INVESTIGATION OF AN OUTBREAK IN LAMBS ASSOCIATED WITH *ESCHERICHIA COLI* O95 SEPTICAEMIA

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ABSTRACT

A disease outbreak in lambs caused by *Escherichia coli* O95 during winter season at an organized farm in arid region of Rajasthan was investigated and controlled by effective therapeutic measures. Of 89 lambs, 17 (19%) died in a week with history of clinical signs such as sudden death, frothy mouth, fever, shivering, unable to walk and lying prostrate. On gross and histopathological examination upper respiratory tract and lungs were found to be the major organs affected with acute vascular changes accompanied by moderate to severe vascular alterations in other visceral organs due to septicaemic nature of the disease. *Escherichia coli* O95 serotype was isolated from all the tissues collected during post-mortem examination. Pathogenecity of the *E. coli* isolate and lung tissue suspension was tested in rabbits. The gross and microscopic lesions observed in the present outbreak and in the experimentally *E. coli* infected rabbits compared well. It was concluded that sudden change in environment, cold stress, dew and frost and poor ventilation might have exposed lambs to *E. coli* septicaemia with major involvement of respiratory system followed by other visceral organs. The mortality was checked with ciprofloxacin in combination with amoxycillin after determination of antibiotic sensitivity.

Key words**:** *Escherichia coli* O95, lambs, septicaemia

Introduction

Mortality in lambs is the major cause of low productivity in sheep. The high mortality rates greatly reduce the efficiency and profitability of a lamb production enterprise. Several studies have shown that approximately 10-35% of lambs die by 6 months of age in different agro-climatic conditions with neonatal lambs being at the greater risk (Yapi *et al*., 1990; Green and Morgan, 1993; Nash *et al*., 1997; Haughey, 1991; Gama *et al*., 1991). The major causes associated with lamb losses were pneumonia (31.4%), digestive disorders (14.6%), starvation (9.6%), endoparasitism (5.0%), septicaemia and toxaemia (10.1%), accidental (2.1%) and undetermined causes (27.2%) (Mandal *et al*., 2007). Septicaemia is the acute invasion of the systemic circulation by pathogenic bacteria, which may cause sepsis or septic shock with possible localization in various organs. *Escherichia coli* (*E. coli*) is one of the first organisms encountered by newborn farm animals after birth. Lambs acquire infection from contaminated bedding, dirty and overcrowded lambing grounds, skin of the perenium and udder of the dam. Septicaemia caused by *E. coli* has been the most frequently reported in lambs (Radostits *et al*., 2007). The septicaemic strains of *E. coli* were found to be invasive and commonly cause rapid death due to the effects of septicaemia involving multiple body systems.

In the present study, we investigated a disease outbreak in lambs caused by *E. coli* O95 serotype during winter season at an organized farm in arid region of Rajasthan and controlled it by effective therapeutic measures.

Materials and Methods

Farm history: The farm under study is located in Bikaner in arid Rajasthan. The maximum temperature during summer (April-July) ranges from 28ºC to 42ºC but may occasionally goes up to 48ºC. Minimum temperature during winter (November-February) ranges from 4ºC to 20ºC. Average rainfall is about 260-440 mm and occurs mostly in July-August. The farms are well organised, situated approximately two kilometers away from each other, and standard animal husbandry practices are followed. A total of 637 Marwari sheep (adult female-460, adult male-88, lambs-89) were maintained as per the standard managemental practices and vaccinated for sheep pox, Peste des Petits Ruminants (PPR), enterotoxaemia as per the schedule. In winter season due to the sudden downfall in the temperature, lambs and other animals were protected by curtains on windows of the shed so that direct exposure to the cold could be avoided. Suddenly mortality started in 2-3 weeks old Marwari lambs during the first week of February (2010). Of 89 lambs, 17 (19%) died in a week with history of clinical signs such as sudden death, frothy mouth, pyrexia, shivering, unable to walk and lying prostrate. Five lambs in the moribund state showing similar clinical manifestations were treated with tetracycline and gentamicin without any perceptible response. Amikacin and sulphadimidine were found to be of some value in controlling the mortality.

Necropsy and histopathology

Detailed necropsy examination of four lambs died during the outbreak was performed and gross lesions in various organs were recorded. Tissue pieces

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Fig. 1: Lungs of lamb showing severe congestion, note pinpoint haemorrage on spleen.

