

Hemato-biochemical and therapeutic studies on *Escherichia coli* associated with concurrent enteric infection in lambs

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Abstract

Aim: The trials were designed on 30 lambs of age below 2 weeks exhibiting the symptoms of colibacillosis.

Materials and Methods: Isolation of organism and identification of pathogenic *Escherichia coli* was confirmed by standard cultural, microbiological, serological, and biochemical tests. Venous blood was collected aseptically for hematological studies.

Result: Bacteriological examinations of fecal sample were positive for *E.coli* and isolates identified on the basis of morphological, cultural, and biochemical characteristics provided further confirmation. Hematological indices recorded significant ($p > 0.05$) increase in the packed cell volume, total leucocyte count, lymphocyte, and neutrophil percentage, whereas biochemical indices recorded significant decrease in the serum glucose, protein and serum albumin/ globulin levels. Treatment regimen in four different groups, suggested, gentamicin and ciprofloxacin effective drugs followed by neomycin and co-trimoxazole.

Conclusion: Study suggested significant ($P > 0.05$) alteration in hemato-biochemical parameters whereas results indicated gentamicin and ciprofloxacin as most effective drugs.

Keywords: enteric infection, *Escherichia coli*, hemato-biochemical, lambs

Introduction

The success of sheep farming is largely based on the good health status and low mortality rate during earlier life. This is measured in terms of lambs successfully reared in a given season which can be achieved by decreasing early mortality rate over a shorter period of time [1]. Epidemiological studies on diarrhoea in lambs have shown that *Escherichia coli* is an important cause of neonatal mortality [2, 3]. Colibacillosis is one of the important diseases in farm animals caused by pathogenic serotypes of *E. coli* [4]. *Escherichia coli* has been associated with two forms of infections; enteric and septicemic infection. - The enteric form is more commonly observed in 2 to 8 day old lambs, and characterized by varying degree of diarrhoea, dehydration, and huge mortality in untreated cases. The *E.coli* infection has been reported to be associated with diarrhea in neonate farm animals [5]. The pathophysiology of colibacillosis is due to K99 + adhesin antigen and heat stable enterotoxin, the major virulence attributes of enteric strain of *E.coli* in farm ruminants [6, 7]. It is a significant cause of economic loss in raising sheep and one of the important zoonotic diseases [8, 9]. In the present investigation, a detailed

study of hemato-biochemical parameters of diarrheic lambs with pathogenic *E.coli* isolated and evaluation of different therapeutic regimens for treatment of colibacillosis was undertaken.

Materials and Methods

Ethical approval: Study was performed according to the 'Guidelines for Animal Experimentation' approved by the Institutional Animal Care Committee.

The clinical trials were designed on thirty lambs exhibiting the symptoms of diarrhea, dehydration, fever, and anorexia. These animals were divided into five groups (six animals each). Rectal swabs were collected aseptically for the confirmation and identification of pathogenic *E. coli* by standard cultural, microbiological, serological and biochemical tests [10]. Four drug regimens were used in four groups which included four different antibacterial (Ciprofloxacin-group II, Co-trimoxazole-group-III, Gentamicin-group-IV, Neomycin-group-V), whereas group-I of six healthy lambs was kept as healthy control group. Only those antibacterial were used which showed moderate to high *in-vitro* sensitivity against *E. coli* isolates, which were readily available and economically feasible. For hematological studies blood was collected from each animal of all groups in clean sterilized glass vials containing EDTA, as anticoagulant. For separation of serum 10 ml of blood was collected in sterile centrifuge tubes and kept in slanting position for about an hour at

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Table-1. Hematobiochemical values (Mean \pm SE) before and after treatment

