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# Experimental development of caprine enterotoxaemia with *Clostridium perfringens* type D whole culture in natural host and its treatments

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## Abstract

The effects of intraduodenal administration of *Clostridium perfringens* type D whole culture in goats were evaluated to develop a reliable experimental model of enterotoxemia in this species and the eventual evaluation of treatment with different drug preparations was also carried out. A total of 28 conventionally reared healthy unvaccinated black bangle goat kids of 6-12 months of age were dosed intraduodenally with whole cultures of *C pelfringens* type D. Four kids were used as controls and received sterile, nontoxic culture medium intraduodenally. All animals received starch solution into the abomasum. The clinical signs developed within 12 hours of post inoculation that were similar to those observed in naturally occurring cases. Among the clinical signs, diarrhea was most common (96.43%) followed by dyspnea (53.57%) and central nervous system (CNS) signs (25.0%). The most striking postmortem findings consisted of necrotizing pseudomembranous colitis (100.0%), lung edema (69.23%) and fluid filled intestines (61.53%). The protocol thus provided a reasonable model of naturally occurring enterotoxemia in goats, producing a range of clinical signs and postmortem changes similar to those observed in the natural disease. Beside this, treatment trial with different drug preparations showed penicillin combined with antitoxin was most effective (100.0%), followed by combination of oxytetracyclin with antitoxin,

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and combined preparation of antitoxin and sulfur drugs both showed 75% recovery rate. On the other hand, treatment with antitoxin, penicillin and oxytetracycline singly could protect goat enterotoxaemia only 25.0%, 50.0% and 50.0%, respectively. Thus in the present study, it was observed that antisera in combination of antibiotics gave better recovery rate than the antitoxin or antibiotics alone.

Key words : *Clostridium perfringens* type D, Experimental enterotoxemia, Goats, Antitoxin, Treatment.

## Introduction

Enterotoxemia caused by *Clostridium perfringens* type D is a disease of great economical and sanitary importance for sheep and goat farming worldwide [1,2], and is probably the most important cause of sudden death in goats of different ages[3]. In sheep, the disease is caused by epsilon toxin, an important toxin produced by *C perfringens* type D[1], and it is believed that the same toxin is responsible for the disease in goats[4]. The persistence of *C perfringens* in the environment is the result of previous cases of enterotoxaemia or of the constant fecal contamination by various animal species that harbor the microorganism as part of their normal intestinal flora[4,5]. Normally, epsilon toxin can be produced in small amounts in the intestine of animals carrying *C perfringens* type D; in this circumstance, the toxin does not cause any deleterious effect and stimulates the formation of antibodies[6]. Several factors have been cited as predisposing to the occurrence of enterotoxaemia, with the most important including sudden dietary changes and a reduction in intestinal transit[4]. This condition has been most thoroughly studied in sheep. Important differences seem to exist between the disease in sheep and in goats, and the condition in the latter is not yet fully understood. The disease in sheep is characterized by central nervous signs with characteristic and consistent histopathologic brain lesions, whereas in goats enterocolitis is the most common postmortem finding[7]. Under experimental conditions, the disease can be induced in goats by the intraduodenal infusion of whole cultures of *C perfringens* type D[7]. The symptoms and some lesions of the disease have been reproduced in goats by the intravenous injection of *C perfringens* type D epsilon toxin[8]. In this study an attempt has been made to produce the disease in its natural host by intradudenal administration of whole cultures of *C perfringens* type D organisms to develop a reliable experimental model that might help diagnosis in natural cases and the eventual treatment with different drug preparations was carried out in order to determine the suitable treatment model against naturally occurring disease.

## Materials and Methods

### Experimental production of disease in goat

Animals: A total of 32 conventionally reared healthy unvaccinated black bangle

goat kids of 6-12 months of age were selected for the experiment irrespective of sex. They were born to mothers that had never been vaccinated against enterotoxaemia. The kids were weaned at age of 10-14 weeks, placed in a warm, dry room and fed with sufficient grasses and grains and water *ad libitum* for 2 weeks before the experiment. All the animals were between 12-16 kg body weight. The experimental procedures involving experimental animals were carried out according to the recommendations and approval of the Bangladesh Livestock Research Institute.

