# IMMUNE STATUS OF CALVES VACCINATED WITH *BABESIA BOVIS* EXOANTIGENS DURING 6 MONTHS

*By*

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## ABSTRACT

*Babesia bovis* exoantigens produced protective immunity extended up to 6 months post vaccination. Two groups (1&3), each consisted of 3 calves, were vaccinated with exoantigens derived from Egyptian isolate. Two other groups (2&4), each of 2 calves, were inoculated with the adjuvant alone as controls.Challenge with the $1X10^9$ infected RBCs of virulent strain after 3 or 6 months of vaccination showed similar specific protection in immunized calves (groups1&3). The control calves (groups 2&4) were manifested by the severe clinical picture of the disease (fever, Jaundice, severe reduction in packed cell volume of erythrocytes and high parasitaemia). The vaccinated calves eliminated the parasite from circulation within 6-8 days while non-vaccinated controls showed continuous detectable parasitaemia for more than 3 weeks. Animals of non-vaccinated control (group 2) and none of immunized (group 1) were detected as carriers for the parasite, 3 months post challenge. Carrier status was confirmed by *in-vitro* cultivation of parasite in the blood of suspected animals using microaerophilous stationary phase (MASP). Pattern of humoral immune response for *B. bovis* was evaluated during a period of 6 months post vaccination in animals of different groups challenged either after 3 months (groups 1&2) or after 6 months (groups 3&4).

## INTRODUCTION

The protozoan *Babesia bovis* is a causative agent of bovine babesiosis, a heamotropic disease of cattle. The disease is endemic in Egypt due to the presence of the tick vectors *Boophilus spp.* The disease is accompanied with great economic impact in the development of livestock industry.

Exoantigens derived from *B. bovis* culture vaccine has proved efficacios with protozoan vaccines as it potentiates the humoral response to cell surface parasite antigens (**McColm *et al.*, 1982**). Furthermore, its use is practical and acceptable because of its ease in formulation due to high water solubility and because no tissue inflammation is evident at inoculation site (**Patarroyo *et al.*, 1995**). In Egypt exoantigens showed protective immunity against challenge a month post the booster dose (**Romany *et al.*, 1999**).

This report extends the previous vaccine study by detecting the humoral immune response during a period of 6 months post vaccination, the protection against challenge among immunized animals after 3 or 6 months and the carrier status among vaccinated and non-vaccinated animals after 3 months of challenge.

## MATERIAL AND METHODS

### 1- *B. bovis* Egyptian strain:

It was isolated from blood of naturally infected cattle at Damanhour, Behira, Egypt and identified microscopically beside Indirect immunofluorescent Antibody Technique (IFAT) using *B. bovis* specific IgG. The parasite was propagated in cell culture to produce exoantigen (immunogens) and to infect a susceptible calf for production of infected blood used for challenge of animals.

### 2- Production of Immunogens (Exoantigens) :

The parasite was cultivated according to the method of (**Levy and Ristic, 1980**) and the exoantigens vaccine was prepared as mentioned by (**Romany *et al.*, 1999**).

### 3- Animal vaccination:

Ten susceptible, 12-months old calves were free from any hemoparasite as proved by two successive Giemsa stained smears two weeks interval and IFAT. The calves were divided into 4 groups. The first and third groups (1&3) were consisted of 3 animals each and inoculated subcutaneously with 10.0 ml *B. bovis* (Egyptian strain) exoantigens containing 1.0 mg saponin as adjuvant, while the second and fourth groups (2&4) were consisted of 2 animals each and inoculated with 1 mg saponin in 10.0 ml PBS as adjuvant control. Booster dose was repeated after 3 weeks.

### 4– Evaluation of the humoral immune response using IFAT:

Serum samples were collected from each animal of all groups before & every weeks post vaccination and after challenge at a week intervals and used for detecting the specific antibody response of the animals. IFAT was applied according to (**Leeflang and Perie, 1972** and **Romany, 1996**) using 6 - 8% *Babesia bovis* infected RBCs as antigens and Fluorecin rabbit anti-bovine IgG (Cappel) diluted 1:100 in PBS as conjugate.

