

## Research Article

# Prevalence of enterotoxemia and antibiogram of *Clostridium perfringens* isolated from diarrheic goat in the vicinity of district Tharparkar, Sindh, Pakistan

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### Abstract

Goat is amongst the earliest small ruminant species to be domesticated and have been reared for meat and milk purpose. Enterotoxemia is a bacterial disease, also said to be pulpy kidney disease or over-eating disease, occurs in sheep and goat throughout the world. In Caprine this disease is caused by a bacteria called *Clostridium perfringens* type D, which is a gram positive, spore forming, anaerobic, and rod shaped. 100 faecal samples were obtained from diarrheic goats from four Taluka of District Tharparker Sindh, Pakistan. These samples were collected aseptically in ultra violet treated contamination free polyethylene bags which were contained double volume of phosphate buffer saline (PBS) and samples were brought to the laboratory (Sindh Poultry Vaccine Centre Korangi, Karachi, Sindh, Pakistan), in portable cooler containing an ice pack, then samples were refrigerated at 4°C until being processed. A chain of laboratory techniques, including colony characteristics, biochemical analysis, gram staining and growth on Robertson cooked meat (RCM) media was performed. Furthermore, all positive samples were subjected to check their antibiogram through 2-fold Micro Broth Dilution Method by using Minimum Inhibitory Concentration (MIC) method. Among 100 diarrheic samples the percentage of positive cases was (44.44%) in Talulka Dahali followed by Chachro (26.67%), Islamkot (17.78%) and Mithi (11.11%). Among 45 samples, a total of 01 sample was shown their resistant at the concentration of 16µg/ml against Amoxicillin. Although for Chloramphenicol, a total of 01 sample was shown their resistant at the concentration of 16µg/ml and 01 samples was shown their resistant at 32µg/ml concentration. Furthermore, there was no any sample show its resistant at the different concentration of Trimethoprim/Sulphamethaxzol used in this study.

**Keywords:** Antibiogram; Antibiotic; *Clostridium perfringens*; Enterotoxemia; Prevalence

### Introduction

Goat is amongst the earliest small ruminant species to be domesticated and have been reared for meat and milk purpose, since 2500 B.C. in the Middle East [1]. At present there are >300 breeds of goats are found in different areas of the world [2]. It has been observed that maximum production can be obtained by protecting them from different prevalent diseases like enterotoxemia [3]. Enterotoxemia is a bacterial disease, also said to be pulpy kidney disease or over-eating disease, occurs in sheep and goat throughout the world characterized by diarrhoea and dysentery, mostly occurs in animals which are supplying with well feed [4]. In Caprine this disease is caused by a bacteria called *C. perfringens* type D, which is a gram positive, spore forming, anaerobic, and rod shaped [5]. *C. perfringens*, a rapid-growing pathogen known to secrete an arsenal of >20 virulent toxins [6], and at the start of monsoon season, frequent disease outbreak of enterotoxaemia in sheep and goat have been encountered every year in Pakistan by *C. perfringens* Type D [7]. Almost all types of *C. perfringens* have produced alpha and epsilon toxin, *C. perfringens* type D also produce these toxins to develop enterotoxaemia in goat and sheep [8]. Factors which are responsible for exposing the animal towards the enterotoxaemia are stress, over feeding and high parasitic load in the intestine, it will create the disturbance in microorganisms which are present in the intestine [9, 10]. Antibiotics are the chemical substances which are used to stop the growth or to kill the microbes [11], as some of the chemotherapeutic agents are bacteriostatic and some are bactericidal. Antibiotics are specific for specific microorganisms such as antimycotics, antibacterial, antiprotozoal and antiparasitic, it will make the antibiotics to be different from disinfectants or other antimicrobials [12]. The ability of microorganisms to grow

even in the presence of chemotherapeutic agents create a disturbance in treating various infectious diseases in animals as well as in human being [13]. Therefore, it is very much necessary to reduce the problem of drug resistant against multiple microorganisms by applying some preventive measures like to understand the mode of action of various drugs against different microbes, proper use of antibiotics and by avoiding self-medication, hence the present study is designed to determine the effect of various antibiotics against the isolates.

### Materials and Methods

#### Sample Collection and Isolation of *Clostridium perfringens*

##### Sample collection

All faecal samples were collected from diarrhoeic goats in district Tharparkar, Sindh, Pakistan. The clinically ill goats were selected in this study and samples were collected from different Veterinary clinics and in the vicinity of district Tharparkar.

