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CHARACTERIZATION OF TRYPANOSOMA EVANSI ANTIGEN BY SDS-PAGE AND WESTERN BLOT.

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SUMMARY

SDS-PAGE of T.evansi antigen after staining with commassie blue stain showed the presence of 26 bands ranging between 222.56 - 9.0 KDa. Western blot analysis revealed immunoprecipitation only with 2 proteins components with camels positive serum and had molecular weight of 98.976 and 16.678 KDa. While with mice positive sera revealed only one immuoprecipitation with protein component had molecular weight 16.678 KDa.

Keywords: Trypanosoma evansi; SDS-PAGE; Western blot; Camel

INTRODUCTION

Trypanosoma evansi is one of economically important and widely distributed haemoflagellate parasites which affect a wide range of animals causing heavy morbidity and mortality. In spite of antigenic multiplicity in a single stock, there may be certain common antigens or non variable antigens (Lukins,1988).The present study was attempted to provide some information through polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting of the T.evansi isolated from camels in Libya.

2. Material and methods

2.1. Mice inoculation

Blood sample was collected from each camel

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(220 camels) in sterile tube containing anticoagulant from Tripoli abattoirs. 0.3 ml was inoculated intra peritoneally in each of two albino Swiss mice (Mus musculus) to isolate parasite strain. Wet blood films were prepared daily from the tail vein of each inoculated mouse and examined microscopically at x 400.

2.2. Preparation of Trypanosoma evansi antigen:

The blood from T.evansi experimentally infected mice (which inoculated with blood from examined camels) was subsenquently used for sub passing in mice (0.3 ml for each).Wet blood smear were examined daily from each inoculated mouse and examined with high power. The experimentally infected mice were sacrified at high parasiteamia and the blood was collected in tubes with anticoagulant. Then centrifuged at 3000 r.p.m. for 15 minutes, the plasma was removed and several washing using phosphate buffer solution pH. 7.2 were accomplished. The erythrocytes were lysed. The suspension was centrifuged at 3000 rpm for 15 minutes and washed several times with distal water. The supernatant was discarded and the sediment was kept in ice bag, then the sediment was sonicated with ultrasonicator in ice bag for 3 minutes with 30 second interval then centrifuge for 45 minutes at 4 ∫C at 14000 r.p.m. The supernatant was used as antigen and stored frozen until used. The concentration of protein in the antigen was determined by the method described by (Lowry et al., 1951) .

2.3. Characterization of T.evansi by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE):

Determination of molecular weights of different polypeptides components of the T.evansi antigen by SDS-PAGE in the present study was carried out according to (Laemmli, 1970) . The sample of antigen (10µg / well) was treated with the reducing buffer in the ratio of 1 : 2. The treated sample was immersed in a water bath for 2 minutes to insure protein denaturation. Unstained protein molecular weigth marker (Amersham company) was applied to the first well and used as standard for SDS-PAGE. A voltage of 100 V was applied and the run was terminated when the bromophenol blue reached the bottom of the gel. After the end of the run, the gel was soaked overnight in 150 ml Commissaie Blue stain. The gel was washed several times with destaining solution till the background become completely clear.

2.4. Electrophoretic transfer of protein from SDS-PAGE to a nitrocellulose sheet.

The protein bands were electrophoretically transferred from SDS-PAGE to a nitrocellulose sheet using the modified technique of (Towbin, 1979) . A sheet of nitrocellulose (0.45 um pore size), was briefly wetted with a transfer buffer [25 mM Tris base,192mM glycerine 20% (vol./vol.) methanol at pH 8.3] and laid on a scouring pad (Scotch-Brite), which is supported by a stiff plastic grid. The gel to be blotted was put on the nitrocellulose sheet and care was taken to remove all air bubbles

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between them. A second pad and plastic grid were added and rubber bands were fitted around all layers. The gel was evenly passed against the nitrocellulose sheet. The assembly was put to an electrophoretic chamber containing a transfer buffer with the nitrocellulose sheet facing the anode. The electrophoretic chamber was kept at 4∫C and a voltage gradient of 100 V is applied for one hour.

2.5. Immunodetection of antigen on nitrocellulose sheet:

The nitrocellulose sheet was soaked in blocking buffer (5% bovine serum albumin) for 2 hours then washed in buffer (0.3% PBS-T) 2 times for 5 minutes. Antibodies solution (4 camels positive serum and 2 rates positive serum experimentally infected) were diluted at 1: 25 in o.3% PBS-T containing in 5% BSA then nitrocellulose sheet was exposed to the diluted sera for one hour. The nitrocellulose sheet was washed 2-3 times for 5 minutes in washing buffer. The nitrocellulose sheet was exposed to Protein A Peroxidase labeled antibody diluted in o.3% PBS-T containing 5% BSA (1 : 500) for one hour. The sheet was

Table (1): Electrophoretic analysis of *T.evansi* antigen by SDS-PAGE.