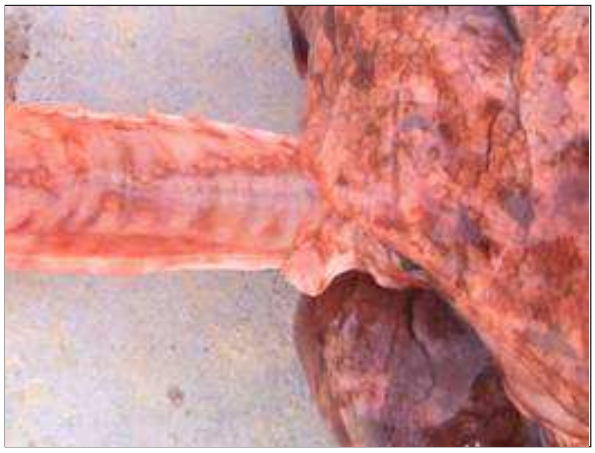

Fig. 2: Severe congestion in lungs, trachea and heart in septicaemic lamb.

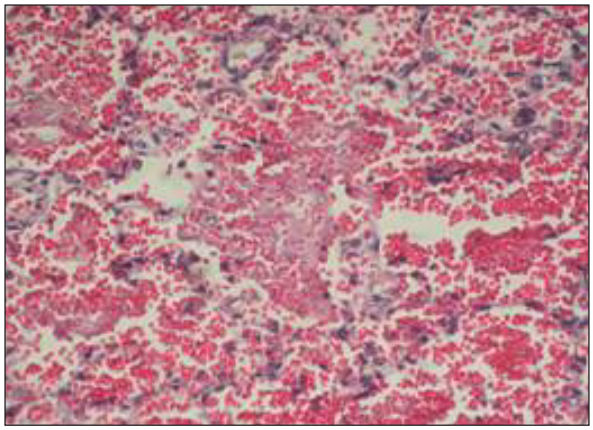

Fig. 3: Lung showing severe haemorrhage with fibrinous exudate in alveoli

from lungs, liver, kidneys, spleen, heart and intestines were collected and divided in two: one for bacterial culture that was brought to laboratory on ice, and other preserved in 10% neutral buffered formalin for histopathology.

Tissues after 24 hr of fixation were further cut into the 3-4 mm thickness, and preserved for another 4-7 days. Prior to dehydration, the tissues were washed in running tap water for 4-6 hr. The tissue sections were dehydrated with ascending grades of ethanol, cleared in xylene and finally embedded in paraffin wax (overnight). Sections were prepared at 5 µm thickness and stained routinely with haematoxylin and eosin (Culling, 1968).

Bacterial isolation

Tissue homogenates (20% w/v) were made in sterile PBS and enriched in bovine heart infusion (BHI) broth at 37^{0}C for 48 hr followed by streaking on to the sheep blood agar. The bacterial colonies were further streaked on Mac-Conkeys and eosin methylene blue (EMB) agar for identification of *E. coli* (Cruickshank *et al*., 1975). The biochemical tests including IMVic pattern i.e. indole, methyl red, Voges Proskauer and citrate utilization tests were performed and the isolates were sent for serotyping at National Salmonella and Escherichia Centre, Kasauli.

Pathogenicity testing

The pathogenicity of the isolated *E. coli* was tested in three New Zealand white rabbits procured from rabbit unit of the institute. Rabbit number N-101M was injected with 2 ml lung suspension prepared in sterile PBS intramuscularly in thigh region, rabbit number N-103M with 2 ml 24 hrs broth culture of *E. coli* and rabbit number N-136M (control) was injected with 2 ml PBS only intramascularly in the thigh region. The infected and control rabbits were observed for clinical signs and rectal temperature were recorded daily for up to 7 days.

Antibiotic sensitivity assay

Antibiotic sensitivity testing was carried out using 21 antibiotic disks (Himedia) such as Cloxacillin (CX), Colistin (Methane sulphonate) (CL), Novobiocin (NV), Doxycycline hydrochloride (DO), Enrofloxacin (EX), Furazolidone (FR), Nitrofurantoin (NF), Norfloxacin (NX), Erythromycin (E), Gentamicin (G), Ammoxycillin (Am), Ampicillin (A), Chloramphenicol (C), Chlortetracycline (CT), Ciprofloxacin (CF), Penicillin-G (P), Polymixin-B (PB), Rifampicin (R), Streptomycin (S), Trimethoprim (TR) and Ofloxacin (OF) to determine as to which antibiotic will be the most effective in the treatment and control of the present outbreak. Briefly, a suspension of the isolate was prepared and spread uniformly onto a Muller Hinton agar plate. Disks of different antibiotics were placed onto the surface of the agar with a multichannel disk dispenser. The zone of inhibition around the disks was measured with the help of the compact zone scale (Himedia). Using the reference table size of zone, results were recorded as whether the organism is susceptible (S), intermediately susceptible (I), or resistant (R) to a particular antibiotic.

