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# DEVELOPMENT OF AN INDIRECT ELISA FOR ENHANCED DETECTION OF ANTI-BRUCELLA ANTIBODIES IN ASYMPTOMATIC INDIVIDUALS

**Dr Lalitha Devi S***

Assistant Professor, Department of Biochemistry, Katuri Medical College & Hospital, Chinakondrupadu, Guntur, India

**ABSTRACT**

Brucellosis, a reemerging zoonotic disease caused by the Gram-negative coccobacillus Brucella, poses diagnostic challenges due to nonspecific clinical manifestations. This study aimed to develop an indirect ELISA utilizing smooth lipopolysaccharide antigens from Brucella abortus 99 for anti-Brucella antibody detection. Serum samples from 1304 asymptomatic individuals were screened using the plate agglutination test (RBPT) and standard tube agglutination test (STAT), followed by indirect ELISA. Compared to RBPT and STAT, indirect ELISA demonstrated higher sensitivity, detecting 20 positive samples, highlighting its potential for improved brucellosis diagnosis.

**Key words**: Brucellosis, ELISA, Diagnostic tests, Antibody detection, Asymptomatic individuals.

## INTRODUCTION

Humans can contract Brucellosis from domestic and wild animals (zoonoses). The disease is widespread in some countries. Occupational exposure and food contamination are the main causes of infection [1]. In addition to causing morbidity, Brucellosis is also a major cause of morbidity with worldwide distribution. Economically and socially, it is important. The Brucella species, especially Brucella melitensis and Brucella suis, are potential biological terrorism agents (2-3). There has been Brucellosis for millennia (4). It is well known that certain geographic areas have a high prevalence. The World Health Organization estimates that there are over 500,000 new cases each year. (5) The international tourism era has made brucellosis an imported disease in developed countries [6]. Without prompt treatment, Brucellosis can cause debilitating conditions. Brucellosis is clinically enigmatic. Diagnoses can be difficult because symptoms are insidious and undulating. Nigerian researchers confirmed brucellosis' endemicity, particularly bovine brucellosis, in slaughtered cattle that have been kept in abattoirs, which presents an occupational risk to workers. As a consequence of unpasteurized food obtained from diseased animals, public health education should focus on the zoonotic aspect of this disease. Brucellosis was first reported in India in the previous century and has since spread to most of the country [8]. According to Vaishnavi and Kumar's study [9], 6.36% of 292 blood donors were seroprevalent to brucellosis. Infections from milk and milk products are common. When milk is stored at optimal conditions, Brucella may survive longer. Additionally, milk neutralizes gastric acid and protects ingested bacteria. Unevenly heated milk, raw milk, and clotted cream can harbor Brucella [3]. Brucellae survive for prolonged periods in the environment, so dust may contain viable organisms. Sources of infection include blood transfusions, bone marrow transplants, and kidney transplants. Semen may transmit sexually [8, 10]. Turkish immigrants are at risk of brucellosis. Among patients in this group, the infection is linked to delayed diagnosis, relapses, chronic courses, and focal complications [9]. In Thassos of Greece, brucellosis was found to be of public health priority [11].

Corresponding Author: - **Dr Lalitha Devi S**

Brucellosis presents with a wide range of clinical symptoms. In brucellosis, hepatic involvement is not uncommon, but kidney or cardiac involvement is rare. Demyelinating syndromes can occur in brucellosis [7]. It is common to have arthritis in one or more big joints in low back pain. Physical findings may be minimal, and symptoms may be intermittent or continuous. Many cases of brucellosis are either misdiagnosed as unknown pyrexia because they are invisible or are complicated. Human brucellosis can be diagnosed with a wide range of serological tests, although each has important limitations. Thus, the present study examines the seroepidemiology of human brucellosis in different parts To investigate the endemicity of human brucellosis and also to standardize an indirect (ELISA) for detecting human brucellosis serodiagnosis in India, to improve disease diagnostics and conduct a comparative study between standardized indirect ELISAs and conventional techniques.

## METHODS AND MATERIALS

A total of 1304 serum samples were collected from individuals who had backache, shoulder pain, arthritis, etc., as their predominant symptoms. Individual histories included names, ages, occupations, types of work, consumption of raw milk previously, fever symptoms and complaints of joint pain, if any, were collected. 333 sera were RA-negative, 186 were negative for ASLO, and 143 were negative for CRP. Anti-streptolysin O, C-reactive protein, and Rheumatoid Arthritis tests were negative.

Three phases of analysis were conducted on serum samples. RBPT was performed as part of the first phase. STAT analyzed the seropositive samples in the second phase. Institute of Animal Health and Veterinary Biologicals provided antigens, for both tests. Thirdly, Brucella abortus 99 smooth lipopolysaccharide was used in indirect ELISA. To determine if age and sex play a role in the transmission of brucellosis, data were collected and analyzed. Furthermore, the disease's endemicity was determined in different parts of the state based on the data.

### Indirect ELISA Procedure

Antigen: smooth lipopolysaccharide)SLPS extracted with hot phenol water from Brucella abortus 99. Strong positive serum controls were obtained from a Medical College from confirmed cases of brucellosis. Negative control serum was taken from healthy individuals. Strong positive serum was diluted in negative serum (1 : 20 diluted).