A total of 100 faecal samples were collected from diarrhoeic goats, The samples were collected aseptically in Ultra Violet (UV) treated contamination free polyethylene sachets which were contain double volume of phosphate buffer saline (PBS) and samples were brought to the laboratory Sindh Poultry Vaccine centre Korangi Karachi, in portable cooler containing an ice pack and then samples were refrigerated at 4°C until being processed within 48hr of collection for isolation and identification of *C. Perfringens* by chain of laboratory technique including colony characteristics, gram staining, biochemical tests and growth on Robertson cooked meat (RCM) media.

##### Isolation and identification

All the fecal samples were inoculated into (RCMB). The inoculated RCMB tubes were placed in water bath for a period of 10-15 mins at 80°C to remove the non-spore forming aerobic bacteria. Finally, the

RCMB tubes were incubated in anaerobic jar at 37°C for 24 to 48 hours, then for sub culturing with the help of sterilized wire loop, picked up the bacterial colonies from (RCMB) tubes and has streaked on prepared RCM agar medium in petri dishes, then petri dishes were placed in incubator at 37°C for 24 hours, the processing of sub culturing continue until the pure growth was observed.

#### **Antimicrobial susceptibility test**

The Minimum inhibitory concentration of bacterial isolates was done in Sindh Poultry Vaccine Centre Korangi Karachi, Pakistan. The antibiogram of *C. perfringens* isolates were determined through Minimum inhibitory concentration test by using (Micro broth 2-fold dilution method) on Muller Hinton (MH) agar (Oxide, UK) and 1:1000 dilution was prepared for minimum inhibitory concentration (MIC) test for that 6µl of bacterial culture of *C. perfringens* in RCM broth were added into 6ml of Muller Hinton broth.

96 well plates of micro titer plates were used for MIC test. Two wells of micro titer tray were used for positive and negative control. Well number 11 of the micro titer tray was used as positive control, having the bacterial culture and media. Well number 12 was used as a negative control, have only contains the media. The wells' optical density values were recorded by enzyme-linked immunosorbent assay (ELISA) reader at 524nm. After 24 hours of incubation at 37°C, the optical density values again recorded. The lowest concentration showed that inhibition of growth by decrease in optical density (OD) value was considered to be minimum inhibitory concentration of antibiotic against test organisms. A concentration 1280 µg/ml of Amoxicillin, 1270µg /ml of chloramphenicol and 1260µg/ml of Trimethoprim/sulphamethaxzole (Sigma, USA) was used for MIC against *C. perfringens*.

*C. perfringens* isolates culture were added in each well of micro titer tray. In first well

180µl of culture was added and in remaining all wells 100µl of bacterial culture was added. Then 20µl of Amoxicillin (Sigma, USA) were added in first well and mixed by push up of Eppendorf tube. After that 100µl of culture should be introduced from first well to next and repeat till last well. The final subculture of bacterial Isolates was discarded. Then MIC plates were placed in incubator at 37°C for 24 hours. Results of MIC test have been obtained by performing it 3X.

Same procedures were applied for chloramphenicol and Trimethoprim/sulphamethaxzole in order to determine their minimum inhibitory concentration against *C. perfringens* isolates.

#### **Statistical analysis**

The data obtained was proceeded for statistical procedure as summary stat to observed the descriptive measures by using computerized statistical package i.e. Student Edition of Statistics (SXW), version 8.1 (copyright 2005, Analytical software, USA).

#### **Results**

##### **Percentage prevalence of enterotoxaemia among diarrhoeic goats on taluka basis district Tharparkar, Sindh, Pakistan**

Total 100 diarrhoeal samples were collected from goats. All samples were cultured on different media to isolate the pure colony of *C. perfringens* then pure culture of organism was confirmed through grams staining and by biochemical properties. 45 samples were found positive with *C. perfringens* in diarrhoeal goats, while 55 samples were found negative out of 100 samples. Results have indicated that Dahali was highly infected with enterotoxaemia amongst four Taluka of district Tharparkar. These results shows that highest percentage (44.44%) found in Dahali Talulka followed by Chachro (26.67%), Islamkot (17.78%) and Mithi (11.11%) respectively as shown in (Table 1).