## Preparation of the inoculation

Organism in pure culture, isolated from goats died of suspected enterotoxaemia by conventional culture based methods and identified by ELISA as *C perfringens* type D, was used as inocula for the study. The organism was inoculated in to the freshly prepared thioglycollate medium (Difco, England) containing 0.5% glucose and incubated overnight at 37℃ under anaerobic conditions. A 10% inoculum was seeded in to 500 ml bottles of the same medium, which were further incubated anaerobically for 10 hours at 37℃. The liquid phase of the culture was filtered through sterile gauze and the filtrate was used as inoculum for the experiment.

The purity of the culture was tested on 5% sheep blood agar plate by incubating anaerobically at 37℃ for 24 hours and by examining Gram's stained smears. The dose of the inoculum was 100 ml of a freshly grown culture in thioglycollate medium with 20 ml of 40% commercial dextrose solution through the intra duodenal route. The control goats were administered with 100 ml of freshly prepared sterile thioglycollate medium and 20 ml of 40% dextrose solution.

## Duodenal cannulation

For implantation of duodenal cannulate, kids were kept fasting for 24 hours before the operation. All the kids were inoculated with a solution of starch in the abomasum.

Experimental procedures involved tranquilization of the animals by intravenous injection of xylazine hydrochloride (Rompun®, Bayer Pharma) 0.1 mg/kg body weight. After 10-15 minutes, the animals were secured at left lateral recumbency. The area caudal to the right costal arch was prepared for aseptic surgery. The abdominal cavity was approached through the skin incision that was made in the right flank posterior to the last rib under local infiltration of 2% lignocaine hydrochloride with adrenaline (Jasocaine-A®, Jayson Pharmaceutical, Bangladesh, Ltd.). The pyloric area of the abomasum and the first portion of the duodenum were exposed. Two hundreds milliliters of a 20% solution of Wheaten comflour®(Fielder, White Wings Foods, Smithfields, NSW, Australia) in 0.85% saline was injected in to the abomasum of all animals. Then 100 ml of freshly grown inoculum in thioglycollate medium was inoculated in to the duodenum through a drip over a period of approximately 10 minutes. The abdominal incision was closed by separate muscles and skin sutures with silk. All the animals were clinically examined before and periodically after infusion and regular dressing of the wound was done as per standard procedure.

### Post inoculation observation

The goats were observed at 3 hours intervals during the first 24 hours post inoculation and subsequently at 6 hours intervals. The time elapsed between the beginning of intra duodenal infusion and onset of clinical signs, as well as, the time elapsed between the beginning of infusion and deaths of the animals were recorded carefully. Dead animals were immediately necropsied and the gross pathological changes in the internal organs were recorded. Re-isolation of *C perfringens* was attempted from duodenum and colonic mucosa following conventional culture methods.

### Experimental design for treatment:

The goats were randomly divided in to 8 groups comprising of 4 goats in each group.

Group-I : Healthy control,

Group-II : Infected control,

Group-III : Infected group treated with 25 ml of *C perfringens* types C and D antitoxin (Equine Origin, Cat. # 63703, Colorado Serum Co, Denver) subcutaneously twice daily along with 1000 ml normal saline (Normasol®, Libra Infusions Ltd, Bangladesh) intravenously and intra muscular injection of vitamin B complex (Combi-vet®, ACI, Bangladesh Ltd.) 2 ml daily.

Group IV : Infected group treated with 25 ml of *C perfringens* types C and D antitoxin subcutaneously twice daily, fortified procaine penecillin plus benzyl penicillin sodium (Pronapen®40 lac, Reneta Bangladesh, Ltd.) 8 lac daily through deep intramuscular route along with 1,000 ml normal saline (Normasol®, Libra Infusions Ltd, Bangladesh) intravenously and intramuscular injection of vitamin B complex 2 ml daily.

Group V : Infected group treated with combined penicillin preparation (fortified procaine penecillin plus benzyl penicillin sodium, i.e. Pronapen®40 lac, Reneta Bangladesh, Ltd.) 8 lac daily through deep intramuscular route along with 1000 ml normal saline intravenously and intramuscular injection of vitamin B complex 2 ml daily.

Group VI : Infected group treated with subcutaneous injection of 25 ml of *C perfringens* types C and D antitoxin twice daily, oxytetracycline hydrochloride (Renamycin-100, Reneta® Bangladesh Ltd.) 200 mg daily through intramuscular route along with 1,000 ml normal saline intravenously and intra muscular injection of vitamin B complex 2 ml daily.

