement with treatment including antibiotic (Ampicillin) and fluid therapy. Groutides and Michell, (1990) reported better immunoglobulins status of survived calves after diarrhoea. The decrease in serum total proteins and immunoglobulins concentration that occurs due to diarrhoea (Aly *et al.*, 1996; Joshi *et al.*, 1997) was observed at the day one with a subsequent increase in these parameters during later stages in calves treated with antibiotic+electrolytes (Tables 1). The hyperproteinaemia mentioned earlier due to diarrhoea (Radositis *et al.*, 1994) was observed during present study, there was an increase in serum total proteins and globulins at day one with subsequent decrease afterwards. These findings suggest probable proportionate increase in serum total proteins and globulins due to diarrhoea during first 1-2 days in two groups of calves as an effect of loss of fluid from blood vessels. However, decrease in serum total proteins in calves treated with antibiotic+electrolytes (Table 1) was due to lower globulins, while albumin was relatively higher than other groups at same day and than zero day value. The decrease in globulins might occur due to loss of this protein fraction from the vessels or due to some other unknown reason. However, during

Table 1: Serum total proteins, albumin, globulins, immuneglobulins and A:G ratio of calves of treatment groups and control group in overall and at different days.

Groups	Days							
	0	1	2	3	4	5	6	Overall
Total Proteins								
1	b 8.19 ±0.08	b 6.55 ±0.83	b 12.77 ±4.38	a 8.16±1.52	b 8.08 ±2.30	b 7.17±3.53	b 7.13 ±3.80	AB 8.59±3.29
2	6.86±0.66	8.34±02.17	8.88±1.04	7.38±0.32	7.65±1.77	8.09±0.56	7.21±0.01	B 7.92±1.40
3	8.30±2.93	8.92±4.17	13.01±4.60	9.18±2.99	8.71±1.36	9.76±2.92	7.08±2.10	A 9.69±3.65
4	7.64±0.92	6.76±0.49	9.31±1.98	8.57±0.67	7.98±0.33	7.45±1.25	7.15±1.25	B 7.59±1.26
Albumin								
1	3.77±0.39	A 4.12±1.04	A 4.97±1.19	3.79±0.39	B 4.22±0.78	4.36±1.73	B 3.98±0.25	A 4.22±0.97
2	c 3.10±0.12	Bc 3.08±0.29	Ab 5.17±0.69	c 3.93 ±0.12	Ac 6.27±0.07	c 3.45±0.71	Ab 4.98±0.01	AB 4.03±1.14
3	3.19±0.41	AB 3.64±0.44	AB 3.86±0.79	3.98±1.27	B 4.02±0.30	4.09±0.12	B 4.26±0.08	AB 3.84±0.73
4	3.55±0.52	B 3.04±0.14	B 3.50±0.58	3.17±0.42	B 3.71±0.49	3.68±0.24	B 3.58±0.24	B 3.53±0.45
Globulins								
1	ab 4.41±0.46	b 2.43±0.79	a 7.79±4.65	ab 4.37±1.77	ABab 3.86±1.92	ab 2.80±4.88	ab 3.15±4.06	AB 4.38±3.29
2	ab 3.76±0.59	a 5.25±2.14	ab 3.72±1.52	ab 3.46±0.44	Bb 1.38±1.69	ab 4.64±0.16	ab 2.26±0.01	B 3.89±1.73
3	5.12 ±3.17	5.28±3.96	9.15±4.07	5.19±3.76	A 4.69±1.64	5.67±2.92	2.82±2.18	A 5.86±3.65
4	4.08±0.74	3.75±0.58	5.81±2.45	3.39±1.04	AB 4.27±0.27	3.57±1.08	3.57±1.08	B 4.06±1.28
ZST-units								
1	52.20±18.36	138.67±102.24	66.15±27.33	40.87±12.36	62.90±43.17	84.33±98.13	79.45±29.06	76.94±64.15
2	53.37±11.02	103.17±30.30	60.83±23.09	68.93±30.56	105.55±80.12	84.45±38.11	90.20±10.20	60.38±35.09
3	68.97±29.86	83.83±31.15	59.43±40.90	64.65±28.74	51.90±40.73	36.20 ±84.36	31.05±22.27	56.57±44.82
4	53.10±19.22	52.17±8.98	65.00±35.37	60.17±14.02	63.83±1.83	56.20±11.44	56.20±11.44	58.09±15.46
A:G ratio								
1	0.87±0.18	A 2.09±1.67	1.19±1.34	1.27±1.26	B 1.38±0.91	1.76±1.68	1.55±1.26	1.46±1.29
2	c 0.84 ±0.11	Bc 0.69±0.35	bc 1.56±0.59	c 1.15±0.19	Aa 3.43±1.39	c 0.74±0.18	b 2.29±0.02	1.28±0.92
3	0.85±0.63	AB 0.90±0.42	0.47±0.18	1.82±2.10	B 0.94±0.39	0.86±0.41	2.16±1.69	1.09±1.13
4	0.89±0.18	AB 0.81±0.14	0.69±0.35	1.03±0.47	B 0.88±0.17	1.16±0.36	1.15±0.35	0.95±0.31

