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Research Article

Prevalence of pulpy kidney disease and antimicrobial sensitivity profile of *Clostridium perfringens* type D recovered from enteric intoxicated diarrheic goat of different age and gender in District Tharparkar, Sindh Pakistan

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Citation

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Abstract

Present study has been carried out to assess the occurrence of pulpy kidney disease and assessment of antimicrobial sensitivity profile of *Clostridium perfringens* type D against various antibiotics. Total 100 fecal samples were collected from diarrheic goat in aseptic polyethylene sachets, and then further processed for identification and isolation of *Clostridium perfringens* type D by different laboratory techniques. Positive samples were processed for detecting their antibiotic susceptibility pattern, through 2-fold micro broth dilution method by using MIC. From total collected samples only 45 samples were found positive. The age wise occurrence of pulpy kidney disease was 11.11 % in 1 month, 33.33% in 6 months, 40.00 % in 1 year, and 15.56 % in 2 year of goat. However; males were 44.44% while females were 55.56% positive for pulpy kidney disease. Among positive samples, 01 sample shown resistant at 16µg/ml and 01 sample at the concentration of 32µg/ml against Gentamycin. Although for Tylosin titrate, 01 sample shown resistant at 16µg/ml, 01 sample at 32µg/ml and 01 sample at the concentration of 64µg/ml. Moreover, for Trimethoprim/Sulphamethaxzol all samples were detected sensitive. Likewise, 01 sample shown resistant at 32µg/ml and 01 sample at the concentration of 64µg/ml concentration of Amoxicillin. In the same way 01 sample shown resistant at 32µg/ml concentration of Chloramphenicol used in this study. This study has been evaluated the occurrence of pulpy kidney disease and also provide the best treatment options and preventive measures against the outbreak of this fatal infection which will prove beneficial for farmers to save their animals from this fatal intoxication.

Keywords: Antimicrobial sensitivity; *Clostridium perfringens* type D; Enteric intoxicated; Goat; Pulpy kidney

Introduction

Goat is considered as a poor man's cow and an earliest small ruminant animal species to keep in houses and reared for milk and meat production in the Middle East since 2500 B.C [1]. It has been reported that there are round about 300 breeds of goat found from different parts of the world [2]. Prevention from seasonal fatal infectious diseases like enterotoxaemia can be very helpful to get maximum milk and meat production [3].

Enterotoxaemia or pulpy kidney disease is a bacterial illness of gastrointestinal tract characterized by hyperthermia, diarrhoea mixed with blood, paleness of mucous membrane, grinding of teeth, blurred vision and reduction in milk production, systemic lesions observed on kidney as it causes nephritis that's why this abnormality called as pulpy kidney disease. Mostly this abnormality takes place in those animals who expose to excessive grain over load that will disturb the normal microbial flora of GIT of animal leads towards toxin production & inflammation of intestine that's why this disease is also said to be over-eating disease this infectious disease reported in caprine and ovine worldwide, animals supplying with nutritive diet are most susceptible [4] in caprine and ovine animal species this disease causes huge economic losses every year at the time of start of moon soon in Pakistan [5] gram positive anaerobic, spore farming & rod shaped pathogen named *Clostridium perfringens* type D is responsible for causing this illness in caprine and ovine [6]. Pathogen secrete epsilon, alpha and iota toxin that will causes enteritis & toxaemia in host [7]. The risk Factors to facilitate the growth of disease causing agents in the intestine of host are included sudden changes in environment, changing in diet plan, burden of pathogen in intestine [8, 9]. Antibiotic are the substances having chemical nature which will be either bacteriostatic or bactericidal [10]. They have been classified on the basis of their mechanism of action like protein synthesis inhibitor included streptomycin & tylosin, cell wall inhibitor included penicillin, DNA gyrase inhibitor like enrofloxacin, ciprofloxacin & antimetabolites included trimethoprim [11]. They have also been classified as narrow and broad spectrum on the basis of covering different range of pathogen [12].

When growth of bacteria observed in spite of having exposure to antibiotics it will create disturbance in subsiding the infection from diseased animals [13]. Therefore, it is very much necessary to apply some preventive measures and strategy like providing diseased animals with specific antibiotics with proper dose and dosage, and understanding possible mechanism of action of different antibiotics, hence this study have been carried out to determine the beneficial effect of the different chemotherapeutic agents against the disease causing pathogen.

