

EFFICACY OF BABESIA BIGEMINA EXOANTIGEN VACCINE FOR PROTECTION AGAINST BABESIA BIGEMINA INFECTION

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ABSTRACT

30 fattening calves 1 year old were divided into 3 groups (10 in each), 1st group injected with B. bigemina exoantigen vaccine (240 mg of total protein combined with 2 mg saponin/dose) 2 doses with one month interval then Inoculated with 1×10^9 of Babesia bigemina infected RBCs after 90 days of 2nd dose of vaccine, 2nd group injected with B. bigemina exoantigen vaccine 2 doses with one month interval, while 3rd group injected with 2 mg saponin 2 doses with one month interval then Inoculated with 1×10^9 of Babesia bigemina infected RBCs after 90 days of 2nd dose of vaccine. Significant increase in antibody titers against B. bigemina at day 21 after 1st and 2nd dose of vaccination while no antibodies were detected in the non vaccinated group as measured by ELISA. Lymphocyte transformation index at day 15 after 1st and 2nd dose of vaccination was higher in vaccinated than non vaccinated animals. Immunized cattle with B. bigemina exoantigen vaccine were protected against virulent homologous challenge with 10^9 virulent b. bigemina infected RBCs and elicited higher antibody titer without showing any clinical signs when compared with control animals.

Keywords: Babesia, Babesia bigemina, Exoantigen, ELISA, Cattle.

INTRODUCTION

B. bovis and *B. bigemina* are economically important parasites of cattle that are transmitted by Boophilus ticks and widespread in Asia, Latin America and Africa. These intra-erythrocytic parasites cause destruction of host cells, which results in fever, haemoglobinuria and anemia (*Bose et al., 1995*).

Attempts to prevent bovine babesiosis by immunoprophylaxis using live, blood-derived vaccines entails some problems, including, the possible spread of silent pathogens such as bovine leukemia virus; difficulties in standardizing the vaccine dose; the risk of reversion of virulence; maintenance of carrier animals, which might serve as reservoirs for tick transmission; and quality control of vaccine production, maintenance and transportation to the end user, including the necessity for a cold chain (*Bock, R. et al. 2004*). The use of crude and soluble Babesia antigens would overcome the difficulties inherent in production of live anti-Babesia vaccines (*Rojas, C. et al. 2006*), but immunity against heterologous challenge imparted by these vaccines have not been well documented, with the possible exception of Babesia divergens (*Edelhofer, R. 1998*). Success has been reported for soluble parasite antigens from Babesia canis and Babesia rossi in vitro cultured parasites, which are used for vaccination of dogs (*Schettters, T. et al. 2006*). Also, success has been reported for non living vaccine as cell culture derived exoantigens of *B. bigemina* and *B. bovis* was (*Levy and Ristic, 1980, Toro et al., 1990, Montenegro-James et al., 1992 and Zwegarth et al., 1995*). The aim of the present work is to Evaluate efficacy of babesia bigemina exoantigen vaccine for protection against babesia bigemina infection.

MATERIALS AND METHODS

3.1. Animals:

30 fattening calves 1 year old were divided into 3 groups (10 in each), 1st group was injected with with *B. bigemina* exoantigen vaccine (240 mg of total protein combined with 2 mg saponin/dose) subcutaneously, 2 doses with one month interval then Inoculated with 1×10^9 of *Babesia bigemina* infected RBCs after 90 days of 2nd dose of vaccine (*Patarroyo et al., 1995*), 2nd group was injected with *B. bigemina* exoantigen vaccine 2 doses with one month interval, while 3rd group (control) injected with 2 mg saponin subcutaneously, 2 doses with one month interval then Inoculated with 1×10^9 of *Babesia bigemina* infected RBCs after 90 days of 2nd dose of vaccine. At the beginning of the experiment, all animals were clinically normal and Giemsa stained blood smears of these animals revealed absence of any blood parasites and ELISA on serum of these animals indicate absence of antibodies against *babesia bigemina*. These animals belong to a fattening farm at Alexandria Governorate.

All animal were subjected to clinical observation, immunological and parasitological examination

after vaccination and after challenge.

3.2. Samples:

Serum samples were collected from animals before 1st dose of vaccine, 21 days after 1st dose, 21 days after 2nd dose of vaccine from all

animals, 21 days after challenge from groups 1&3 and 90 &180 days after 2nd dose of vaccine from 2nd group to determine humoral immune response. Blood smears were collected before vaccination, after vaccination and after challenge to detect parasitaemia especially from feverish animals. Also citrated blood was collected 15 days after 1st dose and 2nd dose of vaccine and 15 days after challenge to detect cell mediated immune response (Table 1).