Values in each row with different small letters and in each column with different capital letters are statistically different (P<0.05). Each figure represent mean±standard deviation.

Table 2: Serum sodium and potassium of calves of treatment groups and control group, in overall and at different days.

Groups	Days 0	1	2	3	4	5	6	Overall
Sodium								
1	125.60 ±8.23 ab	123.87±12.76 Abab	133.00±17.06 a	126.62±14.45 ab	121.93±10.06 Abab	101.53±32.03 b	115.05±7.14 ABab	123.39±16.87
2	124.17±1.71 ab	133.20±1.91 Aa	124.85±24.57 ab	121.00±15.26 ab	106.95±6.57 Bab	116.95±0.08 ab	102.70 ±0.01 Bb	122.98±14.20
3	128.43±1.74	115.80 B±12.24 B	111.00±10.49 A	116.68±19.99 A	127.93±12.38	113.43±6.91	122.15±3.61	117.74±13.05
4	126.90±3.03	121.20±4.68 AB	122.93±7.23 A	126.73±1.29 A	127.50±2.72	127.77±2.75	126.73±4.10	125.68±4.19
Potassium								
1	7.13±1.98 a	6.03±0.99 abc	4.30±1.41 Bc	5.22±1.19 Babc	6.46±0.69 ab	4.63±0.91 Bbc	5.54±2.32 abc	5.48±1.37 B
2	5.82±0.54 ab	6.57±1.17 a	4.75±0.86 Bbc	4.42±0.49 Bbc	6.09±0.03 ab	4.29±0.16 Bbc	3.97±0.02 c	5.37±1.19 B
3	7.31±1.11 a	6.17±1.33 ab	4.18±0.37 Bc	5.47±1.18 ABabc	6.93±1.74 ab	5.87±0.16 ABabc	5.13±1.49 bc	5.71±1.41 B
4	5.70 ±0.37	6.10±0.69	7.44±1.64 A	7.07±1.53 A	6.51±1.07	7.74±1.83 A	6.67±1.36	6.75±1.29 A

Values in each row with different small letters and in each column with different capital letters are statistically different (P<0.05). Each figure represent mean"standard deviation.

Table 3: Serum creatinine, urea and blood pH of calves of treatment groups and control group in overall and at different days.

Groups	Days 0	1	2	3	4	5	6	Overall
Creatnine								
1	1.57±0.53	1.65±0.28	1.42±0.32	1.37±0.17 AB	1.42±0.14	1.16±0.08 B	1.48±0.11	1.45±0.28 B
2	1.87±0.40	1.57±0.25	1.39±0.31	1.67±0.23	1.81±0.18 A	1.48±0.06 AB	1.47±0.01	1.60±0.25 AB
3	1.62±0.19	1.66±0.27	1.45±0.34	1.42±0.25	1.24±0.20 B	1.40±0.32 AB	1.59±0.11	1.49±0.28 B
4	1.82±0.06	1.58±0.51	1.53±0.48	1.65±0.22	1.68±0.25 A	1.76±0.10 A	1.88±0.16	1.70±0.28 A
Urea								
1	51.23±17.83 b	50.13±10.74 Bb	83.85±11.93 Aa	97.17±24.00 a	38.43±8.73 Bbc	16.27±11.70 Bc	32.05±3.75 bc	60.99±30.72
2	63.87±37.42 ab	100.10±49.27 Aab	89.03±24.47 Aab	129.40±70.85 a	28.45±11.10 BCb	27.65±14.35 ABb	16.70±11.02 b	81.58±53.53
3	70.57±7.23 a	83.87±25.94 ABa	71.38±21.29 ABa	102.12±44.84 a	13.47±7.15 Cb	21.67±11.00 Bb	21.30±17.54 b	65.65±39.91
4	74.33±26.52	68.90±36.78 AB	45.97±21.63 B	84.60±17.54	63.57±8.39 A	56.10±20.40 A	51.63±23.10	63.59±23.44
Blood pH								
1	6.90±0.20	6.77±0.21 B	6.72±0.19	6.83±0.20	6.80±0.26	6.73±0.21	6.70±0.01	6.78±0.19 B
2	7.03±1.53 ab	6.63±0.12 Bc	6.80±0.18 abc	6.68±0.21 c	6.55±0.07 c	6.70±0.28 bc	7.10±0.01 a	6.75±0.22 B
3	6.76±0.12	6.72±0.22 B	6.77±0.19	6.82±0.22	6.80±0.10	6.80±0.26	6.90±0.14	6.78±0.18 B
4	6.93±0.25	7.03±0.15 A	6.87±0.15	6.93±0.25	6.83±0.11	6.77±0.11	6.93±0.21	6.90±0.18 A