Groups	Healthy	Infected and untreated	Ciprofloxacin	Co-trimoxazole	Gentamicin	Neomycin
Hb						
0 day	12.45 \pm 0.47 ^{aA}	11.98 \pm 0.43 ^{abAB}	11.37 \pm 0.35 ^{abBC}	11.70 \pm 0.32 ^{bBC}	11.73 \pm 0.36 ^{abA}	13.42 \pm 0.57 ^{aA}
6 day	12.62 \pm 0.33 ^{aAB}	11.03 \pm 0.32 ^{bBC}	11.70 \pm 0.55 ^{abBC}	11.790 \pm 0.55 ^{abBC}	11.17 \pm 0.33 ^{bC}	12.45 \pm 0.51 ^{abAB}
PCV						
0 day	36.75 \pm 0.60 ^{aA}	40.30 \pm 1.92 ^{abAB}	41.33 \pm 1.77 ^{abBC}	34.24 \pm 1.73 ^{CA}	43.17 \pm 1.42 ^{bcB}	39.00 \pm 1.34 ^{abA}
6 day	35.89 \pm 0.54 ^{aA}	43.67 \pm 1.96 ^{ab}	44.58 \pm 1.49 ^{bB}	38.00 \pm 1.83 ^{bcA}	39.67 \pm 1.09 ^{cd}	37.00 \pm 1.53 ^{baD}
TLC						
0 day	5.41 \pm 0.91 ^{abA}	7.13 \pm 0.23 ^{abC}	6.68 \pm 0.18 ^{bcC}	6.20 \pm 0.25 ^{cAC}	6.62 \pm 0.26 ^{bcC}	5.79 \pm 0.29 ^{CA}
6 day	6.00 \pm 0.29 ^{baC}	7.10 \pm 0.23 ^{ab}	7.08 \pm 0.31 ^{bcC}	7.11 \pm 0.33 ^{bC}	7.58 \pm 0.19 ^{bC}	6.67 \pm 0.20 ^{bb}
Neutrophils						
0 day	25.66 \pm 0.67 ^{abBC}	26.16 \pm 1.10 ^{abC}	20.00 \pm 1.39 ^{bB}	22.83 \pm 1.35 ^{cAB}	25.67 \pm 0.80 ^{ba}	24.83 \pm 1.54 ^{abAB}
6 day	26.50 \pm 0.99 ^{aA}	26.00 \pm 1.36 ^{aA}	22.83 \pm 1.08 ^{ba}	25.67 \pm 0.56 ^{baBC}	25.00 \pm 0.68 ^{abC}	21.67 \pm 1.63 ^{bb}
Lymphocytes						
0 day	49.16 \pm 0.48 ^{bcA}	69.33 \pm 2.62 ^{abB}	54.83 \pm 1.38 ^{aC}	53.83 \pm 1.33 ^{aC}	57.17 \pm 1.60 ^{aC}	60.33 \pm 3.25 ^{ac}
6 day	50.17 \pm 0.48 ^{CA}	65.67 \pm 3.02 ^{abbB}	47.00 \pm 1.24 ^{ba}	50.33 \pm 0.67 ^{ba}	49.17 \pm 0.61 ^{ba}	50.00 \pm 1.59 ^{ba}
TEC						
0 day	5.38 \pm 0.18 ^{ab}	7.10 \pm 0.36 ^{aA}	5.90 \pm 0.28 ^{bc}	6.53 \pm 0.21 ^{bcB}	6.56 \pm 0.31 ^{bcB}	6.29 \pm 0.22 ^{aC}
6 day	6.69 \pm 0.20 ^{ab}	7.55 \pm 0.19 ^{aA}	6.68 \pm 0.35 ^{aA}	7.26 \pm 0.35 ^{aA}	7.44 \pm 0.31 ^{aA}	6.20 \pm 0.24 ^{bcB}
Blood glucose						
0 day	78.49 \pm 2.39 ^{aA}	46.34 \pm 1.65 ^{ab}	56.16 \pm 2.62 ^{ab}	52.8 \pm 3.52 ^{ab}	51.17 \pm 2.85 ^{ab}	64.07 \pm 2.96 ^{abc}
6 day	81.45 \pm 2.20 ^{aA}	51.00 \pm 1.29 ^{ab}	73.31 \pm 3.37 ^{ba}	77.57 \pm 5.07 ^{CA}	77.55 \pm 4.58 ^{CA}	80.87 \pm 2.71 ^{CA}
Serum protein						
0 day	8.09 \pm 0.16 ^{aA}	5.12 \pm 0.24 ^{ab}	5.57 \pm 0.33 ^{ab}	5.81 \pm 0.34 ^{ab}	5.12 \pm 0.29 ^{ab}	5.41 \pm 0.28 ^{ab}
6 day	7.99 \pm 0.33 ^{aA}	5.39 \pm 0.34 ^{ab}	6.96 \pm 0.29 ^{bc}	8.28 \pm 0.21 ^{cd}	7.30 \pm 0.47 ^{cAC}	8.37 \pm 0.22 ^{cd}
Albumin						
0 day	3.11 \pm 0.19 ^{aA}	1.89 \pm 0.19 ^{ab}	1.92 \pm 0.22 ^{ab}	2.61 \pm 0.34 ^{aA}	2.09 \pm 0.16 ^{ab}	2.36 \pm 0.22 ^{ab}
6 day	2.96 \pm 0.19 ^{aA}	2.44 \pm 0.22 ^{abAB}	3.13 \pm 0.10 ^{CA}	3.38 \pm 0.22 ^{aA}	3.30 \pm 0.16 ^{CA}	3.65 \pm 0.09 ^{CA}
Globulin						
0 day	4.81 \pm 0.24 ^{aA}	3.37 \pm 0.28 ^{ab}	3.55 \pm 0.13 ^{ab}	4.16 \pm 0.20 ^{bb}	3.65 \pm 0.22 ^{abB}	4.02 \pm 0.40 ^{abbB}
6 day	5.05 \pm 0.19 ^{aA}	2.95 \pm 0.47 ^{ab}	3.83 \pm 0.34 ^{aC}	4.90 \pm 0.25 ^{CA}	4.00 \pm 0.32 ^{bcA}	4.72 \pm 0.29 ^{ba}

