

Research

Comparison of Thioglycollate Broth with Liver Extract (BQ Vaccine Medium) with Traditionally Used Culture Media for Mass Scale Production of *Clostridium perfringens* Type D

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ABSTRACT

Thioglycollate broth with Liver Extract (BQ vaccine medium) was used to produce mass scale yield of *Clostridium perfringens* Type D. Upon growth of *Clostridium perfringens* in different culture media the OD value of bacterial growth in BQ vaccine medium, Reinforced Culture Medium (RCM), Cooked Meat Medium (CMM) and Nutrient Broth (NB) was 2.260, 2.184, 2.096 and 1.984 respectively. Mice Lethality result of growth in BQ vaccine medium was equal to that of RCM while growth in CMM and NB had very less lethality. ELISA percentage positivity for Epsilon toxins in the supernatant of growth in BQ medium at 450 nm wavelength at dilution of 320 and 640 was higher than that of all the culture media under comparison. It was concluded that BQ was an excellent culture medium for the development of Enterotoxaemia vaccine producing maximum concentration of epsilon toxin than any culture media used before.

Keywords: BQ vaccine medium, Thioglycollate broth, *Clostridium perfringens* Type D, RCM, CMM.

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INTRODUCTION

Enterotoxaemia is an acute and fatal disease of sheep and goat caused by *Clostridium perfringens* type D [1,2]. *Clostridium perfringens* has been divided into five groups (A-E) based on toxin types such as alpha, beta, epsilon and iota [3]. This bacterium is a normal inhabitant of GIT of caprine [4,5]. The over growth of the bacterium in GIT occurs due to ingestion of excessive quantities of partially digested carbohydrates especially grains, potatoes and wheat at their peak harvest season which serve as a suitable substrate for rapid clostridial proliferation and the production of alpha and epsilon toxins. Among different types of toxins of *Clostridium perfringens* type D, epsilon toxin is the most lethal bacterial toxin for small ruminants especially for sheep [6,7] and lead to toxemia with development of clinical signs including dullness, tympany, diarrhoea, staggering, convulsions and terminal coma [8,9]. The acute nature of disease especially in sheep does not allow enough time to use therapeutic interventions to save the animal. The only feasible option to control this disease and prophylaxis are based on effective vaccination with proper feeding and management. The toxoid vaccines are prepared by inoculating bacterium in a suitable medium, inactivated with formaldehyde, filtered and adjuvanted. The quality of toxoid is directly dependent on the production of the potent epsilon toxins in the culture medium. The different culture medium with different composition to achieve more potent toxoids have been

used. The present study is aimed to evaluate a new culture medium and compared with traditionally used preparations by measuring cell mass, epsilon toxin production and Pathogenicity in albino Swiss mice. The present article has been also meant for the mass scale of epsilon toxin for more effective vaccine production Production in less resource in terms of culture media.

MATERIALS AND METHODS

Seed for *Clostridium perfringens* Type D

Lyophilized culture of *Clostridium perfringens* Type D was procured from Veterinary Research Institute (VRI) Lahore, Pakistan and revived in Reinforced Clostridium Media (RCM) incubated at 37°C for 18 hrs. The growth of *Clostridium perfringens* Type D was used for the preparation of working seed culture.

Culture media

Following four different culture media were used for mass scale growth of *Clostridium perfringens* Type D.

1. Thioglycollate Broth with Liver Extract (BQ Vaccine Medium)
2. Re-In forced Culture Medium (RCM)
3. Cooked Meat Medium (CCM)
4. Nutrient Broth (NB)

Table 1: Values of O.D (Optical Density), Mice Lethality, ELISA % Age positivity.

Sr No.	Culture Media	O.D Value	Mice Lethality(Dilution ratio)						ELISA % age Positivity (Dilution Ratio) –wavelength 450 nm						
			10	20	40	80	160	320	10	20	40	80	160	320	640
1	CMM	2.096	+	+	+	+		-	97	95	94	94	95	51	32
2	RCM	2.184	+	+	+	+	+	+	98	97	97	98	96	50	28
3	BQ	2.26	+	+	+	+	+	+	96	95	95	97	97	98	72
4	NB	1.984	+	+	+	+		-	97	94	94	95	71	36	15

These media were prepared in 6 L flasks in duplicate and cultured with working seed @10% inoculum. The flasks were sealed with liquid paraffin and incubated at 37°C for 24 hrs. After the stipulated time, 15 ml of culture was taken from each flask and O.D value for each sample was taken. Centrifugation was done @3000 rpm for 10 min and supernatant was used after making dilutions for the detection of epsilon toxin by ELISA. Simultaneously mice were inoculated by preparing different dilutions (1:10 to 1:320) for pathogenicity testing [10].

RESULTS

Three types of tests including O.D (Optical Density), Mice Lethality at different dilution ratios and ELISA % age positivity for epsilon toxin in the supernatant of different dilutions of all of four broths at 450 nm wavelength were applied. Results were noted as depicted in the following Table 1.

DISCUSSION AND CONCLUSION

For the mass scale growth of *Clostridium perfringens* Type D, conventionally used culture media including CMM, RCM and NB were compared with BQ medium to conclude the best culture medium for efficient, abundant and heavy growth of *Clostridium perfringens* Type D. The inoculum containing equal count of *Clostridium perfringens* Type D was dispensed in all of the four culture media and results as given in the Table revealed the highest the OD value for growth in BQ medium as 2.26 even higher than given by RCM which is considered the best culture medium for Type D. Mice Lethality of growth from BQ medium was found equal to that of RCM but the concentration of epsilon toxin produced by bacterial growth in BQ medium was very much higher than produced even in RCM. It was seen that at the dilution of 320 and 640 of supernatant of culture broth of BQ the concentration of epsilon toxin was 98 and 72 respectively very much higher than that of RCM which is considered the best medium with respect to the production of epsilon toxin. In the production of Enterotoxaemia Vaccine the highest concentration of epsilon toxin is considered as the surety of vaccine quality and it has been well seen in this study that BQ medium has immense

potential to produce highest concentration of epsilon toxin even much higher than that of RCM.

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