COMPARATIVE EFFICACY OF THREE PROTOCOLS FOR THE TREATMENT OF HAEMORRHAGIC SEPTICAEMIA IN BUFFALOES AND CATTLE

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ABSTRACT

A total of 50 animals (39 buffaloes and 11 cattle) suffering from haemorrhagic septicaemia (H.S.), selected from the field, were divided into 3 groups (A, B, C) and assigned randomly to either of three treatment protocols viz. A, B and C. Group A (20 animals) was treated with protocol A i.e. norfloxacin (Norfloxillin; Tarobina) + diclofenac sodium + Novacoc forte (Richter Pharma). Group B (20 animals) was treated with protocol B which was essentially the same as protocol A except that Novacoc forte was omitted. Group C (10 animals; control) was treated with one of conventional therapies of H.S. i.e. gentamicin + dipyron. The disease severity index of study animals under each treatment protocol was recorded at 0, 12, 24, 48, and 72 hours from the start of treatment. All the treatments were started after application of cold water on head for 15 minutes. The treatment was repeated at 12, 24 and 48 hours after start of treatment as the situation warranted. The survival per cent was 85, 80 and 30 amongst animals treated respectively with protocol A, B and C. Three animals in group A and 2 in group B died after recovering completely. Excluding these animals from the recovered ones translated into net survival (percentage) of 70, 70, and 30 in group A, B, and C, respectively. In terms of reduction of severity of disease, there was significance difference ($P \leq 0.05$) between protocol C and A and between protocol C and B at 12 and 48 hours after treatment but there was non-significant difference at hour 24. Both treatment protocols A and B exploiting the use of a quinolone (norfloxacin) plus a non-steroidal anti-inflammatory drug (diclofenac sodium) with or without a toxin neutralizer and circulatory-stimulant (Novacoc forte) were more effective than one of the conventional treatments i.e. gentamicin + dipyron for the treatment of H.S.

Keywords: Haemorrhagic septicemia, pasteurellosis, chemotherapy, *Pasteurella multocida*

INTRODUCTION

In terms of morbidity as well as mortality, haemorrhagic septicaemia (H.S.) is one of the economically important diseases of buffalo and cattle (Ajmal *et al.*, 1988). The pathogenicity of the aetiologic agent of H.S. (*Pasteurella multocida)* is mediated by endotoxins. The circulating organism and endotoxins trigger an immunological and inflammatory cascade and release of a number of host-derived mediators of inflammation such as prostaglandins and leukotrienes, which are responsible for clinical signs, pathological changes and death (Carter and De-Alwis, 1989). Studies have demonstrated that selective inhibitors of arachidonic acid metabolism (non-steroidal anti-inflammatory drugs; NSAIDs) can have beneficial influence on the patho-physiologic consequences of endotoxaemia (Slauson and Cooper, 1990; Eades, 1993; Barragry, 1994; Cunningham and Lees, 1994). However, there is no published report on the use of NSAIDs in the treatment of H.S.

Quinolones is a new group of antibiotics which is specifically effective against Gram-negative bacterial infections even at low MIC levels. Field results show that this class of antibiotic is exceptionally useful in the treatment of bovine respiratory disease (Barragry, 1994). Giles *et al.* (1991) documented a 100% recovery rate with the use of danofloxacin (a quinolone) among housed beef cattle suffering from pneumonic pasteurellosis. Although quinolones have been available for veterinary use in developing countries for some years now, published work on the efficacy of these drugs in the treatment of H.S. is limited to a solitary incomplete report (Muhammad, 1994). The need for such data is very pressing in view of the appallingly low (26.67 to 27.3%) recovery rate reported with the use of sulphadimidine (Khan *et al.*, 1983; Sharif, 1993) which has been one of the most widely used drugs for the treatment of H.S. until recently.

In view of the septicaemic nature of H.S. and peripheral circulatory collapse, the affected animals theoretically appear to be the good candidates for

such adjunct drugs as Novacoc forte (a circulatory stimulant). This therapeutic cocktail is claimed to be effective in the treatment of diseases associated with high fever, circulatory collapse and systemic intoxication. However, the role of this drug as a therapeutic adjunct in the treatment of H.S. has not been evaluated thus far. The present study was, therefore, designed to evaluate the comparative efficacy of norfloxacin (a fluoroquinolone) plus diclofenac sodium (an NSAIDs) with or without Novacoc forte in the treatment of H.S.