Results

Clinical Signs

The climate of Bikaner is very diverse in the summer as well as in the winter seasons. The mortality in the present outbreak in Marwari lambs was 19%. The clinical symptoms were frothy mouth, fever, shivering, difficult movement and lying prostrate.

Necropsy findings

On post-mortem examination, carcasses were found to be healthy and good, the oral cavity was wet with frothy/ watery contents. Mucinous nasal discharge was observed with crest formation in all the lambs. Lungs were patchily congested and haemorrhagic. The ecchymoses were predominantly seen in both the diaphragmatic lobes (Fig.1). Trachea was severely congested with the presence of linear haemorrhages on the mucosa (Fig.2). Blood-tinged frothy exudate was found in the bronchi. Liver was slightly enlarged and congested. Kidneys were highly congested in two animals, small intestinal mucosa was mildly congested and had mucus mixed yellowish contents. In the heart and spleen, petechial haemorrhages were observed.

Histopathology

In lungs, blood vessels were severely engorged with extravasation of erythrocytes in alveolar septae at places. Some of the alveoli were filled with erythrocytes and fibrinous exudates (Fig.3). Inter alveolar septae were mildly thickened with fibrinous exudates and infiltration of mononuclear cells. Tracheal mucosa was severely congested. In the liver, hydropic degeneration of hepatocytes, sinusoidal engorgement with erythrocytes and perivascular haemorrhages were seen. Kidneys showed degeneration of tubules, oedema in the pelvis and congestion in the cortical area. Intestine of the one case revealed acute enteritis characterized by infiltration of inflammatory cells predominantly neutrophils in the mucosa and congestion of blood vessels. Epicardial muscles and spleen showed extravasation of erythrocytes and engorged blood vessels and at times extravasated erythrocytes.

Pathogenicity testing

Pathogenicity of the *E. coli* isolate and lung tissue suspension was tested in rabbits. Rabbit No.N-103M, infected with broth culture died after 20 hrs of infection without showing clinical symptoms. On necropsy, lungs were patchily congested and haemorrhagic with frothy exudates. Tracheal mucosa was severely congested with the linear haemorrhages which were reflected in histopathology also. The alveoli were filled with fibrinous exudates. Infiltration of mononuclear cells was observed in the inter alveolar septae. Grossly, liver, kidneys, spleen and heart showed petechial haemorrhages which were detected microscopically also. Intestines and stomach of this rabbit were apparently normal on gross and microscopic examination. Second rabbit (No. 101) injected with lung suspension was dull, depressed and showed fever up to 106°F on second day and subsequently became normal by fourth day PI. The rabbit was sacrificed on 7th day PI. On necropsy, mild congestion in diaphragmatic lobes was observed. Microscopically, scattered engorged blood vessels were seen in the section of lungs and trachea. The uninfected control rabbit (No. 136) was apparently normal throughout the experimental period of 7 days without any necropsy and histopathological findings of septicaemia.

Bacterial isolation

The enriched tissue homogenates inoculated on blood agar showed medium sized, round, smooth and whitish-grey colonies and few of them revealed partial haemolysis. These colonies on Mac-Conkeys agar appeared as pink (lactose fermenting). Lactose fermenting colonies were further streaked on EMB agar showed dark greenish colonies with metallic sheen. On biochemical tests, the isolates showing indole positive, methyl red positive, Voges Proskauer negative, citrate utilization test negative were tentatively identified as *E. coli*. The *E. coli* isolates were further serotyped at National Salmonella and Escherichia Centre, Kasauli and confirmed as *E. coli* O95.

Antibiotic sensitivity assay

Out of 21 antibiotics tested for the sensitivity, *E. coli* was found to be susceptible for enrofloxacin, ciprofloxacin, levofloxacin, cotrimoxazole and amoxicillin. The organisms were intermediately susceptible for norfloxacin, erythromycin, amoxicillin, penicillin-G, polymixin-B, rifampicin, streptomycin, trimethoprim and ofloxacin and were resistant for rest of the antibiotics.

Discussion

Colibacillosis in lambs commonly occurs in septicaemic and peracute forms though some cases exhibit enteric signs and lead to chronic disease. Lambs of 1-2 days and 3-8 weeks old have been reported to be more susceptible and found dead without premonitory signs in peracute form. Acute cases collapse and show occasionally signs of acute meningitis manifested by stiff gate in the early stages, followed by recumbency with hyperaesthesia and titanic convulsions. Chronic cases usually show arthritis.