Materials and Methods

Collection of sample

From diarrheic goat total 100 number of fecal sample were collected in aseptic polyethylene bags filled with saline of phosphate buffer twice the volume of fecal sample and then samples were brought to the laboratory in ice packed portable cooler, & collected samples kept in refrigerator at the temperature of 4^0C which will be further process up to in 48 hours for isolation & identification of type D *Cl. perfringens* bacteria by various laboratory techniques like biochemical characteristics, morphological characteristics & growth pattern on media of Robertson cooked meat (RCM).

Identification and isolation of type D *Cl. Perfringens*

The Robertson's cooked meat broth (RCMB) tubes were filled with diarrheic sample. The poured RCMB tubes were transferred into water bath for to expel out aerobic and non-spore forming bacteria at

80^0C for a period of 10-15 min. then, tubes having RCM broth and culture were kept in anaerobic jar and jar was kept in incubator at 37^0C for 24 to 48hours, then for further confirmation and accuracy bacterial culture was taken from RCM broth with help contamination free wire lope and spread on RCM agar, streaked petri dishes were kept at 37^0C in incubator for the time length of 24 hours for to get the pure colonies of the pathogen.

Test of antimicrobial sensitivity

Procedure

The antibiotic susceptibility pattern of pathogen analyzed through Minimum inhibitory concentration test through 2-fold (MBDM) on Muller Hinton agar (Oxide, UK). For MIC a dilution of 1:1000 was prepared in which 6µl culture of isolates were mixed into 6ml of broth of Muller Hinton.

Micro titer plate tray having 96 wells was use for MIC test. Well number 11 was selected for positive control having culture of disease causing agent and media while well number 12 of micro titer tray have been selected for negative control which was having only media. The optical density values of wells were measured at 524nm in ELISA reader. After keeping the micro titer tray at 37°C for a time length of 24 hours in incubator, the density values of wells were noted again. The low concentration indicated that reduction of growth by decline in optical density value was measured to be reduction of growth at minimum concentration of antimicrobial against isolate. 1250 µg/ml of Gentamycin, 1240 µg/ml of Tylosin titrate, 1260µg /ml of Trimethoprim/sulphamethaxzole, 1280 µg/ml of Amoxicillin and 1270µg /ml of chloramphenicol (Aldrich Sigma) was used for MIC against isolates

The confirmed culture of isolates was filled in micro titer tray's wells. A culture of volume 180µl added in well number 1st and in remaining wells a culture of 100µl were added and then 20µl volume of Gentamycin (Sigma, Aldrich) filled in well 1st of micro titer tray and then thoroughly dissolved by Eppendorf tube and then 100µl volume of culture from 1st well was transferred into next well and repeated till the last well of microtiter tray. The remaining proportion of culture was evacuated after that micro titer tray were kept in incubator at 37^0C. For 24 hours. The obtained results were verified by performing the test for three times repeatedly.

For remaining antibiotics same methodology was used to check MIC against the pathogen.

Statistical analysis

The results were analyzed by using computerized statistical package i.e. Student Edition of Statistics (SXW), version 8.1 (copyright 2005, Analytical software, USA).

Results and Discussion

The purpose of conducting this study is to check the occurrence of pulpy kidney disease in diarrheic goats in the study area. During this study from total 100 number of samples the positive number of samples was 45 in diarrheic goats (Table 1). Similarly, **[14]** have also been determined high occurrence of pulpy kidney disease (60%), 15 out of 25 samples detected positive with type D *Cl. perfringens*. High occurrence of pulpy kidney disease in the study area is due to lack of awareness in farmers, improper timing of treatment & schedule of vaccination against the disease in study area. Likewise, **[15]** also experimentally observed the 26% occurrence of pulpy kidney disease. Among 100 diarrheal samples 26 were detected positive with type D *Cl. perfringens.* Furthermore **[16]** was also reported the occurrence of pulpy kidney disease as (27.38%) in affected goat. Apart from this **[17]** also observe the (22%) prevalence of enterotoxemia among diarrheic goats. This high prevalence of enterotoxemia among diarrheic goats was observed due the various risk factors such as feed, water, parasitic infestation and over feeding etc. Age wise distribution of pulpy kidney disease was observed. Among