### 5- Protection against experimental challenge:

The calves of groups 1&2 were challenged 13 weeks after the booster dose by i/v inoculation of each calf with 1X $10^9$ *B. bovis* (Egyptian strain) infected RBCs (**Montenegro-James *et al.*, 1992**). Animals of groups 3 and 4 were challenged after 24 weeks. The responses of animals to the challenge were measured by the following parameters: 1- Duration and the percent of

parasitaemia by Giemsa stained blood smears. 2- Duration of fever and the maximal body temperature of calves. 3- The reduction in Packed cell volume (PCV) was measured.

### 6- Detection of *B. bovis* carriers by *in-vitro* cultivation of the blood of vaccinated and non–vaccinated animals:

The defibrinated blood of tested animals (group 1&2) were exposed to *in-vitro* cultivation of parasite using MASP system according to the method described by **Holman *et al.*, (1993),** briefly, the blood was centrifuged and decanted plasma and leukocyte coat. Erythrocytes were washed 3 times in PBS. Ten percent of RBCs packed cell volume was mixed with 90% complete medium. The complete medium is mixture of 58% medium 199 (the medium in Earls buffer + L-glutamine), 2% 1 M TES (N-tris-methyl-2-aminoethan-sulfonic acid) buffer, 40% bovine serum and an antibiotic mixture of 100 ug streptomycisn, 100 IU penicillin per ml, sterilized through 0.45 micron. The cultures were incubated at 37°C in a humidified 5% $CO_2$ incubator. Feeding was conducted for 15 days at 24 hrs intervals by removal of the supernatant medium and replaced with fresh prepared medium. Ten microlitres of cell layer were smeared daily and stained with Giemsa stain for detection of the parasite microscopically.

## RESULTS

Animals immunized with *B. bovis* exoantigens vaccine showed protection against challenge with $1X10^9$ of virulent *B. bovis* infected RBCs per animal after 3 months (group1) or after 6 months (group 3) compared with the non-vaccinated control groups (2&4). The body temperature of vaccinated animals was within normal bovine body temperature (38-39.5°C). While the controls showed a period of fever (>39.5 °C) extended up to 8 –9 days with maximal temperature (40.8 – 41.3°C). Also the animals immunized with exoantigens (groups 1&3) eliminated the parasite from circulatory blood faster, within 5-6 days in group 1 and 6-8 days in group 3, than in non-vaccinated animals (groups 2&4) which showed parasitaemia for more than 21 days. The maximal parasitaemia of vaccinated animals were 0.4 – 0.5% in group 1, 1.0 – 1.3% in group 3 while in non-vaccinated animals, it was 7.5 - 9% in group 2 and 7.0 - 7.5% in group 4. Reduction in PCV of animals vaccinated with exoantigens (groups1&3) was less than those of non-vaccinated (groups 2&4). The maximal reduction in PCV of animals was 8 –13% in group 1, 12.8 – 17.5% in group 3, 44 – 50% in group 2 and 51.2 – 52.5% in group 4 (Table 1).

To detect the parasite carriers of the challenged animals either vaccinated (group1) or non-vaccinated (group 2) 3-months post challenge, microscopical examination of thin and thick direct blood smears showed the protozoan in one of non-vaccinated animals was very low (>0.01%) and discontinuous. *In-vitro* cultivation of blood using MASP system confirmed that the animals of non-

vaccinated group were positive for *B. bovis*, while the vaccinated animals were free from the parasite (Table 2).

*B. bovis* specific antibody pattern of animals vaccinated with exoantigens groups (1&3) is illustrated in Tables (1 and 2) and Fig. (1) using IFAT. The titers increased gradually within 7-8 weeks from 1:40 to 1:5120 –10240 (the peak of antibodies level in serum). The IFA titers of vaccinated animals were 2560 in animals of group (1) 13-weeks post booster dose of vaccine (before challenge) and it were ranged from 320 to 640 in group (3) 24-weeks (before challenge). Post challenge, the humoral immune responses of immunized animals increased and were higher than non-vaccinated animals.

## DISCUSSION

Immunogens comprising soluble exoantigens are prime candidates as safe, efficacious and cost effective vaccine against bovine babesiosis (**James, 1989**). The efficacy of exoantigens derived from Egyptian isolate was confirmed one month after booster dose of the vaccine (**Romany *et al.*, 1999 & 2000**). Studies on exoantigens need to continue in order to detect the protection of immunized animals within 6 months post vaccination.