Values in each row with different small letters and in each column with different capital letters are statistically different (P<0.05). Each figure represent mean±standard deviation.

Table 4: Serum ALT, AST and AP of calves of treatment groups and control group in overall at different days.

Groups	Days							
	0	1	2	3	4	5	6	Overall
ALT								
1	26.00±13.75	14.67±6.06	20.67±6.59	19.33±3.67	24.00±6.00	20.67±4.51	20.00±15.56	20.00±7.40^{B}
2	20.67±8.96^{ab}	15.00±4.15^{b}	19.00±6.16^{ab}	22.25±5.91^{ab}	33.00±4.24^{a}	29.00±12.72^{ab}	17.00±0.01^{b}	20.82±7.91^{AB}
3	32.67±11.85^{a}	18.50±2.81^{bc}	16.33±4.89	20.67±10.17^{c}	30.00±5.29^{abc}	21.33±5.13^{ab}	23.00±1.41^{abc}	21.76±8.19^{AB}
4	21.67±8.33	15.00±1.00	17.67±0.01	31.33±14.15	30.00±10.54	31.00±8.00	32.00±13.89	25.52±10.63^{A}
AST								
1	128.00 ±4.58	126.67±20.92	110.17±20.19	114.00±25.39	126.67±17.01^{AB}	129.00±26.00	108.00±11.31	119.72±20.24^{AB}
2	112.33±13.01	136.67±59.98	100.25±23.91	124.25±30.96	167.00±66.47^{A}	143.50±86.97	79.00±0.01	125.23±46.28^{A}
3	91.00±7.81^{b}	106.50±7.53^{ab}	92.67±19.99^{b}	103.67±11.29^{ab}	117.00±1.73^{ABa}	100.33±7.77^{ab}	93.50±0.71^{b}	101.00±13.35^{C}
4	111.33±14.57	115.67± 14.47	118.00±17.06	100.67±22.05	97.00±15.52^{B}	113.33±18.50	07.67±18.58	109.09±16.35^{BC}
AP								
1	742.67±230.93^{a}	357.17±205.34^{b}	307.00±112.53^{ABbc}	293.67±84.77^{Bbc}	184.33±66.43^{bc}	125.00±51.11^{Bbc}	103.50±2.12^{c}	314.14±210.63^{B}
2	394.33±302.99	342.17±129.05	269.50±101.94^{B}	397.25±115.38^{AB}	106.00± 5.66	89.00±22.63^{B}	169.00±23.01	293.73±168.76^{B}
3	630.33±373.05^{a}	377.50±216.47^{abc}	444.83±120.45^{Aab}	406.17±150.31^{ABabc}	106.00±53.33^{c}	137.00±106.33^{Bc}	154.00±79.19^{bc}	355.14±226.50^{B}
4	680.33±407.63	508.30±280.34	471.67±121.11	527.00±200.43	548.30±440.02	456.33±196.31	564.67±411.47	536.67±281.98

Values in each row with different small letters and in each column with different capital letters are statistically different ($P<0.05$). Each figure represent mean±standard deviation.

last day, in all the groups, serum total protein was close to the normal calves and so was globulin.