MATERIALS AND METHODS

Study Animals And Treatment Protocols

A total of 50 animals (39 buffaloes and 11 cattle) of either sex suffering from haemorrhagic septicaemia were selected from the field cases of this disease in and around Faisalabad. Disease was diagnosed clinically on the basis of characteristic features i.e. sudden onset, high rise of body temperature upto 108°F, dullness, anorexia, increased pulse and respiratory rate, oedematous swelling on throat, brisket and upper dewlap region and some time on the face, difficult breathing, nasal discharge, salivation and reluctance to move (Carter and De-Alwis, 1989; Radostits *et al.*, 1994; Jindal *et al.*, 1996). The study period spanned from July to October, 1995. The age spectrum of animals ranged from six to eighty four months. These animals were randomly divided into three groups i.e. A, B and C comprising 20, 20 and 10 animals respectively and were assigned randomly to either of the three treatment protocols given hereunder:

Treatment Protocol A: (15 buffaloes, 5 cattle; mean age 29.15 ± 25.99 months)

i. Application of cold water on head for 15 minutes, repeated 4 times a day.
ii. Diclofenac sodium (Diflosid, Geofman Pharmaceuticals, Pakistan) containing 25 mg/mL @ 1 mL/20 kg body weight administered IM.
iii. Norfloxacin (Norfloxillin, Shin-il, Korea; Tarobina Corporation) containing 50 mg/ml @ 1 mL/10 kg body weight, IM, 15 minutes following cold water irrigation of head, and
iv. Novacoc forte (Richter Pharma, Austria; Waseem Impex Corp.) @ 50–250 mL/animal depending upon the body weight, IV.

Treatment Protocol B: (17 buffaloes, 3 cattle; mean age 26.5 ± 23.71 months)

It was essentially the same as treatment protocol A except that Novacoc forte was omitted.

Cold water irrigation of head was repeated 4 times a day. Norfloxacin was repeated at 12, 24, 48 and 72 hours (as the situation warranted) following the start of treatment.

Treatment Protocol C: (7 buffaloes, 3 cattle; mean age 28.8 ± 25.1 months):

It was one of the conventional haemorrhagic septicaemia therapies already in vogue. This protocol comprised:

i. Application of cold water on head for 15 minutes, repeated 4 times a day.
ii. Metamizole sodium (Dipyron; Hydro Pharmaceutical, Pakistan) containing 500 mg/mL @ 3 mL/50 kg body weight, IM, and
iii. Gentamicin sulphate 10% (Kepro Gentaject; Kepro B.V., Holland; BTI) @ 1 mL/20 kg body weight, IM, following cold water irrigation of head.

Monitoring of Treatment Response

The severity of the 7 different clinical signs (dullness, pyrexia, swelling at throat, drooling of saliva, tachypnoea, nasal discharge and feed intake) were scored on a scale of 2 to 6 as per Ramzan (1995); 2 being for mild, 4 for moderate and 6 for severe. Disease severity index was monitored in each animal before and after treatment and the per cent decrease in severity index of each animal following treatment was calculated. The aggregate clinical score of each animal was calculated by addition of the score of all clinical signs in each animal at a 0 hour and then at hr 12, 24, 48 and 72 post-treatment. The group severity index at a particular time was obtained by addition of the aggregate clinical score of all animals of that group and dividing the sum by the number of animals in that group. An animal was considered cured when:

i. Body temperature returned to normal,
ii. Animal started eating,
iii. Respiratory difficulty disappeared, and
iv. Animal showed normal demeanour.

When an animal died after recovering and remaining normal for 2–4 days of cessation of treatment, it was considered cured even if it died later.