It is evident from the result in Table (1) that vaccinal immunity extended up to 6 months post vaccination and was sufficient to prevent the clinical symptoms and the losses of the disease. Consequently, the duration of protection induced by the vaccine appears sufficiently long to be safe for vaccinated animals even exposed to natural *B. bovis* infected *Boophilus spp.* ticks, vector of the disease, in enzootic zones (**Kuttler *et al.*, 1982**).

The limitation of serological or direct blood examination of suspected animals to detected carrier animals has been demonstrated by **Holman *et al.*, (1993)** guided them to detect the carrier by *in-vitro* cultivation using MASP system. In the present study, the non-vaccinated challenged animals of group 2 were carriers for *B. bovis* while none of vaccinated challenged animals of group 1 was positive (Table 2). This finding showed vaccination with exoantigens limited the spreading of the heamoprotozoan.

The ability of culture derived exoantigens to evoke a specific antibabesial antibodies was demonstrated by IFAT one week after vaccination and increased gradually. Immunological memory to these antigens was displayed by anamnestic response following secondary (booster) dose. This data is in agreement with finding of **Wandurgala, (1990)**. Tables (3 and 4) showed that the peaks of the antibody titers (1/5120 – 1/10240) were detected within 7 –8 weeks post the first dose of vaccination. IFA titers decreased gradually to 1/2560 – 1/5120 and 1/320 – 1/640 15-weeks and 27-weeks post vaccination respectively. Another anamnestic response was demonstrated post challenge

either after 16 or 27 weeks. The role of antibodies induced by parasite killing, reduction of erythrocyte destruction and minimizing other clinical symptoms associated with the disease (**Hall *et al.*, 1968**). In the worker's opinion and according to (**Matijatko *et al.*, 1999**) severe reduction of PCV of blood resulted in great losses in productivity.

In the present study, comparable groups of calves were used for testing duration of *B. bovis* exoantigens derived from Egyptian isolate as immunogens. *Babesia* exoantigens proved useful and effective in protecting highly susceptible cattle against babesiosis up to 6 months. Immunization of cattle is still beneficial and recommended because active immunization enhances the protective immune responses against this economically important disease.

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**Table (1): Protection parameters of animals vaccinated with *B. bovis* exoantigens vaccine and challenged with virulent strain of *B. bovis*.**

| Parameters | Challenge after 3 months | | | | | | | Challenge after 6 months | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of vaccinated animals (group 1) | | | | No. of control animals (group 2) | | | No. of vaccinated animals (group 3) | | | | No. of control animals (group 4) | | |
| | 1 | 2 | 3 | Mean | 4 | 5 | Mean | 6 | 7 | 8 | Mean | 9 | 10 | Mean |
| Maximal body temperature (°C) | 38.4 | 38.3 | 38.5 | 38.4 | 40.9 | 41.0 | 41.0 | 39.2 | 39.4 | 39.4 | 39.4 | 40.8 | 41.3 | 41.1 |
| Duration of fever (days) | 0 | 0 | 0 | 0 | 8 | 9 | 8.5 | 0 | 0 | 0 | 0 | 8 | 8 | 8 |
| Maximal parasitaemia (%) | 0.5 | 0.4 | 0.5 | 0.5 | 9.0 | 7.5 | 8.75 | 1.0 | 1.3 | 1.2 | 1.2 | 7.0 | 7.5 | 7.5 |
| Period of parasitemia (days) | 5 | 5 | 6 | 5 | >21 | >21 | >21 | 7 | 6 | 8 | 7 | >21 | >21 | >21 |
| PCV before challenge | 36 | 38 | 38 | 37.3 | 38 | 36 | 37 | 38 | 40 | 39 | 39 | 40 | 39 | 39.5 |
| Lowest PCV post challenge | 33 | 33 | 34 | 33.3 | 19 | 20 | 19.5 | 32 | 33 | 34 | 33 | 19 | 19 | 19 |
| Maximal reduction in PCV (%) | 8 | 13 | 10.5 | 10.7 | 50 | 44 | 47.3 | 15 | 17.5 | 12.8 | 15.3 | 52.5 | 51.2 | 51.8 |

- Normal bovine body temperature is 38 - 39.5°C, above which is considered fever **(Blood and Radostits, 1989)**.
- Detectable parasitaemia was > 0.1%. PCV = Packed cell volumes of animals.
- Maximal Reduction in PCV (%) = $\frac{\text{PCV before challenge} - \text{lowest PCV after challenge X 100}}{\text{PCV before challenge}}$