Group VII : Infected group treated with oxytetracycline hydrochloride 200 mg daily through intramuscular route, 1,000 ml normal saline intravenously and intra muscular injection of vitamin B complex 2 ml daily.

Group VIII : Infected group treated with intravenous injection of 25 ml of *C perfringens* types C and D antitoxin twice daily, oral administration of combined sulfonamide preparation (sulfadimidine plus sulfapyridine; Diatrim®ACI, Bangladesh Ltd. 1 bol/35 kg body weight at first day followed by half dose for 2nd and 3rd days) along with 1,000 ml normal saline intravenously and intramuscular injection of vitamin B complex 2 ml daily.

## Results

### Experimental production of the disease in goats

Clinical signs : All the animals recovered

Table 1. Time (in hours) elapsed between dosing and onset of clinical signs (1) and between dosing and death (2), main clinical signs, main post mortem lesions in goats infused intraduodenally with *Clostridium perfringens* type D whole culture

| Group | Animal No | Time | | Main clinical signs | Main post mortem lesions* |
|---|---|---|---|---|---|
| | | 1 | 2 | | |
| I | | | | Healthy control | |
| II | 1 | 5 | 7 | Diarrhoea, CNS signs | Lung Edema, Enterocolitis, fluid in int., con Liv |
| | 2 | 7 | 8.5 | Dyspnea, diarrhoea | Lung Edema, Colitis, fluid in int., con Liv. |
| | 3 | 7.5 | 11 | Diarrhoea, dullness and depression | Haemorrhagic Enterocolitis, fluid in int, En Msn LN |
| | 4 | 4 | 5.5 | Diarrhoea, dyspnea | Enterocolitis, lung edema, fluid in int, con Liv |
| III | 1 | 6 | Recovered | Diarrhoea, dyspnea | - |
| | 2 | 6.5 | 12.5 | Diarrhoea, dyspnea | Lung Edema, Colitis, con Liv. |
| | 3 | 9.5 | 16 | Diarrhoea, dullness and depression | Enterocolitis, lung edema, fluid in int |
| | 4 | 5 | 10.25 | Diarrhoea, CNS signs, dyspnea | Enterocolitis, lung edema |
| IV | 1 | 8.5 | Recovered | Diarrhoea, dyspnea | - |
| | 2 | 11.5 | Recovered | Diarrhoea, dyspnea, dullness and depression | - |
| | 3 | 6.5 | Recovered | Diarrhoea, dyspnea | - |
| | 4 | 9 | Recovered | Diarrhoea, dyspnea | - |
| V | 1 | 7.5 | 9.25 | Diarrhoea, dyspnea | Enterocolitis, lung edema, fluid in int |
| | 2 | 12 | Recovered | Diarrhoea, CNS signs, | - |
| | 3 | 8 | Recovered | Diarrhoea | - |
| | 4 | 6 | 10 | Diarrhoea, dyspnea | Enterocolitis, lung edema, con Liv, En Msn LN |
| VI | 1 | 12 | Recovered | Diarrhoea | - |
| | 2 | 9.25 | Recovered | Diarrhoea, dyspnea, dullness and depression | - |
| | 3 | 7.5 | 10.25 | Dyspnea | Enterocolitis, lung edema, En Msn LN |
| | 4 | 7 | Recovered | Diarrhoea | - |
| VII | 1 | 4.5 | 7.5 | Diarrhoea | Enterocolitis, fluid in int |
| | 2 | 8.5 | Recovered | Diarrhoea, CNS signs, | - |
| | 3 | 6 | 11 | Diarrhoea, dullness and depression | Colitis-ileitis, En Msn LN |
| | 4 | 7 | Recovered | Diarrhoea | - |
| VIII | 1 | 5 | Recovered | Diarrhoea | - |
| | 2 | 7.5 | 22 | Diarrhoea, dyspnea, CNS signs | Enterocolitis, fluid in int, En Msn LN |
| | 3 | 8.5 | Recovered | Diarrhoea, dyspnea | - |
| | 4 | 6 | Recovered | Diarrhoea | - |

* Fluid in int: Fluid in intestines, con Liv: Congested liver, En Msn LN: Enlarged mesenteric lymph nodes

completely from anesthesia 1 hour after the end of surgery. The time elapsed between the beginning of intraduodenal infusion and onset of clinical signs as well as the time elapsed between the beginning of infusion and death of animals are shown in Table 1 together with a summary of main clinical and post mortem findings. The most consistent clinical signs were diarrhea (96.83%), respiratory distress (53.57%), central nervous system signs (25.0%), dullness and depression (17.86%) and distended abdomen (14.29 %).