3.3. Giemsa staining:

It was applied according to *Schalm et al. (1986)*.

3.4. Babesia bigemina exoantigen preparation:

It was applied according to (*Holman et al., 1993 and Patarroyo et al., 1995*).

3.5. Preparation of Babesia bigemina exoantigen vaccine:

It was applied according to *Makram (1996)*.

3.6. Cryopreservation of Babesia bigemina :

It was applied according to (*Brigitte and Reinhard, 1993*).

3.7. Enzyme Linked Immunosorbant Assay (ELISA):

3.7.1. Preparation of Babesia bigemina antigen for ELISA:

It was applied according to *O'Donoghune et al. (1985)*.

3.7.2. ELISA procedure:

The ELISA was carried out according to *Voller et al. (1980)*.

3.8. Lymphocyte transformation test (colorimetric method)

It was applied according to (*Rai-L. Balhaa et al., 1985*).

3.9. Statistical analysis:

The following tests were made according to *SAS (1987)*:

- 1- Analysis of variance (ANOVA): For determination of the effect of different treatments on the different variables studied.
- 2- Correlation coefficient: For determination of the degree of relationship between the different variables under study.
- 3- T test: Among the different treatment.

RESULTS

1. Post-vaccinal reactions:

Table (1) show that the maximum body temperatures of all vaccinated animals were 38.3, 38.7, 38.5, 39. No parasitemia was observed in vaccinated and non vaccinated control animals.

Table (1): Clinical observation and blood film examination for detection of clinical babesiosis after vaccination still daily for 15 day.

Groups	Clinical response		
	Maximum body temperatures	Percentages of parasitemia	Drug treatment
Vaccinated animals (group 1&2)	38.3, 38.7, 38.5, 39	no	no
Control animals (group 3)	39.1, 38.1, 38.6, 38.5	no	no

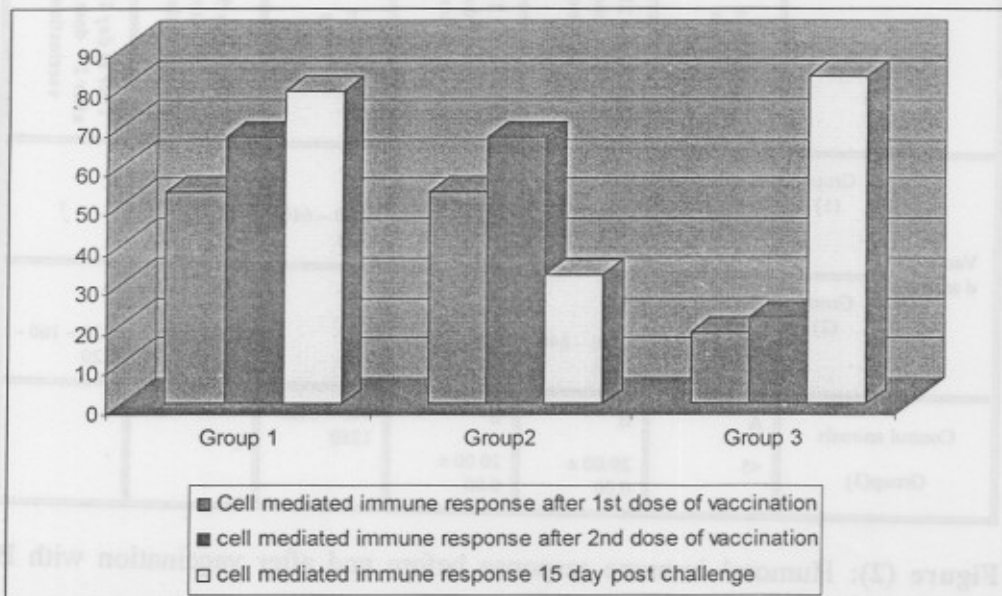
2. Cell mediated immune response to *Babesia bigemina* exoantigen vaccine in fattening animals as measured by Lymphocyte transformation test at day 15 after 1st and 2nd dose of vaccination and after homologous challenge with *Babesia bigemina* infected RBCs at day 90 after second dose of vaccination:

Table (2) and Figure (1) show that lymphocyte transformation index was **53.4 and 67.42** after 1st dose and 2nd dose of vaccination in vaccinated animals respectively, While it was **18.06 and 22.52** after 1st dose and 2nd dose of adjuvant in non vaccinated control animals respectively. Also lymphocyte transformation index was **90.104 – 67.45** and **93.78 – 71.12** after homologous challenge with *Babesia bigemina* infected RBCs at day 90 after 2nd dose of vaccination in vaccinated and control animals respectively.