positive sample, 08 samples in one month, 15 samples in six months, 18 sample in one year and 07 sample were found positive in two years of age among diarrheic goats. The age wise distribution in percentage was 11.11 % in one month, 33.33% in six months, 40.00 % in one year, and 15.56 % in two year of goats, very high prevalence was observing in one year of age (40.00 %) as shown in (Table 1). Similarly, **[15** also observe the age wise percentage prevalence in diarrheic goats 3% in one month, 11.5% in six months, 13% in one year and 3.5% in one year of age respectively. Gender wise occurrence pulpy kidney disease in diarrheic goats in the study area was observed, out of positive samples, 20 in male goat while in female 25 samples were found positive for enterotoxaemia. These results shows that male was 44.44% while female was found 55.56% positive for enterotoxaemia among diarrheic goats in the study area as shown in the (Table 1). Similarly, **[15** also observe the gender wise prevalence of enterotoxaemia among diarrheic goats, males (12.5%) and females (15%). High prevalence of enterotoxaemia among diarrheic female goats due to stress factors like, feed, water, environment, stress of estrus and pregnancy etc. **[16]** also observe the high prevalence of enterotoxaemia in female (17.16%) and in male (11.20%) respectively. This high prevalence observed in female goat also due to the exposure of female animals to the various risk factors were higher than male goat. The disease causing agent of this study was determined by hemolysis activity on sheep blood agar, physical appearance on microscopy & growth pattern. Similarly, **[18]** mentioned that *Cl. perfringens* produced smooth, large, regular convex and slightly opaque colonies and zone of complete hemolysis surrounded by wider zone of incomplete hemolysis **[19]** also observed the inner complete and outer less complete hemolytic zones produced by *Cl. Perfringens*. Apart from this **[14]** also observed hemolysis caused by *Cl. Perfringens* on the agar of sheep blood. Morphologically *Clostridium perfringens* type D was found gram positive bacilli (Rod shaped) **[15** also examined rod shaped *Clostridium perfringens* under electron microscope. The isolates of this study was confirmed by the findings of above studies. The *Clostridium perfringens* type D isolates were confirmed by its biochemical properties. Lecithinase, Methyl Red Test, Geletin Liquefaction Test and Triple Sugar Iron Test were positive for *Clostridium perfringens* whereas Catalase, Oxidase, Urease, Indole, Citrate and Voges Proskauer were found negative (Table 3) **[15** reported the same biochemical test pattern for isolates. The above biochemical results are in agreement with present study. Furthermore **[20]** also conformed the *Clostridium perfringens* isolate through these biochemical tests. Among positive isolates only 02 (4.44%) samples shown resistant of moderate level against Gentamycin (Table 3) **[21]** also observe that out of 51 *Clostridium perfringens* type D isolates, all isolates were sensitive to Gentamycin. Furthermore, **[22]** also observed that out of 100 *Clostridium perfringens* type D isolates, all isolates were sensitive. Apart from this [17] also experimentally observed the beneficial effect of Gentamycin against pathogen. Among positive isolates, 03 (6.66%) samples were shown resistant of moderate level to Tylosin titrate (Table 3) **[22]** also observed that out of 100 *Clostridium perfringens* type D isolates, all isolates were sensitive to Tylosin titrate. Apart from this **[23]** also experimentally observed that Tylosin titrate exert beneficial effect against the isolates. In the same way **[24]** also reported that *Cl. perfrgins* type D shows sensitivity and mild degree of resistant to tylosin. Likewise, **[25, 26]** also observed the excellent chemotherapeutic effect of tylosin against disease causing agents. Among positive samples, all isolates were sensitive to Trimethoprim/Sulphametahxzole (Table 3) **[22]** also observed that out of 100 *Clostridium perfringens* isolates, all