In most cases, the diarrhoea was very fluid, dark green and foul smelling and small pieces of bowel mucosa and/or fibrin were evident in feces. In some cases, strands of blood were also observed in diarrhoeic feces. The CNS signs consisted of recumbency, paddling, bleating, convulsions, increased respiratory efforts and opisthotonus.

The kids without treatment (used as infected control) died within 8 hours of infusion and other 9 goats (belonging different treatment groups) were died between 16-30 hours post inoculation. The healthy control goats showed no clinical signs.

The mean time elapsed between the infusion and clinical signs was 7.4375 ± 2.11435 hours and the mean time elapsed between the infusion and death was 10.8269 ± 4.2603 hours.

## Postmortem changes in experimentally infected kids

Gross changes were only observed in the kids of infected control and in the goats that died in different treatment groups. The gross postmortem changes consisted of severe edema of the airways and interstitium of the lungs (69.23%), together with abundant froth in the trachea and bronchi. The lungs were red, wet and heavy and collapsed only slightly when thoracic cavity was opened. The interlobular septa were markedly widened with fluid. Enterocolitis were most evident (100.0%), there was congestion and hemorrhages of the mucosa, together with adherent whitish pseudomembranes. Fluid with blood and fibrin clots was also noticed in small and large intestines (61.53%). The mesenteric lymph nodes were found enlarged with edema and congestion (38.46%). Necrotic renal tubules and hemorrhages over the kidney capsule were observed in 3 cases. Advance autolysis of therenal tubules, the so-called pulpy kidney lesion was not noticed. The liver was congested and dark (38.46%) and hemorrhages on spleen were also reported in some cases (23.07%). Distended rumen and congestion of ruminal mucosa were also found (23.07%). The abomasal mucosa was congested, hemorrhagic and ulcerative (15.38%).

## Treatment of experimentally produced enterotoxaemia in goat

For the treatment of experimentally produced enterotoxaemia in goat, the goats were divided in 8 groups each containing 4 goats of which group I and II were considered as healthy and infected control respectively and given no drugs. Group III to VIII were the treatment groups in which different drugs were used either in combination or alone (Fig 1, Fig 2).

Group-III : Animals of this group showed clinical signs after 9.5 hours of inoculation. After the appearance of clinical signs, the

goats were given 25 ㎖ of *C perfringens* types C and D antitoxin subcutaneouly twice daily, 1,000 ㎖ of normal saline intravenously and vitamin B complex at the rate of 2 ㎖ intramuscularly daily. One goat of this group appeared less depressed within two hours of treatment, although diarrhoea persisted. The goat's faces became normal 3 days after the continued treatment as above and the goat showed usual signs of health at this time and thus the goat was considered recovered. On the other hand 3 goats belonging this group deteriorated the condition and died within 6.5 hours of treatment.

Group IV : Showed clinical signs within 11.5 hours of inoculation. Goats of this group were treated with intravenous administration of 25 ㎖ of *C perfringens* types C and D antitoxin twice daily, 2 ㎖ of Pronapen® deep intramuscularly daily, 1,000 ㎖ of normal saline intravenously daily and 2 ㎖ of Combi-vet® daily intramuscularly. All the goats of this group showed improvement in condition over the next 4 hours of treatment started at which time the animals were passing pasty faeces and appeared less depressed. At the 2nd day, the goats were eating and passing semi-solid unformed faeces and appeared more alert. At the 3rd day, all the goats became fully alert, ate and drunk normally while passed semi formed but slightly soft faces. The goats were then considered as cured.