Values with Similar superscript (Capital letters -Between groups and small letters -Within groups) do not differ significantly ($P > 0.05$)

room temperature. The blood clot was broken and subsequently centrifuged at 2000 rpm for 30 minutes. Hb, PCV, TLC and TEC were determined using standard procedures as described by [11]. Glucose was estimated by Trinder method [12] and total protein/albumin was determined by Doumas method [13] (Kits supplied: Coral-Clinical System, Crest Biosystems, Goa, India).

Statistical analysis: The results were subjected to statistical analysis as per the ANOVA method [14].

Results

Detailed clinical observations were recorded in all lambs which presented variable clinical symptoms of disease like scant fecal volume to profuse watery-whitish diarrhoea, soiling of perineum and tail, mild to moderate dehydration, rough body coat, dry mouth/muzzle, profound weakness, and slight increase in temperature. In all the groups of lambs with clinical colibacillosis, nonsignificant increase in the values of hemoglobin was recorded. There was significant increase in packed cell volume, total leukocyte count, lymphocyte count, and neutrophil percentage (Table-1). Serum glucose, protein and serum albumin/ globulin value showed declining trend in infected lambs as compared to healthy lambs. In case of treatment groups significant increase was seen in serum glucose, protein and serum albumin/ globulin value after 6th day post

treatment.

In the present study, the treatment of the clinical cases of colibacillosis in lambs was studied on four trail groups of six lambs each. In these trail groups, therapeutic efficacy of four different antibacterial viz. Ciprofloxacin, Gentamicin, Neomycin, and Co-trimoxazole was evaluated (which showed higher *in vitro* sensitivity against *E. coli* isolates) against clinical colibacillosis in lambs. In gentamicin and neomycin treated group, the hemoglobin value showed decreasing trend and by day 6th post treatment, there was significant ($P > 0.05$) decrease as compared to values at '0' day. In ciprofloxacin and co-trimoxazole treated groups progressive decrease was observed in hemoglobin value that was not-significant ($P > 0.05$) at 6th day post treatment as compared to 0 day. In ciprofloxacin, co-trimoxazole, gentamicin and neomycin treated groups there was significant ($P > 0.05$) increase in total erythrocyte count at 144 hours post treatment (6th day). In all infected groups, the mean total leucocyte count was significantly ($P > 0.05$) higher. However, the total leucocyte count was non-significantly lower in case of ciprofloxacin and gentamicin treated groups but significantly lower in case of co-trimoxazole and neomycin treated groups as compared to values of infected untreated control, whereas, values in treatment groups were comparable to healthy control by 6th day post treatment.

Table-2. Therapeutic trials in clinical cases of colibacillosis in lambs

Groups	Name of the antibiotic	Dose	No. of cases treated	Response to Treatment				No. of cases cured	%	No. of cases delayed	%
				Day I	Day II	Day IV	Day VI				
I	Control Group	-	6	-	-	-	-	6	100.00	-	-
II	Control Group	-	6	-	-	-	-	-	-	6	100
III	Ciprofloxacin	4mg/kg (250 mg tab-1/4 th twice daily)	6	+	+	+++	++++	6	100.00	-	-
IV	Co-trimoxazole	15-30 mg/kg BW (Bolus 1/10 th orally)	6	+	++	++	+++	4	66.68	2	33.34
IV	Neomycin	10mg/kg BW (Bolus 1/12 th orally)	6	+	+	+++	+++	5	83.35	1	16.66

+ = Slightly improved; ++ = Improved; +++ = Recovery; ++++ = Complete recovery

The neutrophil values were significantly ($P > 0.05$) lower in ciprofloxacin and neomycin treated, however, non-significantly lower in co-trimoxazole and gentamicin treated groups as compared to both infected untreated group and healthy control at 144 hours post treatment. By day 6th (144 hrs) there was significant ($P > 0.05$) decrease in the lymphocyte percentage in different treated groups and compared to infected untreated control, but comparable to healthy control group. By day 6th (144 hours), the blood glucose levels recorded in treated groups were significantly ($P > 0.05$) higher as compared to infected untreated control but were comparable to healthy control. The protein levels were significantly higher in co-trimoxazole and neomycin treated groups as compared to infected untreated group and comparable to healthy control at 96 hours post treatment. In gentamicin treated group, the protein values were comparable to healthy control only at 144 hours post treatment. By day 6th (144 hrs) albumin levels in different infected groups were comparably similar to healthy control. The globulin levels recorded by day 6th (144 hours) in gentamicin and neomycin treated groups respectively, which were significantly higher as compared to their respective values on zero day. These values were also comparable to healthy control group.