Statistical Analysis

The data collected before treatment, 12, 24, 48 and 72 hours after the treatment was statistically analysed by one way analysis of variance (Steel and Torrie, 1980). In order to assess the therapeutic value of protocol A and B vis-a-viz one of the conventional H.S. treatments (i.e. protocol C), odd ratios between protocol A and C and protocol B and C were calculated. The odd ratio was defined by the formula given hereunder (Dohoo, 1987):

$$\text{Odd ratio A \& C} = \frac{\text{Proportion cured Protocol A / Proportion not cured Protocol A}}{\text{Proportion cured Protocol C / Proportion not cured Protocol C}}$$

$$\text{Odd ratio B \& C} = \frac{\text{Proportion cured Protocol B / Proportion not cured Protocol B}}{\text{Proportion cured Protocol C / Proportion not cured Protocol C}}$$

RESULTS

Homogeneity of Disease Severity Among Different Groups

Pre-treatment severity of the disease varied in each animal. Mean value of cumulative severity scores for animals treated with Protocol A, B and C, respectively were 36.8, 35.9 and 35.4. Thus the individuals in three groups differed non-significantly ($P \geq 0.05$). Animals under Protocol A, B and C had cumulative weighted score ranging respectively from 30–40, 30–42 and 30–40.

Comparative Efficacy of Three Treatment Protocols

Average per cent reduction in cumulative severity score in each group at different hours of treatment varied. This has been presented in Table 1.

The efficacy of 3 different treatment protocols in terms of survival rate is presented in Table 2. The highest survival rate (85%) was observed in animals treated with Protocol A, followed by B (80%), and the least survival rate (30%) was recorded among animals treated with Protocol C. Four, 2 and none of the animal treated respectively with protocol A, B and C recovered with the first treatment. Therefore, most animals required 2–4 rounds of treatment. This repetition of treatment for different treatment protocol is given in Table 3. Three animals in group A and 2 in group B died after recovering completely. Excluding these animals from the recovered ones translated into net survival (percentage) of 70, 70, and 30 in group A, B, and C, respectively.

Pre-treatment mean disease severity scores among animals of 3 different treatment protocols differed non-significantly ($P > 0.05$). These differences among protocol A, B and C were highly significant ($P < 0.01$) at 12 and 48 hours after initiation of treatment. However, at hour 24 and 72 post-first treatment, differences in disease severity index between 3 treatment protocols were non-significant ($P > 0.05$). The net differences in disease severity index over the entire treatment period among 3 treatment protocol were significant ($P < 0.05$).

Summary statistics of comparative efficacy of three treatment protocols tested on 50 cases of haemorrhagic septicaemia has been presented in Table 5.

DISCUSSION

The per cent survival among animals treated with protocol A, B and C was 85, 80 and 30, respectively. Difference in recovery rate between protocol A and B was non-significant. The similarity of protocol A to protocol B (both based on the use of norfloxacin and diclofenac acid) may account for this non-significant difference between these two protocols in terms of survival rate. Nonetheless, a slightly higher (85%) recovery rate with protocol A than protocol B (80%) may be attributed to the inclusion of Novacoc forte (a therapeutic cocktail with circulatory stimulation and intoxication-neutralizing properties) in the former treatment protocol. Survival rates with both protocol A (85%) and B (80%) were significantly higher than with protocol C (30%). Higher recovery rates with protocol A and B may be due to following reasons:

Quinolones are specific for Gram-negative infections particularly those caused by *Pasteurellae* (Giles, 1991; Broome and Brooks, 1992; Raemdonck *et al.*, 1992).

The non-steroidal anti-inflammatory drug viz. Diclofenac sodium used in the present study might have exerted a beneficial influence on the pathophysiologic consequences of H.S. endotoxaemia (Conlon, 1988; Slauson and Cooper, 1990). Metamizol (Dipyron) used in protocol C is a weak inhibitor of arachidonic acid metabolism.

A circulatory stimulant (Novacoc forte) was component of the treatment protocol A. Circulatory stimulant (Novacoc forte) and diclofenac sodium (a NSAID) were not included in protocol C.

