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Research Article

WESTERN BLOT ANALYSIS TO DETECT IMMUNOGENIC PROTEIN OF *Brucella melitensis* 16M SOLUBLE ANTIGEN

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ABSTRACT

Soluble antigen was prepared by treating the *Brucella melitensis* 16M culture of goat origin by ultrasonic waves in an ultrasonicator. This antigen was run on SDS-PAGE along with pre-stained protein molecular weight marker to fractionate antigen according to its molecular weight. SDS-PAGE analysis of *B. melitensis* soluble antigen revealed a total of 29 structural polypeptides, 19 were major and 10 were minor. All of these polypeptides were subjected to Western blotting to detect immunogenic protein. Once polypeptides blotted onto nitrocellulose membrane, then the membrane was treated with hyper immune serum, rabbit anti-goat horseradish peroxidase and diamino benzidine (DAB) substrate respectively. Western blot analysis of *B. melitensis* 16M soluble antigens (cytoplasmic antigens) revealed that 7 polypeptides corresponding to molecular weight 88, 68, 44, 45,30, 25 and 12 kDa were having immunodominant antigens. Therefore, these antigens may be used as a candidate antigen for the development of diagnostic tests for the diagnosis of caprine brucellosis.

Keywords: Western blot, *B. melitensis*, soluble antigen, immunogenic.

INTRODUCTION

Brucellosis is a zoonotic disease endemic in many areas of the world characterized by chronic infections in animals leading to abortion and infertility and a systemic, febrile illness in humans (Paulsen *et al*., 2002). It is the most widespread zoonosis transmitted from animals either by direct contact with blood, placenta, and fetuses or through consumption of infected raw animal products especially milk and milk products. *B. melitensis* from the milk, tissue and blood of goat was identified by using polymerase

chain reaction (Gupta *et al*., 2005, 2006). The economy of nations depends to a large extent upon a healthy and productive livestock; the disease like brucellosis which is related to the reproductive failure is one of the major problems in developing countries like India (Verma, 2013). Interest in *B. melitensis* antigens from veterinary point of view has been mainly directed towards developing better diagnostic test. At present there is no "gold standard" for diagnosis of brucellosis in goats. The major problem in achieving the objective is the lack of well standardized and specific antigens.

In this study, we used *B. melitensis* 16M antigen, because these antigens are twenty times more dominant than 'A' antigen which is dominant in *B. abortus*. This study revealed the western blot analysis of immunogenic protein of *B. melitensis* soluble antigen of goat origin. This is one of the footing step to identify immunogenic antigen which will help further for the development of diagnostic assay for caprine brucellosis.

MATERIAL AND METHODS

Animals

Goat used in this study was provided by Goat Health Division, CIRG, Makhdoom, P.O. Farah, Distt. Mathura, India.

Bacterial culture

B. melitensis used in this study was isolated from stomach content of aborted foetus of goats (Rana *et al.,* 2002). Stomach content was brought to our laboratory under ice and was immediately streaked on *Brucella* agar plates enriched with 5% horse serum. These plates were incubated at 37^{o}C for 48-72 h under microaerophilic condition and were regularly monitored for bacterial growth. The isolates were further characterized by OMP-31 PCR.

Preparation of hyper immune sera

Goat was injected with *B. melitensis* 16M soluble antigen along with Freund's complete antigen followed by booster doses twice in a month. The blood was collected and hyper immune serum was collected and ELISA was performed to check the titer of *anti-Brucella* antibody. This hyperimmune serum was used in western blot analysis to detect immunogenic protein.

Preparation of Soluble Antigens

Logarithmic phase of *B. melitensis* 16M were sedimented by centrifugation at 10,000 rpm for 10 min. at 4^{0}C, washed twice with normal saline solution and re-suspended in 20 ml NSS. The suspended culture was treated with ultrasonic waves in an ultrasonicator for 10 cycles, centrifuged at 10,000 rpm for 10 min at 4^{0}C. Cell debris were removed and supernatant were collected, concentrated in vacuum concentrator and stored at 4^{0}C (Gupta *et al.,* 1994).

Preparation of Polyacrylamide Gel and fractionation of antigen

SDS-PAGE was performed as per Laemmli *et al*., 1970 using separating gel of 10% and stacking gel of 4%.

Western Blotting

Western blotting was performed as per method of Towin *et al.,* 1979.