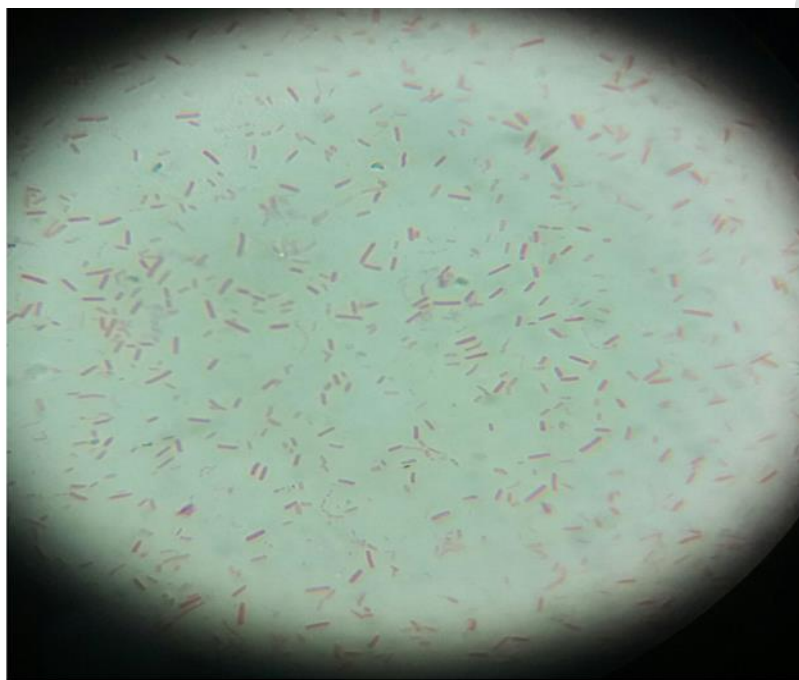
### Morphological characteristics of *C. perfringens* isolated from diarrhoeic goats in the vicinity of district Tharparkar

Morphologically identified sample were subjected to different biochemical tests to confirm the bacterial strains of *C. perfringens* before (MIC). The *C. perfringens* were recorded positive for methyl red, gelatin

liquefaction and triple sugar iron test. *C. perfringens* was found negative for catalase, oxidase, urease, indole, and Voges-Proskauer test. It has been showed A/A properties on triple sugar iron medium, this isolates showing the property of acidic slant and acidic but indicate that the sugar present in the media was fermented (Fig. 1; Table 2).

**Table 1. Percentage prevalence of Enterotoxaemia among diarrhoeic goats in the four Taluka of district Tharparkar**

Taluka	No. of Positive samples	Percentage
Chachro	12	26.67%
Dahali	20	44.44%
Islamkot	08	17.78%
Mithi	05	11.11%



**Figure 1. Biochemical characteristics of *Clostridium perfringens* isolates**

**Table 2. Biochemical characteristics of *clostridium perfringens* isolated from diarrhoeic goats in the vicinity of district Tharparkar**

<i>C. perfringens</i>	Leci.	M.R	GLT	TSI	Cat.	Oxid	Ure	Ind	V.P
	+ ve	+ ve	+ ve	A/A	- ve	- ve	- ve	- ve	- ve

Leci; Lecithinase, M.R; Methyl Red, GLT; Gelatin Liquefaction Test, TSI; Triple Sugar Iron, Cat.; Catalase, Oxid.; Oxidase, Ure; Urease, Ind; Indole, V.P; Voges proskauer,



### Minimum Inhibitory Concentration (MIC; µg/ml) sensitivity and resistant of selected antibiotics against *Clostridium perfringens* isolates from diarrheic goat

In order to detect the prevalence of Enterotoxaemia among goats in district Tharparkar, fecal sample were collected in the vicinity of district Tharparkar from diarrhoeal goats and were brought to Sindh poultry Vaccine Centre Korangi Karachi, Pakistan for isolation and storage of *C. perfringens*. The all isolated *C. perfringens* were further subjected to check their susceptibility and resistance pattern against Amoxicillin, Trimethoprim/

Sulphamethaxzole and chloramphenicol by MIC (2-fold micro broth dilution method). The positive samples were selected for antimicrobial susceptibility testing through Micro Broth Dilution Method by using (MIC) method as described by the clinical laboratory standard institute (CLSI 2011). It has been observed that out of 100 samples (MIC; µg/ml) sensitivity and resistant of selected antibiotics against *C. perfringens* were: Amoxicillin sensitive 44 and 01 resistant, Chloramphenicol 43 sensitive and 02 resistant, and as for Trimethoprim/ Sulphamethaxzole 45 sensitive and 00 resistant (Table 3).

