

GROWTH AND ADAPTATION OF LEPTOSPIRES IN LOW PROTEIN MEDIUM

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ABSTRACT

The present study was undertaken to grow leptospires from high protein (20 per cent BSA) to protein free media (without BSA), which revealed growth at the concentration of 20 per cent to one per cent BSA on the 4th day of incubation. No growth could be observed below one per cent level of BSA. Hence, the leptospires were adapted to grow from high protein (10 per cent) to low protein (upto 0.2 per cent) concentration of BSA. In this adaptation process, growth in low protein medium containing 0.2 per cent BSA was observed to a concentration of $\geq 1.4 \times 10^9$ organisms / ml by day 10 and 11. Hence, the low protein medium containing 0.2 per cent BSA can be used for growing leptospires for vaccine preparation so as to minimize the adverse effects of protein containing media.

KEY WORDS : Bovines - Leptospirosis - Vaccines**INTRODUCTION**

Leptospira causes leptospirosis in 160 mammalian species (Balakrishnan Govindan, 2014). The disease causes enormous losses due to death of animals, decreased milk production, abortion, stillbirth and infertility. It also poses public health related problems. Hence development of vaccines for control of the leptospirosis is mandatory. Leptospires are normally grown in serum containing media which promote the growth of leptospires to higher cell densities. The serum proteins in the media used for growing the leptospires caused adverse reactions; pyrogenicity and dermal toxicities (Bey and Johnson, 1978a), hence unsuitable for vaccine preparation. Variation in the suitability of serum or serum fractions used for cultivation of leptospires and difficulty in sterilizing these media were some of the limitations of protein containing media. To overcome these limitation, low protein medium is preferred (Bey and Johnson, 1978a). Christopher *et al.* (1982) observed that vaccines prepared from leptospires grown in the protein free medium (PFM) were non-pyrogenic and local reactions were minimal and it would be desirable to use this medium for production of bacterins (Bey and Johnson, 1986). The use of serum free medium often required adaptation of the leptospires and only a few serovars could be cultivated in such medium (Kida *et al.*, 1977).

Hence, the present study was undertaken to assess the growth and adaptation of leptospires from medium containing high protein (20 per cent BSA) to low protein or protein free medium (without BSA), in order to use for the preparation of vaccines.

MATERIALS AND METHODS**Growth of leptospires in high protein to low protein medium**

Ellinghausen McCullough Johnson Harris (EMJH) medium containing 20 per cent, 10 per cent, 5 per cent, 2 per cent, 1 per cent, 0.1 per cent and 0.01 per cent of Bovine serum albumin (BSA) (M/s. Sigma, USA) and without BSA were prepared. The media were distributed as three ml aliquots and stored at room temperature and checked for a week to assess their sterility. Five day old cultures of *Leptospira interrogans* serovars grown in EMJH medium containing approximately 2×10^8

organisms per ml were added into EMJH media containing different concentration of BSA and media without BSA. They were incubated at $29 \pm 1^\circ\text{C}$ in BOD incubator for 14 days for growth.

Adaptation of leptospires in high protein to low protein medium

EMJH media containing 10 per cent, 7.5 per cent, 5 per cent, 2.5 per cent, 1 per cent, 0.2 per cent and 0.1 per cent of BSA were prepared. They were distributed into three ml aliquots and stored at room temperature and checked for sterility for a week. Five days old cultures containing approximately 2×10^8 *Leptospira interrogans* per ml were added in EMJH media containing 10 per cent BSA and subcultured once in a week for three times in the media containing 10 per cent BSA and subsequently transferred to EMJH media containing 7.5 per cent BSA and subcultured 3 times then transferred to 5 per cent, 2.5 per cent, 1 per cent, .2 per cent and .1 per cent BSA containing media and the procedure was repeated as followed for 10 per cent BSA containing media.

Growth curve studies

In order to study the growth of leptospires, a low protein medium containing 0.2 per cent Bovine Serum Albumin was prepared following the protocol described by Regional Medical Research Centre (2007), with slight modification (Balakrishnan, 2009). Two milliliter each of the culture of serovars of *Leptospira interrogans* (*australis*, *ballum*, *hardjo*, *hebdomadis* and *pomona*) was suspended in 18 ml of low protein medium and aliquoted in 15 vials at the rate of 1ml / vial. All the 15 vials were incubated for 15 days at $29 \pm 1^\circ\text{C}$. The growth of leptospires was assessed by enumerating the leptospires using Petroff – Hausser counting chamber (Hausser Scientific, USA). Similarly growth curve studies were carried out in the EMJH medium containing 10 per cent BSA.