The therapeutic efficacy of four highly sensitive *in vitro* drugs was evaluated viz. gentamicin, ciprofloxacin, neomycin and co-trimoxazole. Gentamicin at the dose rate of 4 mg / kg body weight and Ciprofloxacin at the dose rate of 4 mg/ kg body weight were found 100 per cent effective at 144 hour post treatment. Neomycin at the dose rate of 10 mg/ kg body weight was 83.35 per cent effective and co-trimoxazole at the dose rate of 15-30 mg/ kg body weight was 66.8 per cent effective at 144 hour post treatment. Treatment regimen used in four different groups of clinical cases of colibacillosis indicated gentamicin and ciprofloxacin most effective drugs followed by neomycin and co-trimoxazole which was comparable with *in vitro* study.

In ciprofloxacin and gentamicin treated groups the rate of recovery (+) during 1st day of treatment was obvious with decrease in dehydration and capillary refill time whereas, there was increase recorded in blood glucose and protein levels post treatment. However by day 6th (144 Hours) rate of recovery was

complete (++++) as compared to day 3rd (+++) and hematobiochemical indices were comparable to healthy control. Lambs treated with oral co-trimoxazole showed delay in recovery and on 3rd day rate of recovery (++) in four cases was recorded with normalizing clinical symptoms. However by day 6th (+++) all four lambs recovered (66.68%) with delay of clinical recovery in two cases. In neomycin treated group, rate of recovery was evident during 1st (+) and 3rd (+++) day with dehydration status returning to normal and obvious increase in blood glucose and protein levels. By day 6th five animals (83.35%) completely recovered (+++) with a delay in one case (Table-2).

Discussion

Increase in haemoglobin values in diarrhoeic lambs might be due to hemoconcentration confirming the fluid loss from vascular compartment [15]. Nonsignificant increase in the values of hemoglobin was recorded [16, 17]. Significant increase in packed cell volume, total leucocyte count, lymphocyte count and neutrophil percentage indicated the presence of intestinal infection [18, 19, 20]. Declining trend of serum glucose, protein, and serum albumin in infected lambs as compared to healthy lambs were reported in diarrhoeic kids [21]; similar findings were reported in scouring calves compared to healthy calves [22, 23, 24]. The drugs helped in normalizing the hematobiochemical changes like hemoglobin, packed cell volume, total leucocyte count, blood glucose and total protein.

The efficacy of the drugs was assessed as per the rate and degree of recovery of clinical symptoms. Gentamicin at the dose rate of 4 mg/ kg body weight (0.3 ml i/m bid) and Ciprofloxacin at the dose rate of 4 mg/ kg body weight orally (250 mg tab-1/4th twice daily) daily for five days proved to be most effective drugs wherein, drugs helped in restoration of the disturbed haematobiochemical changes and abating clinical symptoms. The drugs helped in normalizing the haemato-biochemical changes like hemoglobin, packed cell volume, total leucocyte count, blood glucose and total protein. These drugs showed good results and have been 100 per cent effective for treating enteric colibacillosis. Other group of lambs was treated with another conventional gut acting antibacterial Neomycin at the dose rate of 10 mg/ kg body weight

orally for five days. Five of the lambs clinically responded well with the fecal consistency returning to normal within six days. There was however, a significant increase in the total glucose, total protein and albumin fraction with restoration of hematological values post treatment in the neomycin treated group within six days which suggest that drug was effective for treating colibacillosis. The infected group of lambs treated with Co-trimoxazole at the dose rate of 15-30 mg/ kg body weight orally (1/10th bolus). There was marked improvement in clinical signs in four cases by day six with delay in recovery in two cases even after 144 hour post treatment. These antibiotics help in restoration of disturbed hematobiochemical parameters towards normalcy by day six. Similar efficacy against *E. coli* diarrhea was reported by [25]. As per the present study the clinical cases of colibacillosis in lambs were effectively treated by gentamicin and ciprofloxacin followed by neomycin and co-trimoxazole.

Conclusion

Our study indicated significant alterations in hemato-biochemical parameters with significant increase in packed cell volume, total leukocyte count, lymphocyte count, and neutrophil percentage pre-treatment with significant decrease post-treatment. This was due to the treatment regimen used in four different groups of clinical cases of colibacillosis, which suggested gentamicin and ciprofloxacin as most effective drugs followed by neomycin and co-trimoxazole that was comparable to *in vitro* study.

Authors' contributions

NH, GNS, MMW designed and planned the experiment. The experiment and case study was done by NH and GNS. GNS, HUM and MS revised the final draft of experiment. All the authors interpreted the results, read and finally the manuscript was approved by all after thorough and careful considerations.

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Competing interests

The authors declare that they have no competing interests.

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