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Microbiological studies on diarrhoeic sheep in Southern Rajasthan

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Abstract
The present study was undertaken to find out the bacteriological etiology in 64 sheep affected with diarrhea in southern region of Rajasthan. Bacteria isolated were *E. coli* in 36.92%, *Pseudomonas* 6.15%, *Klebsiella* 9.23%, *Yersinia* 7.69%, and *Citrobacter* 9.23% *Pasteurella* 7.69% and No bacterial growth in 21.87% of the sample collected. *E. coli* serotype identified were O135 in 25 %, O26 in 12.5% and untypable in 62.5%. Molecular confirmation of *E coli* and pseudomonas isolates was done by amplification of *uspA* & *Oprl* gene by using PCR. Antibiogram patterns of different bacterial organisms isolated from various sample of diarrheic sheep showed varying degree of sensitivity to the chemotherapeutic agents. Antibiogram patterns observed for various bacteria will be useful in prevention and treatment of diarrhaea.

Keywords: anitibiogram, bacteriological, diarrhoea, sheep, serotype, PCR

1. Introduction
Diarrhoea is a serious problem in sheep and goat farming, causing great economic losses. Most bacterial enteropathogens of diarrheic sheep isolated and identified were *Escherichia coli*, *Salmonella* species and *Klebsiella* species. [13]. The effective development of any livestock industry mostly depends upon prevention and control of diseases among these animals. Diseases in animals cause heavy economic losses in terms of milk, meat and wool industry. Microbial organisms and parasites play an important role in diseases causing heavy morbidity and mortality as they are important etiological agents causing gastroenteritis in sheep [6].

2. Material and Methods
Samples for present study were collected from Various sheep farms and Veterinary clinics of southern region of Rajasthan. Sheep carcasses submitted to Department of Veterinary Pathology, College of veterinary and Animal Science, Navania, Udaipur died with the history of diarrhoea for post-mortem examination also included in the present study. A total of 64 samples were collected during the period from January 2020 to December 2020.

2.1 Bacteriological studies
2.1.1 Collection of sample
Samples for present study were collected aseptically using sterile cotton swab from the liver and intestine of dead carcass of sheep died with the history of diarrhoea. Faecal swabs were also collected from sheep showing signs of diarrhoea.

2.1.2 Isolation and characterization of concurrent bacterial infections
Bacterial isolation and characterization has been carried out as per the standard techniques [12].

2.2 Antibiotic sensitivity test of concurrent bacterial infections
Different strains of various organisms isolated were subjected to in–vitro drug susceptibility testing using antimicrobials by the disc diffusion method as suggested by [2].

2.3 Molecular confirmation of *E. coli* and Pseudomonas by PCR
Molecular confirmation of *E. coli* was done by PCR amplification of *uspA gene* and Pseudomonas *Oprl* gene the method as described [11].

Table 1: Sequences of different primers used in the present study

Bacteria	Gene	Sequence (5'- 3')	Size (base Pair)	Annealing temp (ºC)
E. coli	*usp A gene*	CCGATACGCGCCAATCAGT ACGCAGACCGTAAGGGCCAGAT	884	50
Pseudomaons	*Oprl gene*	ATGAACAACGTTCTGAAATTCTCTGCT CTTGCGGCTGGCTTTTTCCAG	249	50

2.4 Serological characterization

All *E. coli* isolates showed positive reactions in biochemical tests were sent to National *Salmonella* and *Escherichia* centre Kasauli, Himachal Pradesh, India, for serological characterization.

