



Serotyping and antimicrobial sensitivity of *Escherichia coli* isolated from gastrointestinal tract disorders in sheep

Sourabh Kumar¹, Krishan Kumar Jakhar¹ and Aman Deep Singh²

¹Dept. of Veterinary Pathology, College of Veterinary Sciences, Lala Lajpat Rai Univ. of Veterinary & Animal Sciences, Hisar-125004, India

²Dept. of Veterinary Anatomy, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences Univ., Ludhiana-141004, India

*e-mail: dramandeep287@gmail.com

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Abstract: A total of 30 sheep received for necropsy over a period of 6 months were thoroughly examined. Seventy two tissue samples were collected from 30 sheep showing the gross pathological lesions of gastrointestinal tract disorders. Out of which, 48 confirmed isolation of *E. coli* based on culture characteristics and biochemical tests and 14 sheep were found *E. coli* positive. Infection was prominent in less than one year age groups and more in male sheep. Out of 48, only 18 strains were sent for serotyping. Out of which, 13 belonged to 'O' serogroup, whereas remaining five isolates were untypable strains. The most prevalent serotype was O168(5) followed by O60(4), O1(1), O91(1), O102(1) and O116(1). *In-vitro* drug sensitivity pattern by the single disc diffusion method revealed maximum sensitivity to polymyxin B, ofloxacin, amikacin, ampicillin, colistin, furaxone, amoxycillin+clavulanic acid, cefixime, amoxicillin, amoxycillin+sulbactam, doxycycline, ciprofloxacin, cefpodoxime, co-trimoxazole and nalidixic acid in decreasing order.

Key words: *Escherichia coli*, Drug sensitivity, Intestine, Abomasum, Sheep

Introduction

Escherichia coli, though a normal inhabitant of intestinal tract, is also associated with a variety of pathological conditions in man and animals. Different strains of *E. coli* have different attributes of virulence and thus produce different diseases in sheep (Peer *et al.*, 2001) Birth weight (Yapi *et al.*, 1990) and weather "stress" decreases immunity and predisposes to *E. coli* infection (Starr, 2002). An important member of the normal intestinal microflora of humans and other mammals, *E. coli* has also been widely exploited as a cloning host in recombinant DNA technology. But *E. coli* is more than just a laboratory workhorse or harmless intestinal inhabitant; it can also be a highly versatile, and frequently deadly, pathogen. Several different *E. coli* strains cause diverse intestinal and extra intestinal diseases by means of virulence factors that affect a wide range of cellular processes (Kaper *et al.*, 2004). Healthy ruminants transiently harbour the human pathogen *Escherichia coli* O157:H7 in their gastrointestinal tract; however, the conditions that lead to its acquisition, persistence, and clearance from that site are not clearly understood (Magnuson *et al.*, 2000). Gastrointestinal tract location of *E. coli* was studied in bovines (Luke *et al.*, 2002), but meagre information is available in case of sheep. The present study reports the prevalence of different *E. coli* serotypes at necropsy in sheep showing various pathological lesions and their *in-vitro* antimicrobial sensitivity pattern.

Materials and Methods

A total of 30 sheep were brought for post mortem. Seventy two tissue samples were collected from intestine, abomasum, liver, mesenteric lymph nodes, spleen, lungs, kidneys and pancreas which were showing the gross lesions like catarrhal enteritis, congestion, haemorrhage, necrotic foci etc. Out of which 48 revealed isolation of *E. coli* on M.L.A. Purified cultures were further subjected to biochemical tests (Oxidation-fermentation test, nitrate test, H₂S production on triple sugar iron medium, Indol, methyl red, Voges-Proskauer, citrate utilization test and urease test) and sugar fermentation properties

recommended for confirmation of cultures. Then 18 isolates were sent for serotyping to Kasauli, Himachal Pradesh and 48 isolates were subjected to *in-vitro* drug sensitivity testing using 15 antimicrobials by the single disc diffusion method (Bauer *et al.*, 1966). The results were recorded as per cent sensitivity to antimicrobials.