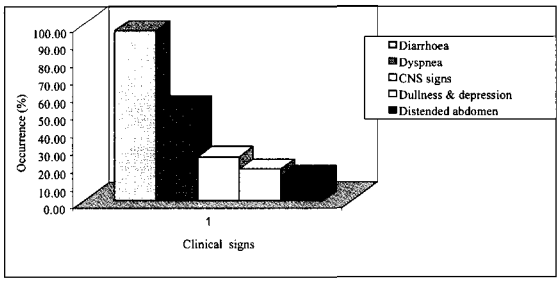


Fig 1. Occurrence of clinical signs in experimental goat enterotoxaemia

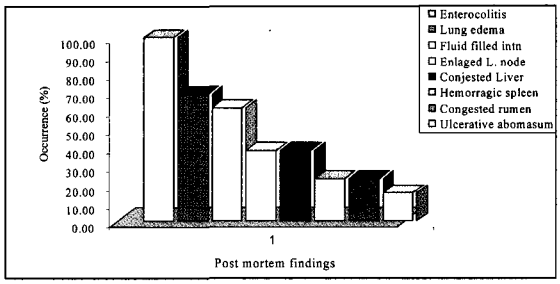


Fig 2. Occurrence of Post morterm lesions in experimental goat enterotoxaemia

Group-V was treated with the same like group-IV except types C and D antitoxin. Two goats of this group showed considerable improvement in clinical condition within 6 hours of treatment started while other two deteriorated the condition over the next 4 hours and died. The other two showing improvement in condition for the next two days with continued treatment. Faces in pasty consistency, weakness and slight anorexia were noticed on 2nd day. But at the 3rd day, the goats were more alert and less depressed although semisolid, unformed faces were going on. The goats started to have eat and drink normally from 3rd day of treatment.

Oxytetracycline hydrochloride (Renamycin® 100) 2 ㎖ IM replaced the drug Pronapen® of group IV was used to treat the animals of Group-VI Three out of 4 goats of this group appeared less depressed within 4 hours of treatment started while the rest one deteriorated the condition over the next 3 hours with consistent diarrhoea, recumbency and died. With the continued treatment, the 3 goats began to eat and

drink normally from 2nd day although faces consistency remained soft and pasty. At the 4th day, the goats were fully alert, ate and drunk normally and passed soft unformed faces and considered as cured.

The treatment protocol in Group-VII was same as for the group VI except types C and D antitoxin. Two goats of this group died in recumbency with consistent fluid diarrhoea, depression and bellowing with in 4 hours of treatment started while the rest two improved their clinical condition over the next 4 hours of treatment. These two appeared more alert, began to eat and drink with a progressive manner with time. At 4th day, although diarrhoea persisted they were taking their feed and water and resembled normal.

Sulfonamide preparation (Diatrim® bolus) 1 bol/35 kg body weight at first day followed by half dose onwards were used in Group-VIII replacing the pronapen®of group IV to treat the goats. Improvements were noticed in 3 goats out of 4 of this group over the next 4 hours of treatment at which time the goats appeared less depressed. At 2nd day, the goats passed semisolid unformed faces and showed a high aptitude to have feed and water. They were fully alert at 3rd day and began to eat and drink normally and passed semisolid roughly formed faces and were considered cured. Another goat of this group did not show improvement with treatment. The goat became more depressed and recumbent with in 7 hours of treatment and become weaker and deteriorated the condition and died on second day of treatment. The summary of different treatment groups is given in Table 2.

## Discussion

The clinical signs observed in experimentally produced enterotoxaemia in goat resembled to those were reported by Blackwell and Butler[3], Smith and Sherman[4], Baxendell[9], Parhi et al[10], Uzal et al[11,12], Phukan et al[7,13]. Three forms of caprine enterotoxaemia have been described; per acute, acute and chronic[3,4,14]. The per acute form typically affects young growing kids and, as in this case, causes death with in hours[14]. The rapid clinical course may or may not be associated with clinical signs, which may include severe pain, vocalization, recumbency, fever, fibrinohaemorrhagic diarrhoea and death[4,14,15]. Acute enterotoxaemia has less rapid clinical course and signs are less severe than those observed with the per acute form[14]. However, if left untreated acute enterotoxaemia usually culminates in death with clinical signs like diarrhoea, abdominal pain and discomfort and severe shock with cold extremities and convulsion[3,4,14,15]. The diarrhoea in this form may initially be yellow-green and pasty but rapidly become watery and mucoid with shred of bowel mucosa and blood[9]. The chronic form has been recognized in adult goats[3,14] and is associated with chronic intermittent diarrhoea often containing blood and mucus, anorexia, weight loss and decreased milk production[3,4,14]. The protocol used here for experimental production of the disease provides a reasonable model of naturally occurring enterotoxaemia in goats, producing enterocolitis, pulmonary edema and CNS signs in frequency similar to those in naturally occurring disease[4,12]. The goats infused intradudenally with a culture of *C perfringens* type D showed clinical signs within 12 hours of dosing that was in close agreement with the result of