**Table (2): Microscopical detection of the carrier animals either vaccinated with *B. bovis* exoantigens or non-vaccinated after 3 months of challenge.**

| Detection of parasite | *B. bovis* carrier animals | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of vaccinated and challenged animals (group 1) | | | | | No. of non-vaccinated challenged animals (group 2) | | | |
| | 1 | 2 | 3 | No. of positive | Percent | 4 | 5 | No. of positive | Percent |
| Before *in-vitro* cultivation | - | - | - | 0/3 | 0% | + | - | 1/2 | 50% |
| After *invitro* cultivation in MASP system | - | - | - | 0/3 | 0% | + | + | 2/2 | 100% |

**Table (3): Specific antibody titers during 6 months of animals vaccinated with exoantigens and challenged with virulent strain of *B. bovis* 13- weeks after the inoculation with the booster dose of vaccine using IFA.**

| Group Inoculated with | Animals no. | *B. bovis* specific antibody titres | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Weeks post vaccination | | | | | | | | | | | | | | | | | Weeks post challenge | | | | | | | | | |
| | | 0 | 1 | 2 | 3* | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| *B. bovis* exo-antigens vaccine (group1) | 1 | -* | 40 | 80 | 160 | 320 | 1,280 | 2,560 | 5120 | 5,120 | 5,120 | 5,120 | 5,120 | 5,120 | 2,560 | 2,560 | 2,560 | 2,560 | 1,280 | 5,120 | 10,240 | 10,240 | 5,120 | 5,120 | 5,120 | 5,120 | 2,560 | 2,560 | 2,560 |
| | 2 | -* | 40 | 80 | 160 | 640 | 1,280 | 2,560 | 5,120 | 10,240 | 10,240 | 5,120 | 5,120 | 5,120 | 5,120 | 2,560 | 2,560 | 2,560 | 1,280 | 10,240 | 20,480 | 20,480 | 10,240 | 10,240 | 10,240 | 5,120 | 5,120 | 5,120 | 5,120 |
| | 3 | -* | 40 | 160 | 160 | 640 | 2,560 | 2,560 | 5,120 | 10,240 | 10,240 | 10,240 | 5,120 | 5,120 | 5,120 | 5,120 | 5,120 | 2,560 | 1,280 | 10,240 | 20,480 | 20,480 | 20,480 | 10,240 | 10,240 | 10,240 | 10,240 | 5,120 | 5,120 |
| | M | -* | 40 | 106 | 160 | 533 | 1,706 | 2,560 | 5,120 | 8,533 | 8,533 | 6,826 | 5,120 | 5,120 | 4,266 | 3,413 | 3,413 | 2,560 | 1,280 | 8,533 | 17,066 | 17,066 | 11,946 | 8,533 | 8,533 | 6,826 | 5,973 | 4,266 | 4,266 |
| Saponin as control (group 2) | 4 | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | 160 | 640 | 2,560 | 2,560 | 2,560 | 2,560 | 1,280 | 1,280 | 1,280 | 1,280 | 1,280 |
| | 5 | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | 160 | 1,280 | 2,560 | 2,560 | 2,560 | 2,560 | 2,560 | 1,280 | 1,280 | 1,280 | 1,280 |
| | M | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | 160 | 960 | 2,560 | 2,560 | 2,560 | 2,560 | 1,920 | 1,280 | 1,280 | 1,280 | 1,280 |

* Booster dose was inoculated at the 3rd week ** Challenge at the 16th week.

M = Mean of titers -* = was negative at dilution 1:40

**Table (4): Specific antibody titers during 6 months of animals vaccinated with exoantigens and challenged with virulent strain of *B. bovis* 24 -weeks after inoculation the booster dose of vaccine using IFA.**