**Table 3. Drugs sensitivity and resistant against *Clostridium perfringens* isolates from diarrheic goat**

Drugs	*Positive		Sensitive		Resistant	
	Number	Percent	Number	Percent	Number	Percent
Amoxicillin	45	45.00	44	97.77	01	2.22
Chloramphenicol	45	45.00	43	95.55	02	4.44
Trimethoprim/ Sulphamethaxzole	45	45.00	45	100.00	00	0.00

\*positive number/percent calculated over total 100 number of collected sample

### Discussion

Enterotoxemia is amongst the most important economic diseases [14], it is an infectious fatal disease of small ruminants caused by anaerobic gram positive bacterium *C. perfringens* [15]. This disease is one of the endemic disease in Pakistan that causes mortality up to 100 percent in goats [16]. We have investigated the occurrence of enterotoxaemia, 45 samples were found positive with *C. perfringens* from 100 faecal samples of diarrhoeic goats (Table 1). High prevalence of enterotoxaemia (45%) was observed among diarrheic goats in the vicinity of district Tharparkar. Similarly, high prevalence of enterotoxaemia (60%) was also reported from 15 samples found positive with *C. perfringens* out of 25 samples [17] and this high prevalence of enterotoxaemia was due to improper vaccination schedule against enterotoxaemia in the study area.

Previously reported that out of 100 faecal samples, 26% prevalence of *C. perfringens* was found among diarrheal goats [18]. Furthermore Taluka, (27.38%) prevalence of enterotoxaemia in diseased goats were also observed in another study [19]. We have investigated Taluka wise enterotoxaemia among diarrhoeal goats in district Tharparkar, Sindh [18], as it was also previously reported study in goats in Khuzdar district Baluchistan. According to results Dahali had high prevalence of enterotoxaemia amongst the four Taluka and the reason behind this might be unawareness, lack of veterinary facilities or improper vaccination schedule [20, 21]. The bacterial isolates of this study were confirmed by their typical growth pattern and colony characteristics, which include the double zone of hemolytic colonies of *C. perfringens* on blood agar. As previously reported that, *C. perfringens* produced smooth, large, regular convex

and slightly opaque colonies and zone of complete hemolysis surrounded by wider zone of incomplete hemolysis [22, 23]. Dual zone of haemolysis created by *C. Perfringens* on the agar of sheep's blood [24, 25]. Positive samples were selected for antimicrobial susceptibility testing through Micro Broth Dilution Method by using (MIC) method [26], Out of 45 isolates of *C. perfringens* only 01 (2.22%) isolate was shown moderate level of resistant to amoxicillin phenotypically, as reported previously [27]. Furthermore, in another study investigated that all isolates were sensitive to amoxicillin from 100 *C. perfringens* isolates [28]. Contra indicatory another study showed an excellent activity of amoxicillin against *C. perfringens* isolates [29].

2 out of 45 isolates of *C. Perfringens* with (4.44%) were shown moderate level of resistant to Chloramphenicol phenotypically in our results. Previous research have indicated that *C. perfringens* isolates were sensitive to chloramphenicol only for few isolates [30]. However, Chloramphenicol experimentally showed an excellent activity against *C. perfringens* isolates [29]. In present study all isolates were sensitive to Trimethoprim/Sulphamethaxazole from 45 isolates of *C. Perfringens*, as reported previously [28, 31].

### Conclusion

It may be concluded that the *C. perfringens* was present in faecal samples of the diarrhoeic goats. A very high prevalence of enterotoxemia found in diarrhoeic goats in the vicinity of district Tharparkar, Sindh, Pakistan. Among three antimicrobials, Amoxicillin, Chloramphenicol and Trimethoprim/Sulphamethaxazol, Trimethoprim/Sulphamethaxazol were found more effective against *C. perfringens* followed by Amoxicillin and Chloramphenicol.

### Authors' contributions

Conceived and designed the study: JK Zaman, MM Rahimoon & A Babar,

Contributed to revise the manuscript: AH Mirani & NH Kalhor, Wrote the paper: JK Zaman, MM Rahimoon & A Babar.

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