isolates were sensitive to Sulphametaxzole/Trimethoprim. Among positive isolates only 02 (4.44%) isolate was shown resistant of moderate level against amoxicillin (Table 3) [21] also observe that out of 51 *Clostridium perfringens* type D isolates, all isolates were sensitive to amoxicillin. Furthermore [22] also observed that out of 100 *Clostridium perfringens* isolates, all isolates were sensitive to amoxicillin. Apart from this [23] also experimentally observed that amoxicillin showed an excellent activity against *Clostridium perfringens* isolates. Likewise, [24] also reported that *Clostridium perfringens* type D is sensitive to amoxicillin. In the same way [25] also observed the excellent chemotherapeutic effect of amoxicillin against disease causing agents. Among positive isolates only 01 (2.22%) samples shown resistant of moderate level against Chloramphenicol (Table 3) [21] also observe that out of 51 *Clostridium perfringens* type D isolates, all isolates were sensitive to Chloramphenicol. Furthermore [27] also observe that all the *Clostridium perfringens* type D isolates shown no resistant against chloramphenicol but some of few pathogens were shown moderate level of resistant against Chloramphenicol. However, [23] also experimentally observed that Chloramphenicol showed an excellent activity against *Clostridium perfringens* type D isolates.

Table 1: Age and gender wise prevalence of pulpy kidney disease among enteric intoxicated diarrheic goats in district Tharparkar

Distribution:	Collected samples (No.)	Positive		Negative		Prevalence among positive samples (%)
		Number	Percent	Number	Percent	
Age-wise						
One month	25	05	20.00	20	80.00	11.11
Six month	25	15	60.00	10	40.00	33.33
One year	25	18	72.00	07	28.00	40.00
Two year	25	07	28.00	18	72.00	15.56
Total	100	45	45.00	55	55.00	100.00
Gender-wise						
Male	50	20	40.00	30	60.00	44.44
Female	50	25	50.00	25	50.00	55.56
Total	100	45	45.00	55	55.00	100.00

Table 2: Biochemical characteristics of type D, *Clostridium perfringens* recovered from enteric intoxicated diarrheic goat in district Tharparkar.

S. No.	Biochemical Tests	Result
01	V.P	-ve
02	Ind.	-ve
03	Ure.	-ve
04	Oxid	-ve
05	Cat.	-ve
06	TSI	A/A
07	GLT	+ve
08	M.R	+ve
09	Leci.	+ve

Whereas:
Leci. = Lecithinase, **V.P** = Voges proskauer, **Ind** = Indole, **Ure** = Urease, **Oxid**. = Oxidase, **Cat.** = Catalase, **TSI** = Triple sugar iron, **GLT** = Gelatin liquefaction test, **M.R** = Methyl Red,

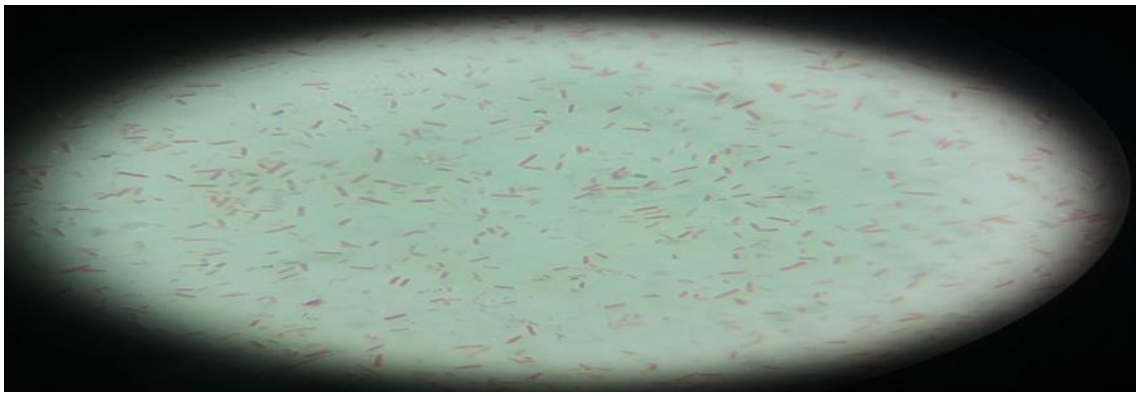

Figure 1: Morphological characteristics of type D, *Clostridium perfringens* recovered from enteric intoxicated diarrheic goat in district Tharparkar