RESULTS AND DISCUSSION

There is no scientific agreement on what should be the nature and characteristics of a universal antigen for diagnosis of

brucellosis due to smooth *Brucella*. Further research is needed to clarify the practical importance and interest of using species specific diagnostic antigen for the different serological tests. There is limited information on the value of outer membrane and inner cytoplasmic proteins for the diagnosis of *B. melitensis* infection. Inner antigens are considered specific for the genus, being useful to differentiate infections due to *Brucella* from those due to bacteria whose LPS cross-reacts with the *Brucella* S-LPS (Diaz and Bosseray, 1974). However cross-reactivity among cytosolic proteins of *B. melitensis* has been reported (Velasco *et al*., 1997). Therefore the present study investigated the immunodominant polypeptides of *B. melitensis* 16 M cytoplasmic antigen.

Polypeptide profile of *B. melitensis* soluble antigen (cytoplasmic antigen) was identified by using SDS-PAGE and western blotting. SDS-PAGE analysis of *B. melitensis* soluble antigen revealed a total of 29 structural polypeptide (pp) bands whose molecular weight ranging from 118 to 11 KDa. Out of total 29 structural polypeptides revealed, 19 pp were major and 10 pp were minor (Fig. 1). The major pp were having molecular weight of 118, 68, 59, 49, 44, 40, 35, 33, 30, 28, 25, 23, 20, 18, 17, 14, 13 and 11 KDa. The 10 minor pp were having molecular weight of 111, 82, 54, 50, 46, 42, 38, 34 and 22 KDa (Table 1).

Western blot analysis of *B. melitensis* 16M soluble antigen was performed to detect anti-Brucella antibodies in hyperimmune serum isolated from goat. The goat used for our experiment was previously immunized with *B. melitensis* soluble antigen along with suitable adjuvant. This analysis revealed 88, 68, 44, 45, 30, 25 and 12 KDa bands (Fig. 2). These bands are major in nature and depicted the status of immunogenic polypeptide in the soluble antigen (Table 2).

Table 1**:** Structural polypeptide profile of *B. melitensis* soluble antigen (cytoplasmic antigen).

S. No.	**Structural Polypeptides (MW in KDa)**	
	Major	**Minor**
1	118	111
2	68	82
3	59	72
4	49	54
5	44	50
6	40	46
7	35	42
8	33	38
9	30	34
10	28	22
11	25	
12	23	
13	20	
14	18	
15	17	
16	15	
17	14	
18	13	
19	11	

Table 2: Showing major immunogenic polypeptide of soluble antigen of *B. melitensis* in western blotting.

Lane No.	Immunogenic Bands (Mol. wt. in KDa)
1	88
2	68
3	44
4	45
5	30
6	25
7	12

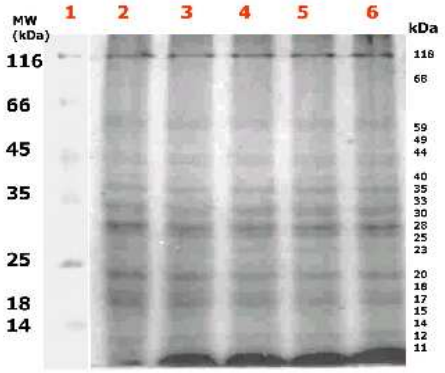

Figure1: SDS-PAGE analysis of cytosolic/ soluble antigens of *B. melitensis* 16 M; Lane-1: MW protein marker; Lane-2, 3, 4, 5, 6: Polypeptide bands

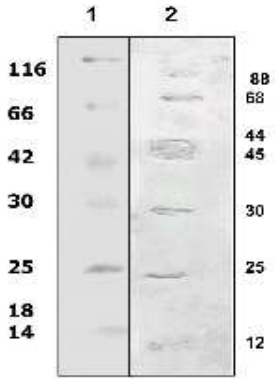

Figure 2: Western blot analysis of *B. melitensis* 16M soluble antigen; Lane-1: Pre-stained protein molecular weight marker; Lane-2: Immunogenic polypeptide bands.

CONCLUSION

Western blot analysis of *B. melitensis* 16M soluble antigens (cytoplasmic antigens) revealed 7 polypeptides corresponding to molecular weight 88, 68, 44, 45,30, 25 and 12 kDa having immunodominant antigens. Therefore these antigens may be used as a candidate antigen for the development of diagnostic tests for the diagnosis of caprine brucellosis.

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