3. Results

3.1 Bacteriological studies

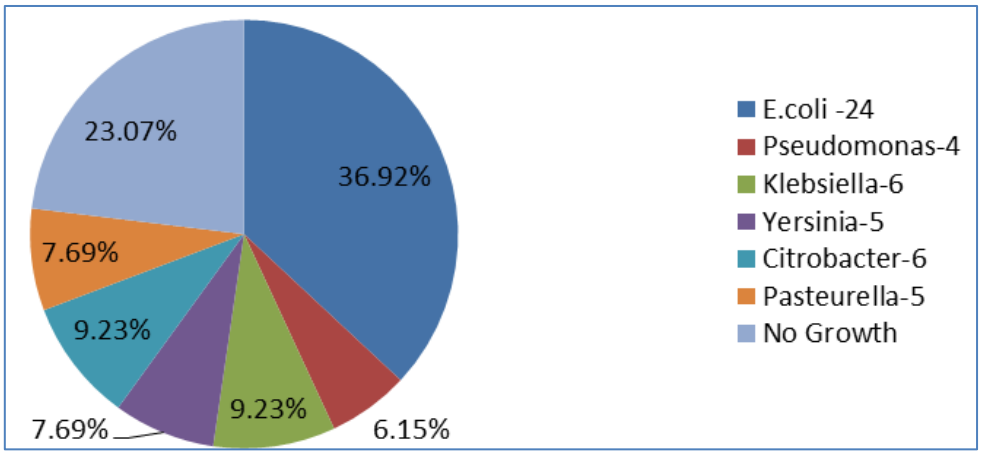

Fig 1: Pie chart showing Percentage of various bectaria isolated from diarrhoeic sheep

Out of 64 sample collected 49 samples revealed the presence of different types of bacteria which may be associated with the causation of the diarrhoea. Bacteria isolated were *E. coli* in 36.92%, *Pseudomonas* 6.15%, *Klebsiella* 9.23%, *Yersinia* 7.69%, and *Citrobacter* 9.23% *Pasteurella* 7.69% and No bacterial growth in 21.87% of the sample collected. Association of theses bacteria in pathology of diarrhoea and isolation of theses bacteria from diarrheic cases were also reported by [9].

[14, 13, 5] In majority of the cases, organisms were isolated, indicating that these bacterial agents might have caused pathological lesions in affected organs and leads to diarrhoea.

3.2 Molecular detection/confirmation of *E. coli* and *Pseudomonas spp.* isolated from sheep affected with diarrhoea

E. coli & *Pseudomonas* isolated from diarrhoeic cases of sheep were subjected to identification, which was done on the basis of cultural, morphological and biochemical characteristics. The PCR based methods are more sensitive and rapid than phenotypic tests performed on individual colonies. Thus, in present study molecular confirmation of *E. coli* and *pseudomonas* isolates was done by amplification of *uspA* & *Oprl* gene by using PCR. The *uspA* genes were successfully amplified using species specific primers. PCR amplification resulted into a single amplicon of 884 bp for *E. coli* and 249bp for *pseudomonas* [11] as illustrated (Figure. 2 & 3). Molecular confirmation of *E. coli* and *Pseudomonas* are in agreement with the studies of previous study by [4].

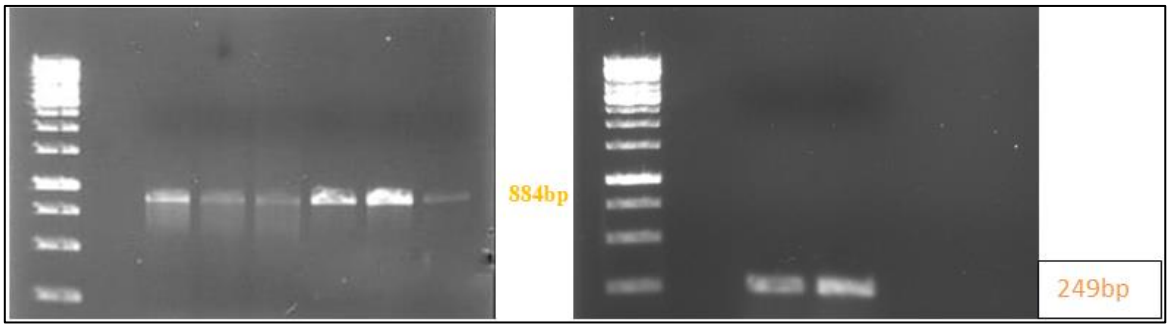

Fig 1 **Fig 2**

Fig 2: Detection of *uspA* by PCR; for detection of *uspA E. coli* isolated from samples were subjected to PCR using gene specific primers of *uspA*. PCR product was run on 1.5% agarose in TAE buffer at 70 volts. Well No. -1 Negative control 3 positive control and 4, 5, 6, 7 and 8 field sample positive for *E. coli.*