Table 3: Number and percentage of Gentamycin, Tylosin titrate, Trimethoprim/Sulphamethaxzole, Amoxicillin and Chloramphenicol Sensitive and Resistant profile of *Clostridium perfringens* type D recovered from enteric intoxicated diarrheic goat

Drugs	Collected samples (No.)	*Positive		Negative		Sensitive		Resistant	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
Gentamycin	100	45	45.00	55	55.00	43	95.55	02	4.44
Tylosin titrate	100	45	45.00	55	55.00	42	93.33	03	6.66
Trimethoprim/Sulphamethaxzole	100	45	45.00	55	55.00	45	100.00	0.00	0.00
Amoxicillin	100	45	45.00	55	55.00	43	95.55	02	4.44
Chloramphenicol	100	45	45.00	55	55.00	44	97.77	01	2.22

Conclusion

It was observed that type D, *Clostridium perfringens* detected in diarrheal samples of the goat. Goats of one year of age were highly affected with enterotoxemia, Female goats were highly affected with enterotoxemia as compared to male goats. Among five antimicrobials, Gentamycin, Tylosin titrate, Trimethoprim/ Sulphamethaxzol Amoxicillin and Chloramphenicol. The Trimethoprim/ Sulphamethaxzol detected beneficial against *Clostridium perfringens* type D followed by chloramphenicol, Gentamycin, Amoxicillin and Tylosin titrate.

Authors' contributions

Conceived and designed the experiments: JK Zaman & MM Rahimoon, Performed the experiment: MM Rahimoon, JK Zaman, NH Kalhoro, AH Mirani & Q Kalwar, Analyzed the data: TA Khokhar, AK Lund, ZA Khoso & H Janyaro, Contributed reagents / Materials / Analysis tools: MA Soomro & HA Kolachi, Wrote the paper: JK Zaman & MM Rahimoon.

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The sample were processed and analyzed in the laboratory of Sindh Institute of Animal Health Karachi Sindh Pakistan.

References

1. Dubeuf JP & Boyazoglu J (2009). An International panorama of goat selection breeds. *Livest Sci* 120: 225–231.
2. Luikart G, Fernandez H, Mashkour M, England PR & Taberlet P (2006). Origins and diffusion of domestic goats inferred from DNA markers. Example analyses of mtDNA, Y-chromosome and microsatellites. In: Documenting domestication. New genetic and archaeological paradigms (edition by M.A. Zeder, D.G. Bradley, E. Emshwiller & BD Smith). pp. 294-305.