Table 2. Summary of different treatment group

| Group | Type of group* | Animal No | No of animal recovered | Time** 1 | Time** 2 | Time** 3 | No of animal died | % Recovery |
|---|---|---|---|---|---|---|---|---|
| I | Healthy control | 1 | | | | | No clinical signs observed | |
| | | 2 | | | | | | |
| | | 3 | | | | | | |
| | | 4 | | | | | | |
| II | Infected control | 1 | None | - | - | - | 4 | - |
| | | 2 | | - | - | - | | |
| | | 3 | | - | - | - | | |
| | | 4 | | - | - | - | | |
| III | Treatment group (AT + NS + Vit-B) | 1 | 1 | 2 | 72 | - | 3 | 25.0 |
| | | 2 | | - | - | 6 | | |
| | | 3 | | - | - | 6.5 | | |
| | | 4 | | - | - | 5.25 | | |
| IV | Treatment group (AT + P + NS + Vit-B) | 1 | 4 | 4 | 66 | - | - | 100.0 |
| | | 2 | | 3.5 | 68 | - | | |
| | | 3 | | 4 | 72 | - | | |
| | | 4 | | 3 | 70 | - | | |
| V | Treatment group (P + NS + Vit-B) | 1 | 2 | 5.5 | 72 | | 2 | 50.0 |
| | | 2 | | 5 | 70 | | | |
| | | 3 | | - | - | 2.5 | | |
| | | 4 | | - | - | 4 | | |
| VI | Treatment group (AT + OTC + NS + Vit-B) | 1 | 3 | 3.75 | 88 | | 1 | 75.0 |
| | | 2 | | - | - | 2.75 | | |
| | | 3 | | 3.5 | 82 | | | |
| | | 4 | | 3.75 | 94 | | | |
| VII | Treatment group (OTC + NS + Vit-B) | 1 | 2 | - | - | 3 | 2 | 50.0 |
| | | 2 | | - | - | 5 | | |
| | | 3 | | 3.75 | 93 | - | | |
| | | 4 | | 4 | 92 | - | | |
| VIII | Treatment (AT + Sulf + NS + Vit-B) | 1 | 3 | 4 | 72 | | 1 | 75.0 |

*: AT=Types C and D antitoxin; NS= Normal saline; Vit-B= Vitamin B complex; P= Penicillin, OTC= Oxytetracycline; Sulf= Sulfur drug

**: time (hours) elapsed between beginning of treatment and response to treatment (1); time elapsed between beginning of treatment and recovery (2), time elapsed between onset of clinical signs and death (3).

Uzal and Kelly[12], Phukan et al[13], Blackwell et al[15], and Phukan et al[16], Dholakia et al[17] and produced disease by intraduodenal administration of *C perfringens* and observed transient diarrhoea with in 12 hours of post inoculation and death occurred with in 12-36 hours, these results also corresponded to the present work. The post mortem findings observed in the present study were more or less similar to those observed by Blackwell and Butler[3], Uzal and Kelly[7], Phukan et al[13], Phukan et al[16] and Shamimuzzaman[18].

In the present study starch was injected into the abomasum in an attempt to imitate the natural conditions predisposing enterotoxaemia, as it has been demonstrated that when large quantities of undigested starch escape in to the small intestine, *C perfringens* grows very rapidly and a high concentration of toxin is produced in the intestine[19]. Enterocolitis was found as the most striking post mortem lesion. The simplest explanation would be that the small intestine in goats is more resistant to the effects of *C perfringens* toxins than is the large intestine. The selective damage in most of these goats could also have been due to rapid proliferation of *C perfringens* in colon versus small intestine. In goats, the transit speed of the intestinal content is 3 hours for the small intestine and 18 hours for the large intestine[4]. This could have accounted for a longer time exposure of the large intestine to *C perfringens* toxins than that of the small intestine. Another possible explanation for the selective damage to the large intestine is that in order to produce intestinal damage, the toxins may need to be modified by the enzymes or other substances present only on the colon of goats.

The hemolytic changes in the lungs, liver and over and/or in other organs might be due to the effect of alpha toxin which has been reported as hemolytic[20], necrotizing[21] and potentially lethal[22] and CNS signs in some cases might be due to the effect of beta toxin which is believed to cause mucosal necrosis and central nervous system signs in *C perfringens* induced diseases in domestic animals[23,24]. No gross changes were observed in the kidneys of any of the goats. Thus the so called pulpy kidney lesion was absent which is in disagreement with Phukan et al[13], Uzal and Kelly[7], Shamimuzzaman[18] and Phukan et al[16] and Islam[25] but showed similarity with the findings of Timothy et al[26] who also reported pulpy kidney as not an important signs for enterotoxaemia in goats. This pulpy kidney lesion in earlier workers' findings might be due to advance autolysis of renal parenchyma or due to delay in postmortem observation.