Table (2): Cell mediated immune response of vaccinated fattening animals with *Babesia bigemina* exoantigen vaccine as measured by lymphocyte transformation test.

Groups		Lymphocyte transformation index at Day 15 after 1 st dose of vaccination X ± S.D	Lymphocyte transformation index at day 15 after 2 nd dose of vaccination X ± S.D	Lymphocyte transformation index at day 15 post challenge X ± S.D
Vaccinated animals	(group 1)	53.4	67.420	90.104 – 67.45
	(group 2)	53.4	67.420	-
Control animals (group 3)		18.06	22.52	93.78 – 71.12

Figure (1): Cell mediated immune response after vaccination with *B. bigemina* exoantigen vaccine as measured by lymphocyte transformation test.



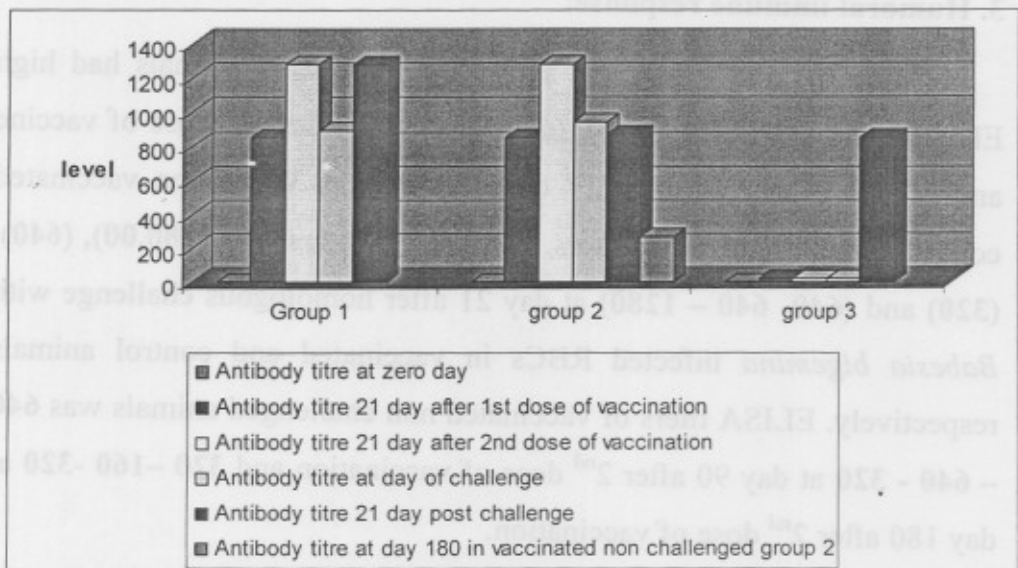
3. Humoral immune response:

Table (3) and Figure (2) show that vaccinated animals had high ELISA titers (1280.00), (640), (640) at day 21 after 1st dose of vaccine and (1280) at day 21 after 2nd dose of vaccine. While non vaccinated control animals showed no titers. Also ELISA titers was (1280.00), (640), (320) and (640- 640 – 1280) at day 21 after homologous challenge with *Babesia bigemina* infected RBCs in vaccinated and control animals respectively. ELISA titers of vaccinated non challenged animals was 640 – 640 - 320 at day 90 after 2nd dose of vaccination and 320 –160 -320 at day 180 after 2nd dose of vaccination.

Table (3): Humoral immune response of vaccinated fattening animals with *Babesia bigemina* exoantigen vaccine as measured by ELISA.

Groups		Titer before vaccination X ± S.D	Titer at day 21 after first dose of vaccination X ± S.D	Titer at day 21 after second dose of vaccination X ± S.D	Titer after challenge X ± S.D	Titer at day 90 after 2 nd dose of vaccination	Titer at day 180 after 2 nd dose of vaccination
Vaccinated animals	Group (1)	A <5	A 1280 – 640 -640	A 1280	A 1280 – 640 -320	-	-
	Group (2)	A <5	A 1280 – 640 -640	A 1280	-	A 640 – 640 -320	A 320 – 160 -320
Control animals Group(3)		A <5	B 20.00 ± 0.00	B 20.00 ± 0.00	640 – 640 - 1280	-	-

Figure (2): Humoral immune response before and after vaccination with *B. bigemina* exoantigen vaccine.