Fig 3: Detection of *Oprl* by PCR; for detection of *Oprl pseudomonas* isolated from samples were subjected to PCR using gene specific primers of *Oprl*. PCR product was run on 1.5% agarose in TAE buffer at 70 volts. Well No. -1 Negative control 3 positive control and 4 field sample positive for *pseudomonas*

3.3 Serotyping of *E. coli* isolate

The following serotype were identified from the *E. coli* isolates from samples of diarrhoeic sheep. Association of these serotypes isolated from the current study on diarrhoea in sheep are reported by many workers [8, 9, 10].

Table 2: Various serotypes of *E. coli* isolate from diarrhoeic sheep

Serotype	Number of isolate	Percentage
E. coli (O26)	1	12.5%
E. coli (O135)	2	25%
E. coli (Untypable)	5	62.5%

3.4 Antibiotic sensitivity test of concurrent bacterial infections

Table 3: Antibiotics sensitive test for concurrent bacterial isolates recovered from samples of dirrhoeic sheep

S. N.	Drugs	*E. coli* (24)			*Pseudomonas* (4)			*Klebsiella* (6)			*Yersinia* (5)		
		S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
1	Ampicillin	60	10	30	66	10	24	56.10	15	20%	45	5%	50
2	Ciprofloxacin	45	5	50	38.43	3	59.30	42	5	53	45	5	50
3	Azithromycin	25.20	10	65.80	45	15	40	35	20	45	40	15	45
4	Rifampicin	38.30	20	40.6	35.38	20	45.36	40	15	45	30	15	55
5	Streptomycin	54	5	41	53	7	40	50	10	40	50	5	45
6	Gentamycin	35	15	50	30	15	55	35	10	55	50	15	35
7	Tetracycline	40	0	60	40	10	50	40	10	50	45	0	55
8	Cefixime	35	15	50	20	15	65	20	10	60	35	15	50
9	Amikacin	52	5	43	49.23	10	40.25	55	5	40	50	10	40
10	Clarithromycin	50.63	10	39	35	15	50	45	10	45	35	5	60

In the present study, 10 different antibiotics were used for antibiotic sensitivity test for the concurrent bacterial isolates recovered from diarrhoeic sample of sheep. The antimicrobial resistance profile of the isolates obtained from diarrhoeic sheep to 10 antibiotics revealed that the most effective/sensitive antibiotic against *E. coli spp. was* ampicillin (60%) followed by streptomycin (54%), amikacin (52%),clarithromycin(50.63%), ciprofloxacin (45%), tetracycline (40%), rifampicin (38.30%), less sensitive towards, gentamycin and cefixime (35%) azithrpmycin (25.20%). *Pseudomonas* aeruginosa was found to be most sensitive to ampicillin (66%), followed by Streptomycin (53%), amikacin (49.23%), azithrpmycin (45%) and least to cefixime (20%) and highest resistance was showed against cefixime (65%) followed by ciprofloxacin (59.30%) gentamycin (55%) and were less resistance towards ampicillin (24%) *Klebsiella spp.* revealed maximum sensitivity to ampicillin (56.10%), followed by amikacin (55%), streptomycin (50%) & least to cefixime (20%). and highest resistance were showed against cefixime (60%) gentamycin (55%), ciprofloxacin (53%) and were less resistance towards ampicillin (20%).*Yersinia spp.* exhibited maximum sensitivity to streptomycin, gentamicin, amikacin (50% each), followed by ciprofloxacin, tetracycline (45% each) whereas it was least sensitive to rifampicin (30%) and highest resistance were stowed against clarithromycin (60%) followed by rifampicin, Tetracycline (55% each) and were less resistance towards gentamycin (35%).These finding were in agreement with the [5, 7].

4. Conclusion

On the basis of this study it is reasonable to conclude that diarrhoea in sheep are mainly caused by bacteria i.e. *E. coli*, *Pseudomonas*, *Pasteurella*, *Klebsiella*, *Yersinia* and *Citrobacter*. Antibiogram patterns observed for various bacteriain will be useful in prevention and treatment of diarrhaea.

5. Acknowledgements

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