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# CHARACTERIZATION OF BABESIA BIGEMINA ANTIGEN (EGYPTIAN STRAIN) ISOLATED FROM AN EXPERIMENTALLY INFECTED CALVES.

BY

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## SUMMARY

Characterization of *B. bigemina* antigen (Egyptian strain) using SDS-PAGE and immunoblotting technique was performed in order to study the protein components of *B. bigemina* (Egyptian strain) which help in accurate diagnosis of the parasite. Running of *B. bigemina* isolate on 10-15% polyacrylamide gradient gel (phast system, Pharmacia, Sweden) was carried out. Staining of the gel with silver nitrate showed 3 major polypeptide components of molecular weights 85,61 and 53 KDa. Other descrinable bands was recorded with molecular weights of 47, 37,33 and 26 KDa. The western transfer analysis using (BIO-RAD, USA) showed that antiserum from animals experimentally infected with *B. bigemina* contained detectable antibodies directed against only 2 proteins at the molecular weights of 61 and 53 KDa.

## INTRODUCTION

Babesiosis is a tick-born disease of domestic and wild animals. Most of the world cattle population of approximately $1.2x10^9$ cattle is exposed to bovine babesiosis (Mc Cosker, 1981). *B. bigemina* has a world wide distribution that corresponds to the distribution of their vectors (Boophilus species) i. e. countries between 32°S and 40°N the equator (purnell, 1981). It is necessary to emphasize that identification of *Babesia parasites* in thin or thick blood films true evidence of infection. However, negative miroscopic examination does not exclude the possibility of infection. In the very early or chronic stages of the disease the detection of *Babesia* parasites in stained blood films is uncommon. So, it is necessary to develop serological method to detect specific antibodies to *Babesia* rather than *Babesia* Organisms (Todorovic and Carson, 1981). Cross reaction between *Babesia* species has been recorded using different serological tests, ELISA (Duzgun et al., 1991) and IFAT (Ordina et al., 1992). The determination of the specfic diagnostic protein in each species would be very useful to differeniate between the different *Babesia* species serologically. In our investigation, characterization of *B. bigemina* antigen using SDS-PAGE and immunoblotting technique was performed in order to study the protein components of *B. bigemian* (Egyptian strain) which help in accurate diagnosis of the parasite.

## MATERIALS AND METHODS

In order to study the protein components of *B. bigemina* (Egyptian strain) which help in accurate diagnosis of the parasite, electrophoresis and immunoblotting were carried out. Electrophoresis of *B. bigemina* lysate was performed (according to Laemmli, 1970) in 10-15% gradient polyacrylamide gel using phast system (Pharmacia, Fine chemicals, Sweden) for silver staining and 12% polyacrylamide gel using BIO-RAD (USA) for immunoblotting.

### 1. Preparation of the antigen

The used *B. bigemina* lysate was prepared from 1 litre of infected *B. bigemina* blood collected from experimentally infected calf when parasitaemia reached 15%. The blood was collected on 4.5% Sodium citrate with the ratio of 1 volume citrate to 7 volumes blood. The blood was washed five

times with 0.01 M PBS pH 7.4 with centrifugation at 3000 RPM for 20 minutes each. The buffy coat was removed carefully from the top of the erythrocytes and the blood was then deep frozen at -20°C. Oxyhaemoglobin free antigen was prepared from infected blood using ammonium chloride, Tris according to Martin et al. (1971). The material was then washed five times with 0.01 M PBS pH 7.4 at X18000 RPM for 45 minutes each and the pellet was used as semipurified *Babesia* antigen. The amount of protein was determined according to Bradford (1976).

**2. SDS-PAGE for silver staining using Phast system, Pharmacia, Sweden.**

The method was applied on gradient gel 10-15% polyacrylamide according to the manual for SDS-PAGE, Phast system, Pharmacia, Sweden, (1991) to detect the molecular weights of the different polypeptides in the prepared *B. bigemina* antigen. The purified antigen was applied at concentration of 0.5 µg/lane. A stained molecular weight standard (BIO-RAD, USA) with molecualr weights of 106, 80, 49.5, 32, 27 and 18 kDa was applied on the same gel to recognize the molecular weights of the different recorded bands. Staining of the gel with silver nitrate was carried out directly after running using phast system (Development unit, Pharmacia, Sweden). The molecular weights of the different recorded bands were determined according to Margolis and Wrigley, 1975.

**3. SDS-PAGE for immunoblotting using (BIO-RAD, USA)**

Electrophoresis was done on 12% separating gel and 4% stacking gel according to Wright et al (1985). The *B. bigemina* prepared antigen was applied on concentration of 20 µg protein/lane. A stained molecular weight standard (BIO-RAD, USA) with molecular weights of 200, 116, 97.4, 66.2, 45, 31, 21.2 and 14.4 KDa) was applied on the gel at the same time. Western transfer using nitro-cellulose paper was performed according to towbin et al. (1979). The paper was placed in multiscan apparatus and the positive and negative control sera were applied carefully in the special channels.

## RESULTS

**1. SDS-PAGE using Phast system with silver staining:**

Running of the isolated *B. bigemina* strain on SDS-PAGE was carried out using (Phast system, Pharmacia). Staining with silver nitrate was performed to detect the molecular weights of different polypeptides components. Analysis of the semi-purifed *B. bigemina* preparation (Egyptian of 10-15% polyacrylamid gradient gel revealed the presence of 3 major polypeptide components. The molecular weights of the major components were 85, 61 and 53 KDa. Other discernible bands occurred at position corresponding to molecular weights of 47, 37, 33 and 26 KDa (Fig. 1).