3. Chandran D, Naidu SS, Sugumar P, Rani GS, Vijayan SP, Mathur D, Garg LC & Srinivasan VA (2010). Development of a recombinant epsilon toxoid vaccine against enterotoxemia and its use as a combination vaccine with live attenuated sheep pox virus against enterotoxemia and sheep pox. *Cli Vacc Immuno* 17: 1013-1016.
4. Jemal D, Shifa M & Kebede B (2016). Review on Pulpy Kidney Disease. *J Vet Sci Technol* 7: 361.
5. Kumar VN, Sreenivasulu D & Reddy YN (2014). Prevalence of clostridium perfringens toxin genotypes in enterotoxemia suspected sheep flocks of Andhra Pradesh. *Vet World* 7: 1132-1136.
6. Uzal FA, Vidal JE, McClane BA & Guraj AA (2014). Clostridium perfringens toxins involved in mammalian veterinary diseases. *Op Toxico J* 2: 24-42.
7. McClane BA, Uzal FA, Fernandez-Miyakawa ME, Lyerly DM & Wilkins T (2006). The enterotoxigenic clostridia, p 698 –752. In Dworkin M, Falkow S (ed), The procaryotes, 3rd edition Springer-Verlag, New York.
8. Javed MT, Irfan M, Mukhtar N & Hussain R (2009). An outbreak of enterotoxaemia at livestock farm during subtropical summer. *Acta Tropica* 112: 225-227.
9. Lewis CJ (2000). Clostridial diseases. In: Diseases of sheep, ed. Martin WB, Aitken ID, 131–142. Blackwell Science, Oxford, England.
10. Brandt KK, Amézquita A, Backhaus T, Boxall A, Coors T, Heberer JR, Lawrence J, Lazorchak J, Schönfeld JR, Snape YG, Zhu & Topp E (2015). Ecotoxicological assessment of antibiotics: a call for improved consideration of microorganisms. *Environ Inter* 85: 189–205.
11. Kümmerer K (2009). Antibiotics in the aquatic environment-A review-part I, Chemosphere 75: 417–434.
12. Bernier SP & Surette MG (2013). Concentration-dependent activity of antibiotics in natural environments, *Front Microbiol* 4: 20.
13. Fernández J, Bert F & Nicolas-Chanoine MH (2016). The challenges of multi-drug-resistance in hepatology. *J Hepat* 65: 1043–1054.
14. Nazki S, Wani SA, Parveen R, Ahangar S A, Kashoo ZA, Hamid S & Dar PA (2017). Isolation, molecular characterization and prevalence of Clostridium perfringens in sheep and goats of Kashmir Himalayas, India. *Vet World* 10(12): 1501.
15. Ajaz-ul-Haq, MKT, Taj I, Arif S, Ahmed A, Muhammad G, Ahmed Z, Ahmed Z, Abbas F & Samad A (2016). Isolation of Clostridium perfringens from Goats and Sheep of the Khuzdar district of Balochistan, Pakistan. *Inter J Biol* 9(5): 156-162.
16. Desh S & Deepak (2017). Molecular pathogenesis of enterotoxaemia in neonatal goat kids. *Ind J of Vet Patho* 41(4): 327-327.
17. Maqbool B, Iqbal MK, Ijaz M, Asalam MB, Ahmed HI & Hussain K (2017). Prevalence and chemotheraupy of enterotoxemia (Clostridium perfringens) in darrheic sheep and goat. *J Innov Biol Res* 1(1): 30-35.
18. Karthik K, Manimaran K, Bharti R & Shoba K (2017). Report of enterotoxemia in goat kids. *Adv Anim Vet Sci* 5(7): 289-292.
19. Uzal FA & Songer JG (2008). Diagnosis of Clostridium perfringens intestinal infections in sheep and goats. *J of Veter Diag Invest* 20(3): 253-265.
20. Miah MS, Asaduzzaman M, Sufian MA & Hossain MM (2011). Isolation of Clostridium perfringens, Causal agents of necrotic enteritis in chickens. *J Bang Agri Uni* 9(1): 97-102.
21. El-Tayab WN & Gomaa FAM (2012). Rapid Identification and Antimicrobial Susceptibility Profiling of Anaerobic Bacteria in Clinical Specimens. Analysis of Interleukin-18

and CD14 Gene Polymorphism in Atopic Patients. *Egy J of Med Microbiol* 21(3): 29.
22. Gad W, Hauck R, Krüger M & Hafez HM (2011). Determination of antibiotic sensitivities of Clostridium perfringens isolates from commercial turkeys in Germany in vitro. *Archiv für Geflügelkunde* 75: 80-83.
23. Llanco LA, Viviane N, Ferreira AJP & Avila-campos MJ (2012). Toxinotyping and antimicrobial succepatbilty of clostridium perfringens isolated from broiler chicken with necrotic enteritis. *Inter J Microb Res* 4(7): 290-294.
24. Silva ROS, Salvarani F, Assis R, Martins N, Pires P & lobato F (2009). Antimicrobial susceptibility of Clostridium perfringens strains isolated from broiler chickens. *Brazi J Microb* 40: 262-264.
25. Martel A, Devriese LA, Cauwerts K, De K, gussem A & Decostere F (2004). Susceptibility ofClostridium perfringens strains from broiler chickens toantibiotics and anticoccidials. *Avi Patho* 3-7.
26. Gholamiandehkordi A, Eeckhaut V, Lanckriet A, Timbermont l, Bjerrum l, Ducatelle R, Haesebrouck F & Vanimmerseel F (2009). Antimicrobial resistance in *Clostridium perfringens* isolates from broilers in Belgium. *Vet Res Commun* 33: 1031-1037.
27. Naidoo S, Perovic O, Richards GA & Duse AG (2011). Clinically significant anaerobic bacteria isolated from patients ina South African academic hospital–antimicrobial susceptibility testing. *S Afr Med J* 101: 732-734.