An invasive strain of *E. coli* invades the tissues and systemic circulation via the intestinal lumen, nasopharyngeal mucosa, tonsillar crypts and umbilical vessels and leads to coliform septicaemia. These strains are able to invade extraintestinal tissues, to resist bactericidal effect of complement, survive and multiply in body fluids, escape intracellular killing and phagocytosis, releases cytotoxins and induce tissue damage (Radostits, 2007).

In the present study, of 89 lambs, 17 (19%) died in a week exhibiting clinical signs such as sudden death, frothy mouth, pyrexia, shivering, inability to walk and lying prostrate. Lungs and trachea were found to be the major

organs affected with the acute vascular changes accompanied by moderate to severe vascular alterations in other visceral organs due to septicaemic nature of the disease. The gross and microscopic lesions observed in the present outbreak and in the experimentally *E. coli* infected rabbit were previously reported by various workers in acute pneumonic and septicaemic forms of the disease in sheep (Ertan, 2006; Lacasta *et al*., 2008; Raji *et al*., 2000). It has been reported that *Mannheimia haemolytica* is one of the most important microorganisms causing respiratory problems in lambs. At times *P. multocida* has also been found to causing respiratory problems (Ewers *et al*., 2004; Moses *et al*., 2004; Gracia Curras *et al*., 2005; Chandrasekaran *et al*., 1991). However, in the present study, *E. coli* was isolated from all the tissues collected during post-mortem examination. Serological typing of these isolates confirmed *Escherichia coli* O95. Other bacteria, including *M. haemolytica, Pasteurella multocida*, *Actinomyces pyogenes, Streptococcus,* and *Staphylococcus* could not be isolated in the present investigation (Malone, 1998; Raji *et al*., 2000), as reported previously. Non isolation of one or other of these bacteria could have been due to treatment of the affected lambs with antibiotics such as tetracycline, amikacin and sulphadimidine to control the mortality before our investigation or lambs were not infected with these at all. Later the antibiotic sensitivity test confirmed that *E. coli* isolated from the tissues of lambs were resistant to these antibiotics. *E. coli* has been reported to be an important cause of septicaemia in lambs (Raji *et al*., 2000; Ertan, 2006; Lacasta *et al*., 2008). It has been consistently isolated from the acute catarrhal bronchopneumonia (56.8%), catarrhal purulent bronchopneumonia (15.8%), fibrinous pneumonia (17.9%) and interstitial pneumonia (50%) with overall incidence of 24.56% in lambs (Ertan, 2006). The septicaemic processes originating in respiratory organs were high (44%) in lambs associated with cold stress and kept in poorly ventilated pens. In a recent study, *E. coli* was isolated from 12 (9.6%) out of 125 pneumonic lungs and septicaemia (Lacasta *et al*., 2008). The ten years data of Zaria (Nigerian state) revealed that *E.coli* was the most important etiological agent isolated from the caprine and ovine lungs (Raji *et al*., 2000).

The adverse climate influences the disease resistance of the lambs (Wassmuth, 2003; Turkson and Sualisu, 2005). In the present report, the outbreak occurred in the winter season (February month), with the temperature ranging from 0 to -4^{o}C, and also there was overcrowding. Therefore, it was suggested that sudden change in environment, cold stress, dew and frost and poor ventilation might have exposed lambs to *E. coli* septicaemia with major involvement of respiratory system followed by other visceral organs.

Long acting tetracycline has been recommended in the treatment of acute bacterial pneumonia in sheep (Martin, 1996). Prior to our investigation, the affected lambs were treated initially with tetracycline to control the mortality. Subsequently, other antibiotics such as amikacin, sulphadimidine and gentamicin were tried alone and in combinations but without promising results. The great diversity of potential pathogenic serotypes encountered in colisepticaemia and the failure of serotype specific antibody to cross-protect against a heterologous challenge in experimental infection have made it difficult to develop vaccines against septicaemic colibacillosis. Based on the results of antibiotic sensitivity test, two separate classes of antibiotics, ciprofloxacin in combination with amoxicillin were used as injectables for treatment that resulted in the control of mortality. The ciprofloxacin was given @ 10 mg/kg body weight, i/m, 12 hourly. Whereas, amoxicillin was injected @ 10 mg/kg body weight i/m, continued for 5 to 7 days depending upon progress of the disease in individual cases. Ancillary treatment included infusion of normal saline, sodium bicarbonate, Ringer's lactate, in some cases high dose of B-complex and antioxidants.

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