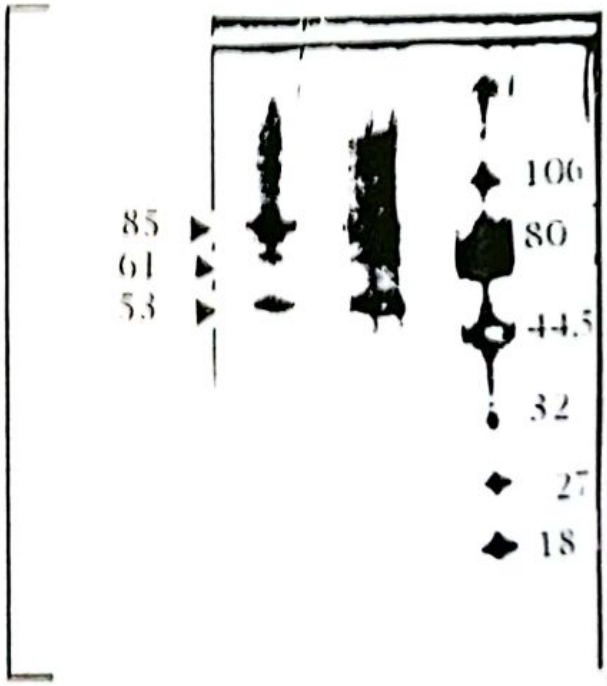
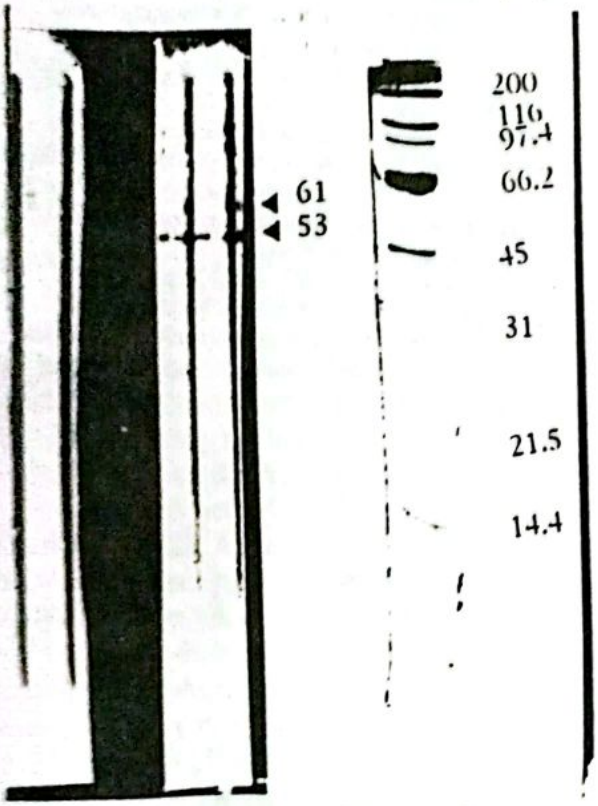



**Fig. (1): SDS-PAGE (10-15% gradient gel) of B. bigemina semipurified antigen using phast system (Pharmacia) stained with silver nitrate demonstrating the three major polypeptid components with molecular weights of 85, 61, 53 KDa. Other descrinble bands occured at position corresponding to 47, 37, 33 and 26 K D a .**

**2. Immunoblotting**

The western transfer analysis using (BIO-RAD USA) showed that antiserum from animals experimentally infected with *B. bigemina*

contained detectable antibodies immunoprecipitated only with 2 of the major protein components. Running of 5 positive and 5 negative control sera against the determined protein components of *B. bigemina* revealed immunoprecipitation only with 61 and 53 KDa with the positive sera. No precipitation was observed with the negative control sera (Fig. 2).




**gen using (BIO-RAD) on SDS-PAGE (12%). Positive *B. bigemina* sera recorded immunoprecipitation at 61 and 53 KDa (a). The negative sera showed no blotting (b).**

## DISCUSSION

Running of *B. bigemina* isolate (Egyptian strain) on 10-15% polyacrylamide gradient gel (phast system, Pharmacia, Sweden) revealed 3 major polypeptide components of molecular weight of 85, 61 and 53 KDa beside 4 other descrinable bands with molecular weights of 47, 37, 33 and 26 KDa. Very close results have been shown by McElwain et al. (1987) who precipitated *B. bigemina* surface antigen at molecular weights 72, 58, 55, 45, and 36 KDa using MAB. On the other hand, Montenegro-James et al. (1989) recorded that the molecular weight ranges of *B. bigemina* exoantigens and merozoites were 92 to 37 and 143 to 24 KDa respectively. Wanduragala (1988) recorded that *B. bigemina* was eluted at relative molecular weight of 60 KDa by Cation exchange chromatography. Twelve proteins of molecular weights vary from 68 and 36 KDa were recorded from 6 geographical different *B. bigemina* stabilates in Mexico (Figueroa et al., 1990).

The present investigation showed that antiserum from animals experimentally infected with *B. bigemina* were directed against only 2 proteins of the molecular weights of 61 and 53 KDa. This result coincide with Wanduragala (1988) who found that western blot analysis of *B. bigemina* exoantigen demonstrated 3 antigens with molecular weights of 64, 60 and 53 KDa. McElwain et al., (1988) identified 72 KDa polypeptides as candidate *B. bigemina* species specific protein. McElwain, et al., (1991) immunoprecipitated 4 *B. bigemian* surface antigen proteins using MAb at molecular weights of 36, 55 and 85 KDa.

The obtained result in this investigation agree with the most previous work on analysis of polypeptide components of *B. bigemina* antigen. The recorded difference with some of the previous mentioned publications might be due to the method of preparation of the antigen or the stabilate antigenic variety between *Babesia* species in different geographical regions.

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