4. Results of challenge infection:

Table (4) show that maximum body temperatures of all vaccinated animals after challenge were 38.6, 38.1, and 38.4 except one animal has increased body temperature 39.9 at day 10 post challenge; percentages of parasitemia were zero in all vaccinated animals except one animal has positive parasitemia at day 10 post challenge. No need for drug treatment of vaccinated animals except one animal.

Control challenged animals had increased body temperature at day 9 post challenge (39.9, 40.1, 40.2), also presence of parasites inside RBCs recorded in 9 animals from 10 animals at day 9 post challenge. 9 control challenged animals need to drug treatment.

Table (4): Clinical observation and blood film examination for detection of clinical babesiosis after challenge still daily for 15 day.

Groups	Clinical response		
	Range of body temperatures	Parasitological reaction	Drug interference
Vaccinated animals(group 1)	38.6, 38.1, 38.4, 39.9	1 / 10	1 / 10
Control animal (group 3)	39.9, 40.1, 40.2	9/ 10	9 / 10

DISCUSSION

Babesia is a tick-borne pathogen that remains an important constrains for the development of cattle industries worldwide. Effective control can be achieved by vaccination with live attenuated forms of the parasite, but they have several drawbacks and thus the development of alternative subunit vaccines (*Florin-Christensen et al., 2007*).

Table (1) showed that maximum body temperatures of all vaccinated animals with *Babesia bigemina* exoantigen vaccine after 1st and 2nd dose of vaccination and non vaccinated ones was 38.3, 38.7, 38.5, 39, also no parasitemia was recorded and no drug interference was needed. Clinical observation and blood film examination for detection of clinical babesiosis after vaccination still daily for 15 day.

The results recorded in table (2) and figure (1) showed that vaccinated animals had higher lymphocyte transformation indexes which were (38.19), (68.56) after 1st dose of vaccination with *Babesia bigemina* exoantigen vaccine plus saponin adjuvant and also higher lymphocyte transformation index (67.420) after 2nd dose of vaccination with *Babesia bigemina* exoantigen vaccine plus saponin adjuvant. While non vaccinated control animals showed 18.06 and 22.52 after 1st and 2nd dose of saponin adjuvant respectively.

Vaccination increases lymphocyte subpopulations and neutrophil phagocytosis, thereby promoting immunity by enhancing phagocytosis of merozoites (Timms *et al.*, 1984).

As shown in table (3) and figure (2) it was observed that, a significant increase in the level of antibodies in the vaccinated animals with *B. bigemina* exoantigen vaccine adsorbed on saponin adjuvant after 21 days from first and second dose of vaccine. Vaccinated animals had higher ELISA titers which were (1280.00), (640), (640) after first dose of vaccination with *Babesia bigemina* exoantigen vaccine plus saponin adjuvant and also higher ELISA titers (1280) after second dose of vaccination with *Babesia bigemina* exoantigen vaccine plus saponin adjuvant. While non vaccinated control animals showed no titers.

Our result supported by (Toro *et al.*, 1990, Montenegro-James *et al.*, 1992, Wright *et al.*, 1992, Hines *et al.*, 1995, Beniwal *et al.* 1997, Alvarez *et al.*, 2004, Varda Shkap *et al.*, 2005, Rodríguez-Vivas *et al.*, 2007 and Shkap *et al.*, 2007).

The presence of antibodies to *b. bigemina* in the sera of cattle in this study is indication for protection against *b. bigemina* infection. On the other hand, animals with no titers to *b. bigemina* were at increased risk of subsequent infection with *b. bigemina* (Smith *et al.*, 2002a and Todorovic 1973). Strong immunity developed in animals vaccinated with soluble *b. bovis* and *b. bigemina* exoantigens containing supernatant fluid in 2 doses at 3 weeks interval (Montenegro- James *et al.*, 1995 and Wright *et al.*, 1992).

Immunized calves with merozoite surface antigens of *b. bigemina* showed significant increase in antibody titer, reduced parasitemia and lower temperature response after challenge in comparison with non vaccinated control calves (McElwain *et al.*, 1988).

The mechanism of protection of antibodies against *b. bigemina* is explained in the basis of the antibodies able to prevent entry of merzoits into RBCs as prevent infection (Todorovic 1973, Smith *et al.*, 2002 and McElwain *et al.*, 1988)

Table (3) showed that ELISA titers of vaccinated fattening animals with *Babesia bigemina* exoantigen vaccine were (1280.00), (640), (320) after homologous challenge with 10^9 *Babesia bigemina* infected RBCs at day 90 after second dose of vaccination and ELISA titers of control animal challenged with 10^9 *Babesia bigemina* infected

RBCs were 640- 640 – 1280. Immunized calves with merozoite surface antigens of *b. bigemina* showed significant increase in antibody titer, reduced parasitemia and lower temperature response after challenge (McElwain et al., (1988).