In the treatment trial, combined therapy of *C perfringens* types C and D antitoxin with procaine penicillin plus benzylpenicillin sodium together with normal saline and vitamin B complex (Group-IV) was found 100 % effective against experimentally induced enterotoxaemia in goat. Combined therapy of *C perfringens* types C and D antitoxin and oxytetracycline hydrochloride (Group-VI) and *C perfringens* types C and D antitoxin with sulfonamide preparation (Group-VIII) were found 75% effective. On the other hand, treatment with *C perfringens* types C and D antitoxin (Group-III), combined penicillin preparation (Group-V) and oxytetracycline (Group-VII) singly could protect goat enterotoxaemia only 25%, 50%

and 50%, respectively. The result of the present study is in agreement with Blackwell and Butler[3] who recorded high degree of efficacy of combined therapy (ie 25 ml of antitoxin SC and 60 ml of a 12% sulfonamide preparation PO) in the treatment of goat enterotoxaemia. Further, the result was also in accordance Oxer[27] who suggested the use of antitoxin in the treatment of enterotoxaemia and mentioned that a combination of serum and drug therapy gave better result that either alone. Roberts[28] mentioned that intravenous administration of antitoxin along with tetracycline orally was found effective against enterotoxaemia in goats with which present study results also found similar. Seventy five percent of efficacy was observed in the treatment Group-VIII that showed agreement with Shanks[29] who treated 3 enterotoxaemic goats and reported that treatment with 50 ml antiserum SC twice daily together with sulfmethatzine 3-4 gram per oral effected cure in enterotoxaemic goats with in 48 hours. But, there was some variation observed in the preset study. In the present study 3 out of 4 goats recovered with this protocol with in 72 hours, which was somewhat delayed then the result of Shanks[29]. This variation might be due to difference in drug preparation and use of less amount of antiserum (25 ml) than he mentioned (50 ml).

Group-V was treated with combined preparation of penicillin and showed 50% efficacy alone, which was similar with the findings of Urban et al[30] who reported penicillins as the most effective drugs for the therapy and prevention of enterotoxaemia. The result was also in agreement with the result of Johnstone et al[31]. The findings also correlated with the most recent study results against goat enterotoxaemia by Phukan et al[16] who reported that combined therapy of hyper immune serum and fortified procaine penicillin was the most effective against goat enterotoxaemia as 83.33% followed by hyper immune serum with oxytetracycline dihydrate (50.0%), fortified procaine penicillin (50.0 %), hyperimmune serum, (33.33%) and oxytetracycline dihydrate (33.33%). Although slight variation was observed with the result of Phukan et al[16], present study showed almost similarity with him (viz; combined therapy with *C perfringens* types C and D antitoxin and combination of fortified procaine penicillin and benzyl penicillin sodium showed 100% efficacy in the study, followed by combined therapy with *C perfringens* types C and D antitoxin and oxytetracycline hydrochloride as 75% and combined therapy with *Clostridium perfringens* types C and D antitoxin and sulfonamide preparation as 75%). These slight variations might be due to variation of drug preparation and sample size of the treatment group.

Group-VII treated with oxytetracycline hydrochloride alone as main drug showed 50% efficacy that was in agreement with Roberts[28], Johnstone et al[31], Anjum et al[32], Phukan et al[33] although slight variation was noticed but disagreed with Phukan et al[16]. This variation might be due to sample size of the study, presence of oxytetracycline resistant strains of the organism etc.

Conclusively, *C perfringens* produces toxins, which primarily responsible for the disease in different animal species and such antibiotics do not act on the toxins. It is a

fact that antibiotics act upon the causal organisms but not on the toxins. But the toxins of the organism produce a disease of per acute nature in the field. It was not surprising that antisera in combination with antibiotic gave better recovery rate than the antibiotics alone in the present study. Therefore, in an endemic area, the unvaccinated animals, which show symptoms of enterotoxaemia, should be immediately treated with *C perfringens* types C and D antitoxin and penicillin to avoid mortality. Antitoxin combined with oxytetracycline or sulfonamide preparation may be second choice instead of penicillin.

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