RESULTS AND DISCUSSION

Growth of leptospires in high protein to low protein medium

The results of the growth and adaptation studies in low protein medium and high protein medium are presented in Table 1.

Growth of leptospires in EMJH media containing 20 per cent, 10 per cent, 5 per cent, 2 per cent, 1 per cent, 0.1 per cent and 0.01 per cent of BSA and without BSA revealed growth at the concentration of 20 per cent to one per cent BSA on the 4th day of incubation. No growth could be observed below one per cent level of BSA.

Adaptation of leptospires in high protein to low protein medium

Five strains of leptospires were adapted to grow from high protein (10 per cent) to low protein (upto 0.2 per cent) concentration of BSA. In this adaptation process, growth in low protein medium containing 0.2 per cent BSA was observed to a concentration of $\geq 1.4 \times 10^9$ organisms / ml by day 10 and 11 (Table-1). Whereas the high protein media containing 10 per cent BSA showed growth of all the 5 strains to a concentration of $\geq 1.9 \times 10^9$ organisms / ml by day 6 and 7 (Table – 1).

The production of vaccine requires large volumes of culture media and most expensive component is the serum albumin. The serum proteins caused adverse reactions; pyrogenicity and dermal toxicities (Bey and Johnson, 1978a). Variation in the suitability of serum or serum fractions used for cultivation of leptospires and difficulty in sterilizing these media were some of the limitations of protein containing media. To overcome these, low protein medium is preferred (Bey and Johnson, 1978a). In the present study, the growth of leptospires in EMJH media containing 20 per cent, 10 per cent, 5 per cent, 2 per cent, 1 per cent, 0.1 per cent and 0.01 per cent of BSA and without BSA revealed growth at the concentration of 20 per cent to one per cent BSA on the 4th day of incubation. No growth could be observed below one per cent level of BSA. Hence the leptospires were adapted to grow from high protein (10 per cent) to low protein (upto 0.2 per cent) concentration of BSA. In this adaptation process, growth in low protein medium containing 0.2 per cent BSA was observed to a concentration of $\geq 1.4 \times 10^9$ organisms / ml by day 10 and 11 (Table-1). Hence, the

Table 1 : Growth pattern of leptospirens at 0.2 per cent and 10 per cent BSA in EMJH media

Incubation periods (in days)	Concentration of BSA	No. of leptospirens in millions per ml				
		<i>australis</i>	<i>ballum</i>	<i>hardjo</i>	<i>hebdomadis</i>	<i>pomona</i>
0	0.2 per cent	9	8.5	9	9.5	10
	10 per cent	9	8.5	9	9.5	10
1	0.2 per cent	18.5	14.5	18	31	13.5
	10 per cent	200	150	200	250	300
2	0.2 per cent	28.5	121.5	31	47.5	85
	10 per cent	550	500	650	650	750
3	0.2 per cent	38	145	46	90	122
	10 per cent	700	600	850	900	800
4	0.2 per cent	100	315	120	360	590
	10 per cent	800	750	1000	1000	1000
5	0.2 per cent	135	320	145	390	870
	10 per cent	800	750	1000	1000	1000
6	0.2 per cent	200	360	225	800	940
	10 per cent	2000	1900	2200	2250	2200
7	0.2 per cent	440	425	480	1300	1150
	10 per cent	2300	1900	2500	2500	2500
8	0.2 per cent	900	615	1050	1530	1250
	10 per cent	3300	2000	3600	4000	3800
9	0.2 per cent	2200	690	2400	1650	2850
	10 per cent	5800	2100	6300	6800	6600
10	0.2 per cent	3050	1400	3300	2850	4000
	10 per cent	7400	2300	7600	7900	7800
11	0.2 per cent	2900	1700	3000	4900	2000
	10 per cent	7800	2300	7700	8000	7900
12	0.2 per cent	1600	1500	2400	1300	1300
	10 per cent	7900	2500	7700	8100	8000
13	0.2 per cent	650	1500	600	750	700
	10 per cent	8000	2500	7700	8100	8000
14	0.2 per cent	200	1200	240	300	450
	10 per cent	8000	2500	7700	8100	8000
15	0.2 per cent	110	1200	130	250	300
	10 per cent	8000	2500	7700	8100	8000

*EMJH - Ellinghausen McCullough Johnson Harris

low protein media containing 0.2 per cent BSA could be used for growing leptospire for vaccine preparation so as to minimize the adverse effect of protein containing media. The results were also compared with high protein (10 per cent) medium which are generally used for inoculation of *Leptospira* organisms. Growth was slower in low protein media compared to high protein media (Table - 1), but growth to the extent of 1.4×10^9 to 4.0×10^9 by 10 days of culture in low protein media was highly promising hence could be used for the preparation of vaccines.

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