Another reason for higher recovery rates with protocol A and B may be repetition of therapy at 12 hour interval which is two order of the frequency recommended by manufacturer. Norfloxacin and diclofenac sodium were repeated at 12 instead of 24 hours intervals because our own observations and those of other practicing veterinarians would indicate that the H.S. cases respond temporarily with partaking of feed and water but worsen again if the repetition is deferred until 24 hours. Animals treated with conventional H.S. treatment (protocol C) received repeat treatment(s) as per manufacturer's recommendations. A higher mortality rate in protocol C may be ascribed in part at least to Jarisch-Herxheimer reaction (Smith, 1986) reported in human patients due to administration of bactericidal drugs in the treatment of Gram-negative bacterial infections.

To assess the therapeutic value of two new H.S. treatments (i.e. protocol A & B) vis-a-viz one of the conventional treatments of this disease (protocol C), odd ratios between protocol A and C and between B and C were calculated. As used in the context of the present study, odd ratio referred to the odds (chance) of the new therapies (i.e. protocol A and B) curing H.S. and dividing that by the odd of the conventional H.S. therapy (protocol C) resulting in H.S. cure (Dohoo, 1987). The advantages of odd ration are two-fold. Firstly it is more likely to hold constant across trial than an absolute difference in cure rates. Secondly,

Table 1: Per cent reduction in cumulative disease severity index in cured animals with 3 different treatment protocols at different hours of initiation of treatment

Hours	% Reduction In Cumulative Disease Severity With Treatment Protocol		
	A	B	C
12	54.59	55.76	27.37
24	71.22	71.09	66.10
48	99.00	98.94	94.35
72	100.00	100.00	100.00

Table 2: Per cent survival among cases of haemorrhagic septicaemia treated with 3 different treatment protocols

Treatment Protocol	Total No. Of Animals Treated	No. Of Recovered Animals	Per cent Survival
A	20	17	85
B	20	16	80
C	10	3	30

Table 3: Number of treatments required to affect a cure among cases of haemorrhagic septicaemia treated with 3 different protocols

Treatment Protocol	Animals Treated	Per cent Animal Requiring			Cumulative % Survival
		2nd Dose	3rd Dose	4th Dose	
A	n = 20	76	59	12	85
B	n = 20	87	63	19	80
C	n = 10	100	100	100	30

Table 4: Mean disease severity scores in each treatment protocol at different hours of treatment among recovered animals

Hours	Mean Disease Severity Score in Treatment Protocol		
	A	B	C
Initial (0)	36.80 ± 0.59	35.90 ± 0.83	35.40 ± 1.04
12	16.71 ± 1.26	15.88 ± 1.23	25.71 ± 0.92
24	10.59 ± 0.61	10.38 ± 0.94	12.0 ± 0.00
48	0.24 ± 0.16	0.38 ± 0.20	2.00 ± 0.00
72	0	0	0

Table 5: Summary statistics of comparative efficacy of three treatment protocols tested on spontaneous cases of haemorrhagic septicaemia

Particulars	Treatment Protocol		
	A	B	C
Animals treated	20	20	10
No. of treatments given	49	51	28
Mean No. of treatments per animal	2.45	2.55	2.80
Median No. of treatment	3	3	3
Range No. of treatment	1–4	1–4	1–4
Per cent mortality	15	20	70
Per cent survival	85	80	30
Odd ratios with Protocol C	13.19	9.30	N.A.

there is a large body of statistical techniques developed for analysing odds ration which enable either variables such as herd effect to be easily taken into account. Odd ratio between treatment protocol A and C was 13.19 and between B and C was 9.30. These figures underscore the superiority of new treatments (protocol A & B) over one of conventional treatments (protocol C).

In the present study, an animal was considered arbitrarily cured when:

i. Body temperature returned to normal;
ii. Respiratory difficulty disappeared;
iii. Animal started eating; and
iv. Animal showed an almost normal demeanour.

The animal which died after an apparent recovery were still considered cured. Including these subjects among the deads results in a considerable decrease in the net survival rate.

In conclusion, both treatment protocols A and B exploiting the use of a quinolone plus an NSAID with or without circulatory stimulant (Novacoc forte) were superior to one of the conventional treatments (gentamicin + dipyron). In view of the preliminary nature of this work, further work is warranted.

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