| Group Inoculated with | Animal no. | *B. bovis* specific antibody titres | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Weeks post vaccination | | | | | | | | | | | | | | | | | | | | | | | | | | | Weeks post challenge | | | |
| | | 0 | 1 | 2 | * 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | ** 27 | 1 | 2 | 3 | 4 |
| *B. bovis* exo-antigens vaccine (group 3) | 6 | -* | 40 | 80 | 160 | 320 | 1,280 | 2,560 | 5,120 | 5,120 | 5,120 | 5,120 | 5,120 | 5,120 | 2,560 | 2,560 | 2,560 | 2,560 | 2,560 | 1,280 | 1,280 | 1,280 | 1,280 | 640 | 640 | 640 | 320 | 320 | 320 | 160 | 640 | 2,560 | 5,120 |
| | 7 | -* | 40 | 80 | 160 | 640 | 1,280 | 2,560 | 5,120 | 10,240 | 10,240 | 5,120 | 5,120 | 5,120 | 5,120 | 2,560 | 2,560 | 2,560 | 2,560 | 2,560 | 1,280 | 1,280 | 1,280 | 1,280 | 640 | 640 | 640 | 320 | 320 | 160 | 1,280 | 2,560 | 10,240 |
| | 8 | -* | 40 | 160 | 160 | 640 | 2,560 | 2,560 | 5,120 | 10,240 | 10,240 | 10,240 | 5,120 | 5,120 | 5,120 | 5,120 | 5,120 | 2,560 | 2,560 | 2,560 | 2,560 | 2,560 | 1,280 | 1,280 | 1,280 | 1,280 | 640 | 640 | 640 | 320 | 1,280 | 5,120 | 10,240 |
| | M | -* | 40 | 106 | 160 | 543 | 1,706 | 2,560 | 5,120 | 8,533 | 8,533 | 6,826 | 5,120 | 5,120 | 4,266 | 3,413 | 3,413 | 2,560 | 2,560 | 2,133 | 1,706 | 1,706 | 1,280 | 1,066 | 853 | 853 | 533 | 426 | 426 | 213 | 1,066 | 3,413 | 8,533 |
| Saponin as control (group 4) | 9 | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | 80 | 640 | 2,560 | 2,560 |
| | 10 | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | 160 | 640 | 2,560 | 2,560 |
| | M | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | -* | 120 | 640 | 2,560 | 2,560 |

* Booster dose was inoculated at the 3rd week
M = Mean of titers

** Challenge at the 27th week.
-* = was negative at dilution 1:40

**Figure (1): Thin smears of *B. bovis* infected RBCs stained with fluorescein isothiocyanate after reaction with:**
**A- Serum of animal vaccinated with *B. bovis* exoantigens.**

**B- Serum of non-vaccinated animal (negative control).**

## الملخص العربي

# الاستجابة المناعية للعجول المحصنة بالبابزيا بوفيز اكسوأنتجين خلال ستة أشهر

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أحدث الأكسوأنتيجين للبابزيا بوفيز حماية مناعية امتدت إلى ستة أشهر بعد التحصين حيث تم تحصين مجموعتان (ثلاث عجول لكل منها) بالأكسوأنتجين المحضر من العترة المصرية بالإضافة إلى مجموعتان استخدمت كمجموعات ضابطة.

أظهر اختبار التحدي بعد ثلاث أو ست شهور والذي أجرى بحقن ١ × ١٠ $^{٩}$ من كرات الدم المصاب بالطفيل عن وجود حماية مناعية خاصة ضد الطفيل في الحيوانات المحصنة (المجموعات ١،٣)في حين أظهرت حيوانات المجموعات الضابطة الصورة الحادة للمرض ممثلة في حمى ، صفراء ، اختزال حاد في كمية كرات الدم (PCV) ونسبة اصابة عالية بالطفيل. الحيوانات المحصنة تخلصت من الطفيل في الدم خلال ٥:٨أيام في حين استمر وجود الطفيل في الدم لمدة أكثر من ٣ أسابيع في دم حيوانات المجموعات الضابطة.

وجدت حيوانات المجموعة الثانية حامله للطفيل بعد ٣ شهور من اختبار التحدي ولم يتم تحديدا أي من الحيوانات المحصنة في المجموعة الأولى كحامل للطفيل وتم التأكد من هذه النتيجة بزرع الطفيل لدم كل الحيوانات المجموعة الأولي والثانية باستخدام الميكرو إيروفيرس ستشنرى فيز

شملت الدراسة تقيم الإدرار للأجسام المناعية الناتجة خلال الستة أشهر بعد التحصين في حيوانات المجموعات المختلفة والتي تعرضت لاختبار التحدي بعد ثلاث شهور (مجموعات١ ، ٢ )أو بعد ستة شهور (مجموعات٣،٤)