The indirect ELISA was developed using commercial reagents. The rabbit antihuman HRP conjugate was used along with O-Phenylene Diamine Dihydrochloride and hydrogen peroxide. A checkerboard titration was used to determine the optimal dilution of rabbit antihuman HRP conjugate, sera control, and SLPS antigen. Dilutions of control and test sera were 1: 100. Brucella abortus 99 SLPS antigen was coated on microtiter plates in carbonate bicarbonate buffer at 100 g per well. Washing buffer containing 0.002 M phosphate-buffered saline (PBS) was used three times. Test sera were poured into respective wells of the plate in duplicate, while control sera were added in quadruplicate, diluted (1:100) in blocking buffer,were added to respective wells of the plate. The plates were then incubated for 1 hour at 37°C and washed. Incubation at 37°C for 1 hr with incubating rabbit antihuman IgG HRP conjugate (1: 3000). After washing, the plates were treated for 10 minutes with freshly prepared OPD solution with H2O2. To stop the enzyme substrate reaction, 100 l of 1 M sulfuric acid was added per well. An ELISA Microtiter plate reader read the plates at 492 nm. A number of methods can be used to determine seropositive or seronegative thresholds. PD ADMAS laboratory screening results involving indirect ELISA indicate a positive result if the ELISA positive-negative ratio is three, and the positive and negative OD values are three standard deviations from the mean. Positive samples are those with OD values three times higher than the negative control, while negative samples have OD values below that.

## RESULT

RBPT and STAT results for 652 samples were negative, but indirect ELISA results for 20 samples were positive.

This category of individuals has a seroprevalence of 3.06% by indirect ELISA. As illustrated in Table 1, seroprevalence is high in 30–40 years , and low in the age group >40 years.

**Table 1: The influence of age on brucellosis seropositive results**

| Age | Seropositive male | Seropositive female | Total | Seronegative male | Seronegative female | Total |
|---|---|---|---|---|---|---|
| 30–40 | 22 | 04 | 26 | 436 | 440 | 902 |
| >40 | 14 | 00 | 14 | 202 | 186 | 402 |
| Total | 36 | 04 | 40 | 638 | 626 | 1304 |

## DISCUSSION

The seropositivity of individuals without a history of fever is 3.06%. No positive results have been detected by RBPT and STAT in this group, indicating the need for tests with higher sensitivity. South Region has 2.14% brucellosis, while North Region has 0.92%. Sero prevalence is 2.3% in males and 0.76% in females; ratio is 2.33:1. Analyses of the samples indicate a high sero prevalence in the 30–40 age group (1.99%), followed by the 40+ age group (1.07%). One of the most challenging

tests of medical knowledge and clinical acumen is the diagnosis of brucellosis. For the diagnosis of brucellosis, ELISA provides a profile of immunoglobulin classes, making it suitable for mass screening and the serological diagnosis of brucellosis [14]. Serological tests may be useful in situations where positive cultures and clinical findings cannot confirm the infection's persistence. Brucella lipopolysaccharide antibodies are crucial for the diagnosis of the disease [15]. In different studies, seroprevalence of brucellosis varies over time and place. States in India have different magnitudes of problems. Even within states where prevalence is known, it varies. Brucellosis diagnosis also depends on antigen type, diagnostic techniques, and antibody titers. When determining the seroprevalence of brucellosis in a particular geographical area, selection criteria for laboratory examination of cases can play an important role. The authors of Chadda et al. [16] found brucellosis among raw meat eaters. A common localized brucellosis complication involves the spine, large joints, or sacroiliac joints. Fever, diaphoresis, lymphadenopathy, anorexia, malaise, and subclinical disease are typical symptoms. The effects of endocarditis or central nervous system damage are very severe in localized disease. Under endemic conditions or through illegal imports, brucellosis can be contracted [17–19]. When suspicion of exposure exists, only look for brucellar arthritis for diagnosis and search. Because brucellosis is a slow-growing organism [20], serological methods are needed to diagnose it quickly due to the delay in isolation. A serological test detects antibodies directed against lipopolysaccharide [21]. Brucellosis can affect any organ or system, and localized symptoms may occur. Localization is most common in osteoarthritis; large weight-bearing joints are most commonly affected. Our study detected seropositivity only through the use of SLPS from Brucella abortus 99. Blood and cerebrospinal fluid can be tested for Brucella-specific IgG, IgM, and IgA antibodies using the ELISA test [22]. There is a worldwide distribution of Brucellosis with varying rates of focal disease. Clinical manifestations and severity may vary according to Brucella species and host region. According to Nagalotimath et al,[23], 30% of diagnosed cases are not related to any high-risk occupation. Mathur reported outbreaks of brucellosis in Indian families and institutes [1], which he attributed to raw milk consumption. Negative results would have been reported if RBPT and STAT were used only. ELISA tests are highly sensitive and specific.

## CONCLUSION

Brucellosis was detected by indirect ELISA. Consuming or handling raw milk or milk products can result in occupational exposure. Even in endemic areas, clinicians and microbiologists must be alert to diagnose and treat brucellosis in close collaboration. There is no history of unpasteurized milk consumption and no risk of brucellosis, but some individuals come from rural areas, so animal contact cannot be ruled out. As the vaccine is limited in efficacy, maintaining hygiene, especially around livestock, and drinking milk and milk products properly are the only ways to prevent it.

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