Immunoglobulins (ZST-units) were relatively higher in all the treatment groups and remained higher throughout the study than control calves (Table 1). However, lower immunoglobulins in the last few days in calves treated with electrolytes alone could not be explained. Higher immunoglobulin level estimated through ZST test has also been reported previously in survived calves due to diarrhoea (Groutides and Michell, 1996). Overall A:G ratio in normal calves was less than one and was similar as suspected by Coles (1980). However, in diarrhoeic/treatment groups, A:G ratio was more than one suggesting either increase in albumin (may be due to dehydration; Kaneko, 1989) or decrease in globulins. Higher albumin and globulins in treatment than control calves is very much suggestive of increase in these probably due to dehydration. The present data of serum proteins including A:G ratio suggests that dehydration could not be corrected in few days rather recovery in clinical picture is early but in biochemical profile is late. Overall serum sodium concentration in all the treatment groups was nearly the same as for control calves (Table 2). However, serum potassium was lower in all treatment than control calves (Table 2). This suggests that sodium concentration due to diarrhoea is not much affected or the correction is early and rapid due to treatment, while it appears that potassium loss is more and/or its correction is rather slow. The hyponatraemia due to diarrhoea (Dalton *et al.*, 1965; Tennant *et al.*, 1972; Deshpande *et al.*, 1993; Radositis *et al.*, 1994; Aly *et al.*, 1996; Doll and Trenti, 1996) was not observed during present study perhaps owing to early treatment. Present findings of hypokalaemia in all treatment groups was similar with those as reported by Groutides and Michell, (1990) in surviving calves, while hyperkalaemia in dying calves, which was also observed during present study with 6.91 mean values in dying calves.

Lower ($P<0.05$) blood pH in treatment than control calves was observed but overall pH was not less than 6.75 (Table 4). However, calves those died were having pH of 6.73. Dalton *et al.* (1965) and Hartmann *et al.* (1997), however, observed 6.85 blood pH in dying cow calves, while they reported 7.38 blood pH in normal cow calves and 7.29 in recovered calves. Tennant *et al.* (1972) observed pH of 7.08 in diarrhoeic calves but they reported range to 6.88 of 7.28. The present findings probably suggests that blood pH in buffalo calves is lower than cow calves.

Most significant affected enzyme was AP, which was lower in all the treatment groups than control group (Table 4). However, AST was higher in antibiotic treated, while lower in electrolytes treated calves and ALT was lower only in antibiotic+electrolytes treated calves (Table 5). These findings of invariably lower AP and ALT suggest the importance of these enzymes as a response of liver to various treatments than AST. The ALT showed gradual increase from day one value, till day four but later on showed invariable results, while AP showed invariable decrease till day six in all the treatment groups. The AP showed significant changes for which further studies are required to suggest usefulness of this enzyme in diarrhoea/treatment. The increase of serum AP, ALT and AST as reported by Groutides and Michell, (1990) and Lechowski, (1996) was not observed during present study. The difference in these studies might be due to the very early start of treatment where involvement of the liver probably has not yet taken place. The decrease in levels of some enzymes observed were probably due to effect of long storage at $-20\ ^{0}C$ (six months storage) or the effect of treatment (as a dilution effect or on the liver). Values of AP & ALT in dying calves were lower, while of AST was higher during present study (Table 4) and are otherwise as reported by Benjamin (1978) in dying diarrhoeic calves. The lower values for these enzymes during present study in dying calves were probably due to the effect of treatment.

Overall serum urea showed no variation in treatment and control calves, but creatinine was lower, both in calves treated with electrolytes alone or in combination with antibiotic (Table 4). However, urea concentration rose initially till day three of treatment, in all the treatment groups, but fell sharply after day four (Table 3). This probably suggests improvement in Glomerular Filtration Rate (GFR) from day four onward or decrease production of urea as an effect of treatment. Serum creatinine decreased invariably gradually in all the treatment groups and was lower in the last two days, in all the treatment groups than the control group (Table 3). This suggests an improved GFR toward later stages of treatment or effect of treatment causing lower levels for both urea and creatinine. The improvement in the GFR in diarrhoeic calves due to treatment with oral rehydration, nutritional ORS has already been reported (Brooks *et al.*, 1997).

The significant relationship of serum sodium with ALT ($r = 0.49$; $P<0.05$), AP ($r = 0.42$; $P<0.05$) and creatinine ($r = 0.52$; $P<0.01$) in normal, while no such relationship in treatment/diarrhoeic calves suggests its importance in evaluating the response toward treatment. This probably suggests that the normalization of these parameters occur much later. The increase in body weight in control calves correlated with better clearance of creatinine ($r = -0.56$; $P<0.01$) and was similar in antibiotic+electrolytes treated calves ($r = -0.56$; $P<0.0001$). This suggests effectiveness of this treatment compared with other treatments.

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