Table (4) showed that clinical response of vaccinated animals with *Babesia bigemina* exoantigen vaccine after homologous challenge with 10^9 *Babesia bigemina* infected RBCs at day 90 after second dose of vaccination. Maximum body temperatures of all vaccinated animals after challenge were 38.6, 38.1, and 38.4 except one animal has increased body temperature 39.9 at day 10 post challenge; no parasitemia detected in all vaccinated animals except one animal at day 10 post challenge. No need to drug treatment in vaccinated animals except one animal. Control challenged animals had increased body temperatures at day 3 and 4 post challenge (39.9, 40.1, 40.2), also presence of parasites inside RBCs recorded in 9 animals from 10 animals at day 9 post challenge. All control challenged animals needed drug treatment. Vaccinated cattle with soluble *b. bovis* and *b. bigemina* exoantigens containing supernatant were protected against clinical babesiosis after challenge with 10^9 virulent with heterologous strains 3 months after the last dose (Montenegro- James et al.,1995 and Wright et al.,(1992).

Immunization of cattle with culture derived experimental vaccine of *b. bigemina* induces protection against virulent homologous challenge with 10^8 of virulent *b. bigemina*. They found that vaccinated animals elicit high antibody titer without showing any clinical signs if compared with control animals as explained by (Shkap et al., 2007).

Table (2) and Figure (1) showed that lymphocyte transformation index after homologous challenge with *Babesia bigemina* infected RBCs at day 90 after second dose of vaccination was **90.104 – 67.45** in vaccinated animals with *Babesia bigemina* exoantigen vaccine and **93.78 – 71.12** in control animal challenged with *Babesia bigemina* infected RBCs.

Table (3) showed that ELISA titers of vaccinated non challenged animals were **640 – 640- 320** at day 90 after 2nd dose of vaccination and **320 –160 -320** at day 180 after 2nd dose of vaccination, titers declined at 6 months post vaccination, so re-vaccination every 6 months is recommended.

Conclusion:

Immunization of cattle with *B. bigemina* exoantigen vaccine elicit a significant cell mediated and humeral immune response which can protect cattle against virulent homologous challenge with 10⁹ of virulent *b. bigemina* infected RBCs, animals should be revaccinated every 6 months.

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كفاءة التحصين المحضّر من الأنتجين الخارجي لطفيل البايبيزيا بايجيمنا في وقاية الأبقار من الإصابة بهذا الطفيل

تم تقسيم 30 عجل بقري عمرها 12 شهر إلى 3 مجموعات، 10 في كل مجموعة، تم حقن المجموعتين الأولى والثانية بجرعتين من التحصين المحضّر من الأنتجين الخارجي لطفيل البايبيزيا بايجيمنا بينهما 21 يوم أما المجموعة الثالثة فقد تم حقنها بمادة الصابونين جرعتين بينهما 21 يوم. تم حقن المجموعتين الأولى والثالثة بكرات دم حمراء مصابة بطفيل البايبيزيا بايجيمنا الضاري بينما تركت المجموعة الثانية بدون إصابة.

وقد أظهرت النتائج زيادة كبيرة في كمية الأجسام المناعية المضادة لطفيل البايبيزيا بايجيمنا في المجموعتين الأولى والثانية بعد 21 يوم من الجرعتين الأولى والثانية من التحصين، أما المجموعة الثالثة فلم يتم اكتشاف أجسام مناعية تذكر بها وذلك باستخدام اختبار الأليزا.

وقد أظهرت النتائج أيضاً زيادة كبيرة في معدل إنقسام الليمفوسيتس في المجموعتين الأولى والثانية عندما تم قياسه بعد 15 يوم من الجرعتين الأولى والثانية من التحصين، أما المجموعة الثالثة فكان معدل الانقسام أقل بكثير.

المجموعة الأولى التي تم حقنها بكرات دم حمراء مصابة بطفيل البايبيزيا بايجيمنا الضاري بعد الجرعة الثانية من التحصين ب 90 يوم لم تظهر عليها أي أعراض للمرض أما المجموعة الثالثة فقد ظهر على معظم حيواناتها المرض بصورة شديدة.

مما سبق يتضح أن استخدام التحصين المحضّر من الأنتجين الخارجي لطفيل البايبيزيا بايجيمنا يوفر حماية كافية للأبقار من الإصابة بطفيل البايبيزيا بايجيمنا.