Results and Discussion

Out of 30 sheep, 72 tissue samples were collected from organs mainly intestine, abomasum, liver, mesenteric lymph nodes, spleen and also from pancreas, kidneys and lungs showing gross pathological lesions of gastrointestinal tract disorders like catarrhal enteritis, congestion, haemorrhage etc. Out of 30 sheep, 14 were *E. coli* positive and 48 out of 72 tissue samples, confirmed *E. coli* on M.L.A. Eighteen *E. coli* strains were submitted for serotyping. Thirteen strains were identified with 'O' serogroup and remaining 5 were untypable. The most prevalent serotype was O168(5) followed by O60(4), O1(1), O91(1), O102(1) and O119(1). O60 serotype was reported in intestine, spleen while O168(3), O60, O1, O91 in liver, O60, O168, O102 in lungs, O116 and O168 from heart blood was recorded (Table-1). O60 was reported from diarrhoeic lambs as most prevalent serogroup by Chatterjee and Kashyap (2006). Although healthy sheep (Kudva *et al.*, 1997), and goats (Chapman, 2000) have been shown to transiently harbour *E. coli* O157:H7 naturally, cattle are the main source of human infections (Besser *et al.*, 1999). The predominant location of *E. coli* O157:H7 persistence was the lower GIT. These findings suggest the colon as the site for *E. coli* O157:H7 persistence and proliferation in mature ruminant animals (Luke *et al.*, 2002).

Peer *et al.* (2001) reported O91 and O168 from intestinal samples and rectal swabs of lambs suffering from colibacillosis. O168 serotype was also isolated from diarrhoeic faecal samples (Wani *et al.*, 2004). These findings suggest the role of O60, O91 and O168 serotypes for causing diarrhoea and various gastrointestinal tract disorders in sheep. Elucidating the relationship between sheep and

Table-1: Organ-wise distribution of *E. coli* serotypes isolated from sheep carcasses

Serotypes	Intestine	Abomasum	Mesentericlymph nodes	Pancreas	Liver	Lungs	Heartblood	Spleen	Kidney
O168	-	-	-	-	3	1	1	-	-
O60	1	-	-	-	1	1	-	1	-
O1	-	-	-	-	1	-	-	-	-
O91	-	-	-	-	1	-	-	-	-
O102	-	-	-	-	-	1	-	-	-
O116	-	-	-	-	-	-	1	-	-
(UT)	-	-	-	-	1	1	2	-	1

E. coli O157:H7 may impact the development of interventions to curb its presence in ruminants and thereby reduce the incidence of human infections with this pathogen (Magnuson *et al.*, 2000). The prevalence of gastro intestinal tract disorders in poor body weight lambs deprived of colostrum was significantly higher leading infection to flare up and produces diarrhoea (Wray *et al.*, 1981).

A relatively high rate of *E. coli* infection was reported in sheep of <1 year age group (83%) than >1 year age (17%) group in the present study. Infection was more pronounced in male (73 per cent) than female (27%). Raza *et al.* (2007) reported more susceptibility to gastrointestinal tract disorders in rams as compared to ewe. These findings may be due to testosterone which is known for its immunosuppressive activity (Seli and Arici, 2002).

All the 48 isolates of *E. coli* showed maximum sensitivity to polymixin B, ofloxacin, amikacin (93.3% each), ampicillin (90.4%), colistin (87.5%), furaxone (75%), amoxycillin+clavulanic acid (63.6%), cefixime (50%), amoxycillin (45.4%), amoxycillin+sulbactam (40%), doxycycline (25%), ciprofloxacin, cefpodoxime, co-trimoxazole and nalidixic acid (12.5% each). However Blanco *et al.* (1996) reported highest percentages of antibiotic resistance were reached in the group of antibiotics (tetracycline, streptomycin, sulphadiazine, ampicillin, kanamycin, neomycin, chloramphenicol, trimethoprim and cotrimoxazole) against O4, O6, O7, O8, O9, O11, O23, O26, O77, O80, O101, O103 and O161 serotypes. They concluded that *E. coli* strains isolated from diarrhoeic lambs belong to a large number of serogroups and may be reason for high variation in antibiotic resistance. Magnuson *et al.* (2000) reported that fast intestinal cell proliferation in the cecum and the distal colon correlates with rapid clearance of *E. coli* O157 from the ruminants GIT. Boerlin *et al.* (2005) reported that the antimicrobial resistance frequency among *E. coli* isolates from swine in Ontario was moderate in comparison with other countries and was higher in isolates from pigs with diarrhea than in isolates from healthy finisher pigs. Due at least in part to gene linkages, the distribution of resistance genes was very different between enterotoxigenic *E. coli* (ETEC) isolates and other porcine *E. coli* isolates. This demonstrates that antimicrobial resistance epidemiology differs significantly between pathogenic and commensal *E. coli* isolates. These results may have important implications with regards to the spread and persistence of resistance and virulence genes in bacterial populations and to the prudent use of antimicrobial agents. The *strA-strB* gene pair was the most frequent resistance determinant in the isolates examined. This study indicates that nonpathogenic *E. coli* from swine may represent a considerable reservoir of antibiotic resistance genes that might be transferable to pathogens (Sunde *et al.*, 1998).

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