Laines: / Rows	Laine 1 (M.W.)	Marker (M.W.)	Laines: / Rows	Laine 1 (M.W.)	Marker (M.W.)
r1	222.56		r14	54.387	
r2	206.19		r15	46.888	47.5
r3	190.59		r16	44.133	
r4	168.76	175	r17	41.071	
r5	154.73		r18	38.622	
r6	130.56		r19	35.434	
r7	111.07		r20	34.031	
r8	98.976	83	r21	32.144	32.5
r9	79.632		r22	29.478	
r10	75.504		r23	25.344	
r11	71.611		r24	22.944	
r12	63.585	62	r25	16.678	16.5
r13	60.013		r26	9	6.5

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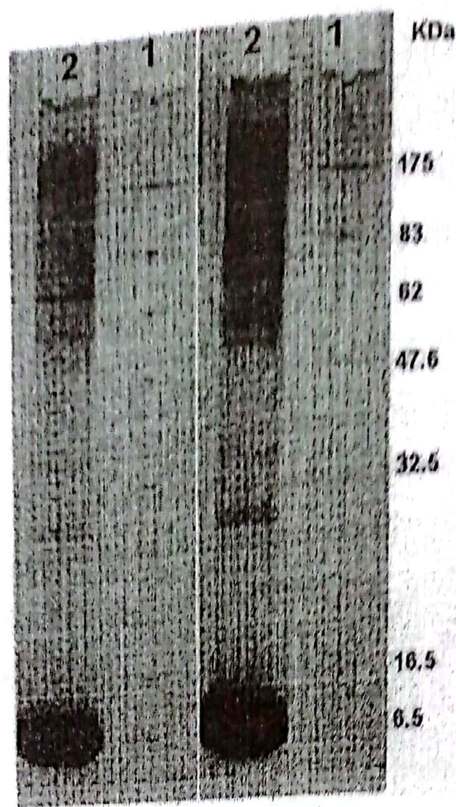

Plate (1): SDS-PAGE of *T. evansi* antigen stained with Commassie Blue stain demonstrating the protein bands of *T.evansi* compared with the marker. (1) Marker (2) Antigen

Table (2): Determination of specific epitopes against camel (laine 2 to 5) and mice (line 6 and 7) anti *T.evansi* sera by Western blot.

Laines: / Rows	Marker 1 (M.W.)	Laine 2 (M.W.)	Laine 3 (M.W.)	Laine 4 (M.W.)	Laine 5 (M.W.)	Laine 6 (M.W.)	Laine 7 (M.W.)
r1	214						
r2	118						
r3		98.976					
r4	92						
r5	52						
r6	35.7						
r7	28.9						
r8	20.8						
r9		16.678	16.678	16.678	16.678	16.678	16.678

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washed 2-3 times for 5 minutes in washing buffer. The nitrocellulose sheet was exposed to the substrate (3 Amino-9- ethyle Carbazole) for 30 minutes. The sheet was then rinsed thoroughly with distilled water to stop reaction.The reaction was read by gel pro-analyzer 3.1.

3. Results

3.1. Electrophoretic and western blot analysis of T.evansi antigen:

Electrophoretic analysis of T.evansi antigen after staining with commassie blue stain revealed the presence of 26 bands ranging between 222.56 ñ 9.0 kDa. The molecular weight (M.W.) of these were 222.56, 206.19, 190.56, 168.76, 154.73, 130.56, 111.07, 98.976, 79.632, 75.504, 71.611, 63.585, 60.013, 54.387, 46.888, 44.133, 41.071, 38.622, 35.434, 34.031, 32.144, 29.478, 25.344, 22.944, 16.678 and 9.000 kDa. (Table 1 and Plate 1). Western blot analysis of antiserum from 4 naturally infected camels (T.evansi positive camels sera by serological tests) and two mice experimentally infected with T.evansi showed immunoprecipitation only with 2 protein components. Their molecular weights were 98.976 and 16.678 kDa (Line No 2). While in line 3 to 7 one immunoprecipitation with protein components had molecular weight 16.678 KDa was detected (Table 2).

DISCUSSION

The characterization of T.evansi antigen was done by studying their electrophoretic pattern by SDS-PAGE. 26 polypeptides were detected ranging between 222.56 ñ 9.00KDa.(The molecular weight of these were 222.56, 206.19, 190.59, 168.76, 154.73, 130.56, 111.07, 98.976, 79.632, 75.504, 71.611, 63.585, 60.013, 54.383, 46.888, 44.133, 41.071, 38.622, 35.434, 34.031, 32.144, 29.478, 25.344, 22.944, 16.678 and 9.000kDa.). The immunoblotting using camel immune serum against T.evansi recognized two bands with molecular weight of 98.976 and 16.678 KDa while only one band (16.678 KDa) was identified using mice immune serum against T.evansi. The number of polypeptides in the present study were less than those obtained by Jithendran and Rae (2001) (39 polypeptides ranges from 20.890 to 199.520). Also, the number of total polypeptides in the present study were more than those obtained by Xie et al. (1997) (14 bands); Pareek et al. (1999) (20 bands ranging from 180 to 24KDa). Also, Giardina et al. (2004) (25 polypeptides bands in the molecular rang 97 ñ 14.8KDa). These variation might be due to the method of preparation of the antigen or to the antigenic and strain variation in different geographical region. This comment was supported by Zheng et al. (1990) who suggested that the different geographical isolates of T.evansi had undergone some variation during evolution. On the other hand some bands resolved in this investigation were found identical to that revealed by Singh et al. (1994) 35.4 and 60.0KDa; also, 75.5KDa found by Uche et al. (1993) ; 71.6KDa by Xie et al. (1997) .

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تصنيف عترة طفيل التريبانوسوما أفنزاى
بإستخدام إختبار البولى أكريلاميد الهلامى والإنتشار الغربى

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بإستخدام عترة التريبانوسوما افنزى الذى تم صباغته بصبغة الكومسياسى الأزرق أوضح وجود ٢٦ حزمة تتراوح ما بين٦٥,٢٢٢ – ٠,٩٠ KDa التحليل الأشعاعى انتج مركبين بروتينيين مع أمصال الجمال الأيجابية وزنها الجزيئ ١٦,٦٧٨ , ٩٨,٩٧٦ KDa بينما مع أمصال الفئران الموجبة وجد بروتين واحد ذو وزن DKa. ١٦,٦٧٨.

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